

BIOCATALYST

3RD YEAR SECONDARY

LIFE SCIENCES
SECTION

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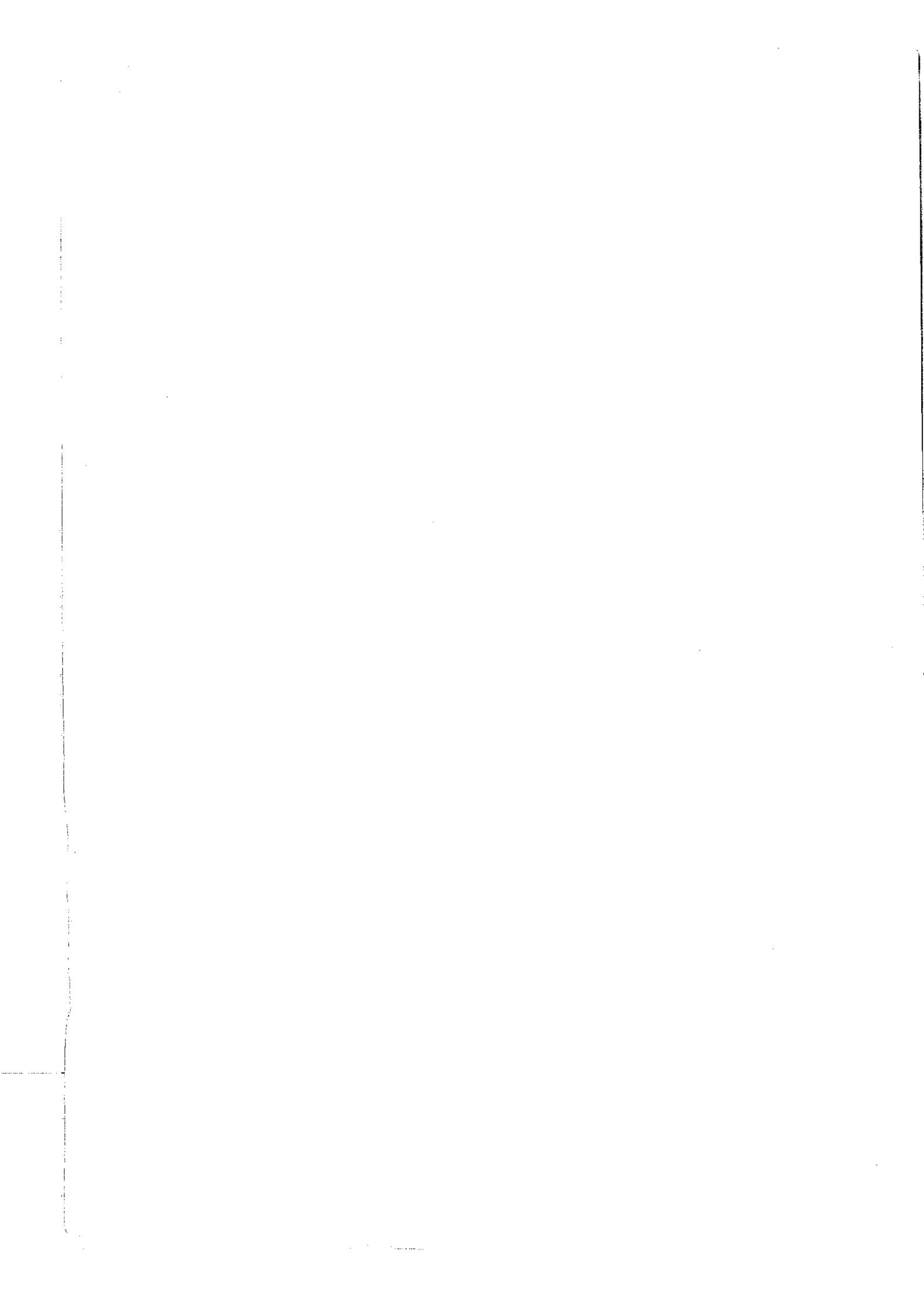
Biocatalyst
3rd secondary year
Life sciences section



Preface

This book has been developed to make it easier for the learners to review courses and to train them in different types of exercises. In addition, it was intended to help teachers prepare course abbreviations, design tests, and provide unsolved exercises that could be given as homework.

On the other hand, this book provides a simplification of action verbs and their application; it also provides a classification of official exercises by theme to facilitate the preparation of worksheets and training for official exams.



What distinguish this book:

-  Simplified course extract.
-  Simple training exercises on the objectives.
-  Non-solved exercises.
-  Official exercises sorted by chapter.
-  A content that corresponds to the new reductions.
-  Exercises and answers respecting the official criteria.
-  Clarification of the action verbs.

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The action verbs

1. Analyze:

- 1.1. Analyze the results of an experiment: Indicate the variations of the results of an experiment when a factor (the variable) is introduced, deleted or changed, without going into the details of the experiment and without indicating the common factors.
- 1.2. Analyze a curve (or a table that can be transformed into a curve): Indicate the intervals of the variations of the ordinate according to the abscissa.
- 1.3. Analyze a histogram (or a bar graph, or a table that can be transformed into a histogram or a bar graph): compare the values of each given measurement in the different situations).

2. Calculate: Write a mathematical operation to find values.

3. State: Express a law or a principle without explanation.

4. Compare:

- 4.1. Compare mechanisms, cells, structures, organs: indicate common points (similarities) and differences between them.
- 4.2. Compare values: use more, less or equal to point out the relation between them.

5. Complete: Fill in what is missing.

6. Conclude: Write a general relationship between a condition and its result, an effect of a factor, an action ...

7. Determine: Make a logical reasoning to reach a decision, use in the reasoning the necessary information of the documents in relation.

8. Describe: Express the details of an observation, experiment, diagram, device using a scientific language with time or location connectors, but not with logical connectors.

9. Show: Specify the arguments that prove something obvious by making appropriate logical reasoning.

10. Deduce: Write a conclusion or a decision based on a limited analysis of related data.

11. Draw out: Take information from relationships between data without any explanation or justification.

12. Distinguish: Indicate a difference that discerns one thing from another.

13. Explain: Base on acquired knowledge or on data to make understand a phenomenon, a result ...

14. Identify: Recognize something from its characteristics (write the characteristic, the given information and the name of the identified element).

15. Interpret: Analyze and write meaning (non-general conclusion in direct relation to results).

16. Indicate: Write the name without justification.

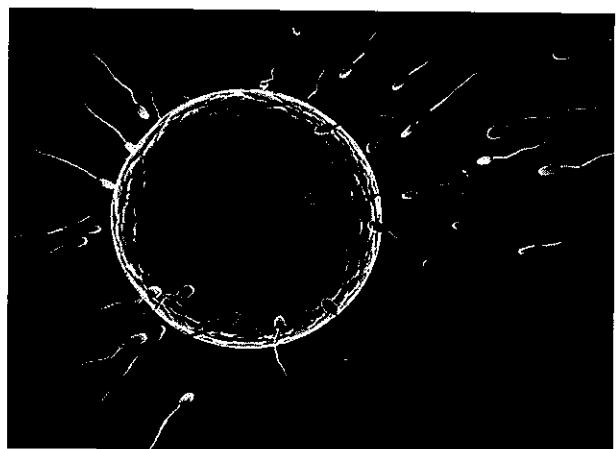
17. Justify: Write an argument to confirm an answer.

18. Specify: Indicate and justify.

19. Pick out: Take information from the data without any explanation or justification.

20. Verify: Confirm with an argument, a reasoning if something is correct or not.

Ch. 1 Reproduction





Reproduction

Course abstract

1. Male and female reproductive systems

	Male reproductive system	Female reproductive system
Gonads	Testes: production of sperm cells and testosterone	Ovaries: production of oocytes and of estrogen and progesterone
Genital tracts	<u>Epididymis</u> : maturation and storage of spermatozoa. <u>Vas deferens</u> : conduction and storage of spermatozoa. <u>Urethra</u> : conduction of sperm and urine to the outside.	<u>Fallopian tube</u> : reception of the released oocytes and site of fertilization. <u>Uterus</u> : site of implantation and development of the embryo.
Accessory glands	Seminal vesicles, prostate, Cowper's glands: secretion of seminal fluid (rich in fructose ensuring survival and motility of spermatozoa).	-
Organs of copulation	Penis	Vagina
Activity	Continuous from puberty till death	Cyclic from puberty till menopause, interrupted by gestation
Number of gametes	300 million per ejaculation	1 oocyte per menstrual cycle

Comparative table between male and female reproductive systems

In both sexes, the primary sex characteristics are determined by the gonads present from birth. On the other hand, secondary sexual characteristics are controlled by the corresponding sex hormones and are manifested from puberty.

2. Diploid and haploid cells

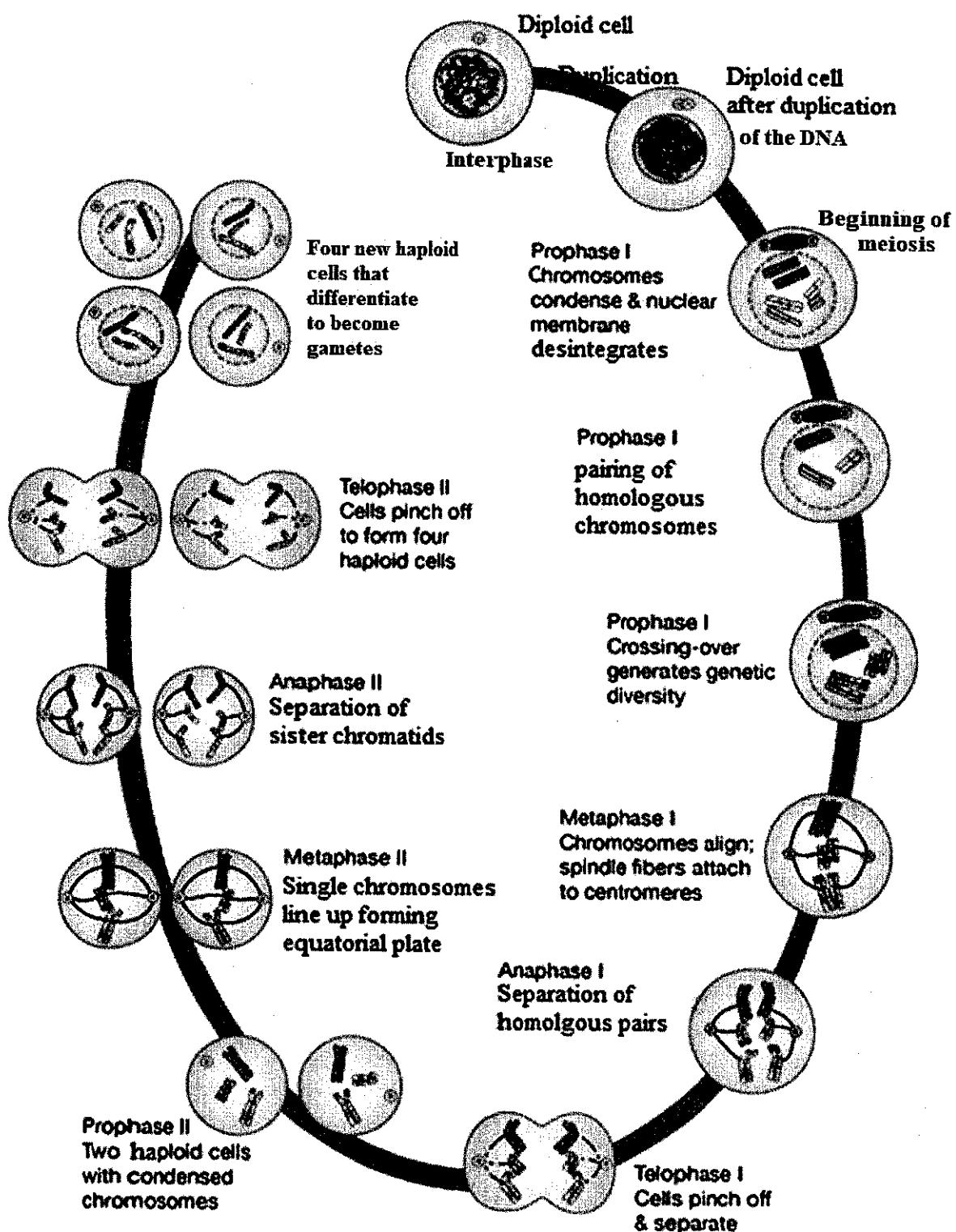
The diploid cell contains $2n$ chromosome (23 pairs of chromosomes in humans) i.e. having two copies of each of the chromosomes, these chromosomes can be with one or two chromatids each. The haploid cell contains n chromosomes (23 chromosomes in humans) i.e. having a single copy of each chromosome, these chromosomes can be with one or two chromatids each.

	Diploid cell 2n of 2 chromatids	Diploid cell 2n of 1 chromatid	Haploid cell n of 2 chromatids	Haploid cell n of 1 chromatid
Female	 1 2 3 4 XX	 1 2 3 4 XX	 1 2 3 4 X	 1 2 3 4 X
Male	 1 2 3 4 XY	 1 2 3 4 XY	 1 2 3 4 X	 1 2 3 4 Y

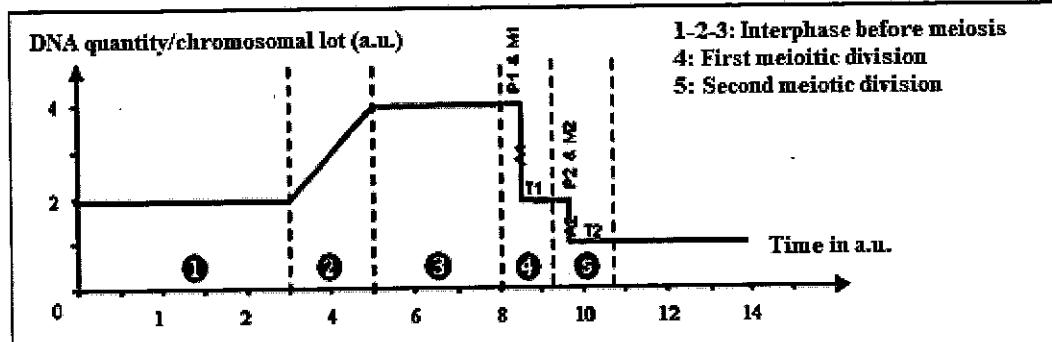
Table showing the karyotypes of diploid for $2n=10$ and haploid cells for $n = 5$

3. Meiosis

3.1. Phases of meiosis



3.2. Variation of the DNA quantity during meiosis



Variation of the DNA quantity per chromosomal lot during an interphase and meiosis

4. Gametogenesis: spermatogenesis and oogenesis

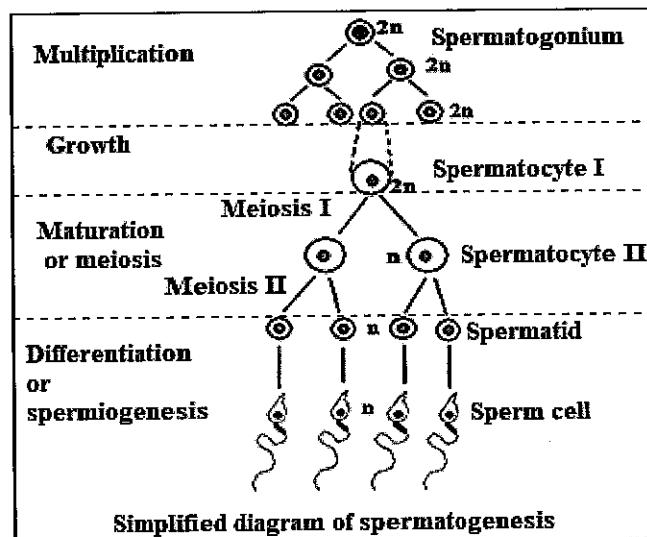
Spermatogenesis and oogenesis are two processes responsible for the formation of male gametes (spermatozoa) and female gametes (oocytes II blocked in MII) respectively. Spermatogenesis finish in the seminiferous tubules in the testes while oogenesis continues in the released oocyte after fertilization to give a fertilized ovum. Each of these processes have 4 successive phases: multiplication-growth-maturation-differentiation. But they have some differences mentioned in the table below.

	Spermatogenesis	Oogenesis
Place	Seminiferous tubules in testes	Ovaries and oviducts
Number	4 sperm cells per each spermatocyte I	1 oocyte II per each oocyte I and 1 fertilized ovum per each oocyte II
Rate of production	Daily (millions/day)	Monthly (1 oocyte II / cycle)
Duration of production	74 days in continuous manner	Undetermined due to blockages
Renewal of germ stem cells	Renew of spermatogonia by mitosis	No renewal of oogonia

4.1. Spermatogenesis

Two types of cells are necessary for the spermatogenesis: interstitial cells (Leydig cells) producing the testosterone that plays a role in the stimulation of the spermatogenesis and the Sertoli cells that support and nourish the descendant cells of spermatogonia.

The adjacent diagram shows the phases of spermatogenesis.



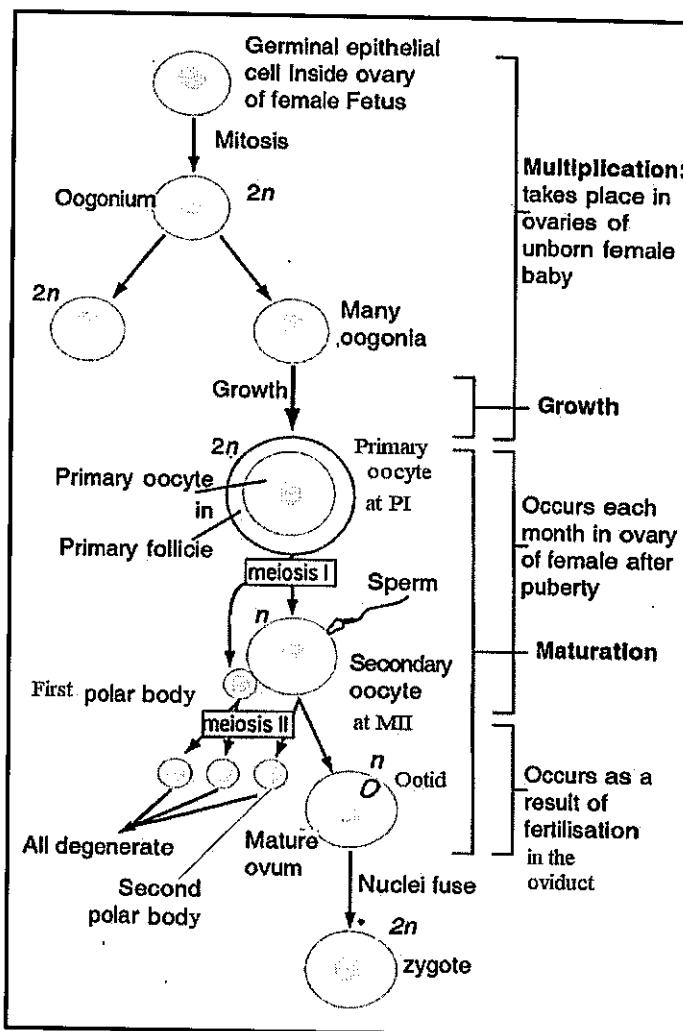
4.2. Oogenesis

The activity of the female reproductive system is cyclic. During each cycle, there is a change at follicle and oocyte level. Before 3-4 months of each cycle, 500 primordial follicles develop but only one follicle develops into a cavitary follicle at the beginning of the corresponding cycle and the others degenerate.

During the follicular phase of a cycle, the cavitary follicle develops and becomes a Graafian follicle (mature follicle) about 14 days later. Few hours before ovulation, the oocyte I blocked in prophase 1 resumes its meiotic activity and blocks in metaphase 2. On the 14th day, the envelope of Graafian follicle breaks and releases the oocyte II blocked in metaphase 2. During the luteal phase, the ruptured follicle turns into a corpus luteum which degenerates 14 days later and the oocyte II degenerates approximately 24 hours after ovulation, if there is no fertilization.

In case of fertilization, the corpus luteum is developed and maintained during the first three months of pregnancy, while the oocyte II completes, in the oviduct, its second meiotic division to be transformed into a zygote after releasing the second polar body.

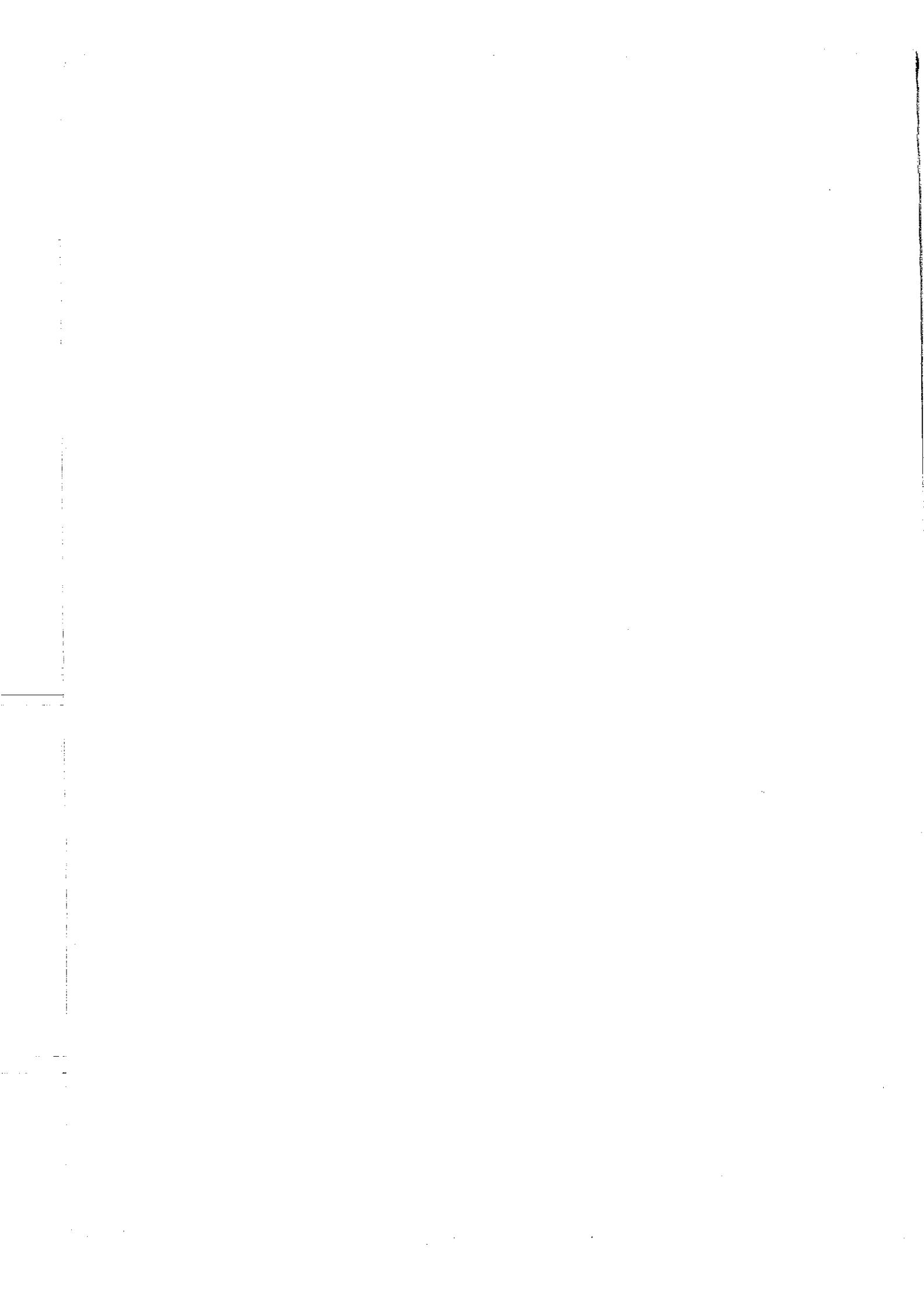
The following diagram represents the phases of oogenesis with their durations.



5. Fertilization

Fertilization takes place in the fallopian tube. It is the union between a spermatozoon (after having acquired its fertilizing capacity in the female genital tract - capacitation) and an oocyte II blocked in MII.

Fertilization reestablish the diploidy of the species after meiosis has been establishing the haploidy.



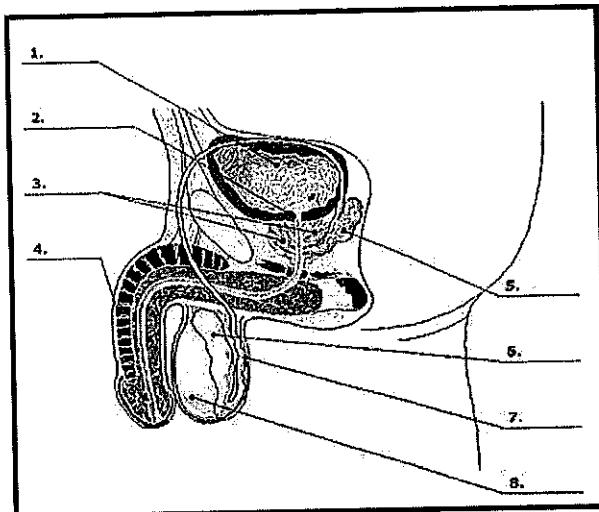
Reproduction Training exercises

EXERCISE 1 Male and female reproductive systems

The figure in document 1 shows a human reproductive system.

1. Name the reproductive system in document 1.
2. Label the numbers indicated on the figure of the document 1.
3. Indicate the pathway of the sperm cells in:
 - 3.1. The male reproductive system.
 - 3.2. The female reproductive system after mating.

In order to make them sterile, the sultans had practiced testes removal (castration) of the males that were in charge to protect and take care of their wives.

**Document 1**

4. Interpret the results obtained after castration.

5. Explain why the castrated men do not show secondary male sexual characteristics.

Sperm cells are taken from the beginning and the ends of the epididymis. They are mixed with oocytes after being subjected to some treatments. Fertilization is observed only if the sperm cells are taken from the end of the epididymis.

6. Explain the obtained results.

Normal sperm cells are produced by the testes of a man, but these sperm cells do not show any ability to make fertilization of the female gametes.

7. Formulate two hypotheses about the possible origins of the problem shown by this man.

A medical test of a sterile woman shows that she has normal release of oocytes, her problem is caused by a blockage in the oviducts.

8. Explain why a blockage of the oviducts leads to sterility in the women.

Solution:

1. Male reproductive system.

2. 1. Urinary bladder. 2. Urethra. 3. Prostate. 4. Penis. 5. Seminal vesicle. 6. Epididymis. 7. Vas deferens. 8. Testis.

- 3.1. Testis – epididymis – vas deferens – urethra (through the prostate then the penis).

- 3.2. Vagina – cervix – uterus – oviduct.

4. The castration of the males by removal of their testes leads to the sterility of them this means that the testes are responsible for the production of gametes.

5. The testes are responsible for the production of male sex hormones that are responsible for the appearance of the secondary sexual characteristics, then after the ablation of the testes no more male hormones are secreted and the secondary sexual characteristics do not appear.

6. The epididymis is the site of the maturation of the sperm cells, sperm cells acquire in it their motility that allows them to move toward the oocyte and fertilize it. Otherwise, sperm cells cannot make fertilization of the oocyte even if they are normal.

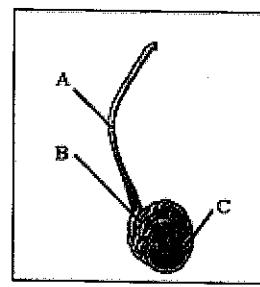
7. Hypothesis 1: A defect in the epididymis makes it unable to realize sperm cells maturation.
Hypothesis 2: The seminal fluid does not contain the necessary nutrients for the survival of the sperm cells.

8. The fertilization needs the meeting of a sperm cell with the oocyte in the fallopian tube. The sperm cells pass from the vagina to the uterus then to the oviduct where fertilization of the oocyte takes place. A blockage of the oviduct prevents the sperm cells from entering in the oviduct and make fertilization.

EXERCISE 2 Sperm cells motility

The structure in document 1 corresponds to a part of a reproductive system.

1. Give a title to this structure.
2. Label the letters A, B and C in document 1.
3. Indicate the role of each part of this structure.

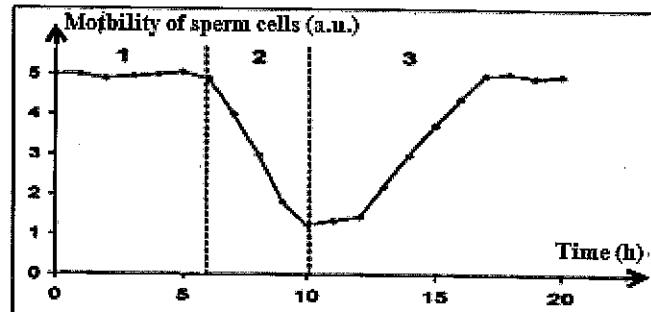


Document 1

Sperm motility depends on the beats of the flagellum that consumes energy in the form of chemical substances called ATP.

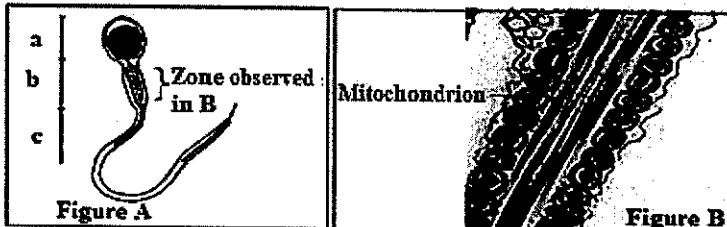
Sperm motility is assessed under three different conditions (Medium 1: ATP supply, Medium 2: no ATP supply, Medium 3: ATP supply). The results are shown in document 2.

4. Interpret the results presented in Document 2.



Document 2

Document 3 represents the organization of a spermatozoon: Figure A shows its general organization; figure B shows the part b of figure A observed under an electron microscope (longitudinal section).



Document 3

5. Label items a, b and c.
6. Explain, with reference to documents 2 and 3, the origin of motility of spermatozoa.

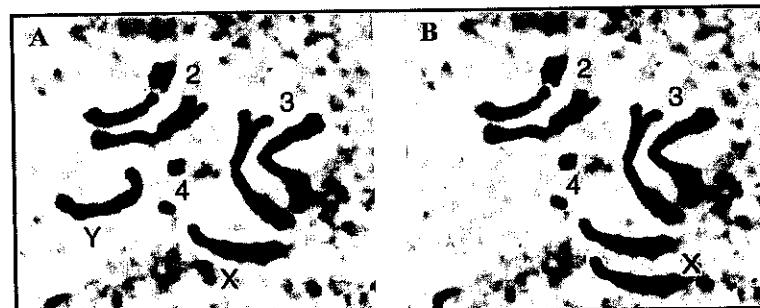
Solution:

1. Part of the male reproductive system.
2. A. Vas deferens, B. Epididymis, C. Testis.
3. A: Storage and conduction of spermatozoa.
B: Storage, conduction and maturation of spermatozoa.
C: Production of spermatozoa and the male sex hormone, testosterone.
4. In the medium 1 in the presence of ATP the motility of the spermatozoa is almost constant at 5 a.u. during 6h. On the contrary, this motility decreases to 1.5 a.u. in medium 2 devoid of ATP for 4 hours. While it increases again to reach 5 a.u. in medium 3 in the presence of ATP, same value as in medium 1. This shows that ATP is necessary for the mobility of spermatozoa.
5. a- Head b- middle piece c- flagella
6. The text shows that the spermatozoon is mobile due to the beats of the flagellum but this motility needs energy in the form of ATP verified in document 2. Document 3 shows that the flagellum is connected to the intermediate part rich in mitochondria which play a vital role in cell oxidation and energy release in form of ATP. The origin of sperm motility is the energy released by the mitochondria presented in the intermediate piece and which ensure the flapping of the flagellum to ensure mobility.

EXERCISE 3 Phases of meiosis

The following drawings represent the karyotypes of two drosophila A and B of different sex with $2n = 8$.

1. Compare the 2 karyotypes A and B in document 1.
2. Identify the sex of each of these drosophilae.



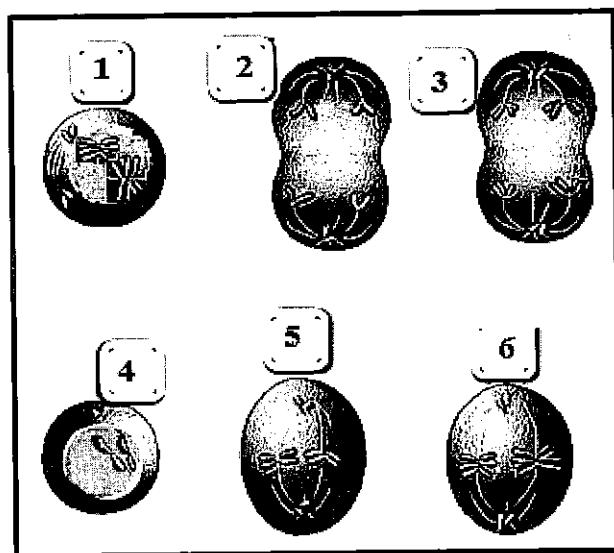
Document 1

We find in the ovaries of a female drosophila the cells that undergo meiosis. The following figure represents several stages of this division. To simplify, only two pairs of chromosomes are represented.

3. Name the phase 1 in document 2. Justify.
4. Justify that the phase 3 is the anaphase 1 of meiosis.
5. Compare the two phases 5 and 6.
6. Arrange in chronological order the figures in document 2.
7. Indicate the DNA quantity in terms of Q in the cells 4, 5 and 6. Justify.

Solution:

1. The two karyotypes A and B contains the same number of chromosomes (8) that are all of 2 chromatids, the karyotype B show two chromosomes X while A shows one chromosome X and one chromosome Y.
2. Since the karyotype A includes two chromosomes X so the drosophila A is a female that is characterized by the presence of two sex chromosomes X, and since the karyotype B includes one chromosome X and one chromosome Y so the drosophila B is a male that is characterized by two sex chromosomes X and Y.
3. The phase 1 is the prophase 1 of meiosis since the homologous chromosomes are paired.
4. In the phase 3 there is migration, towards the poles of the cells, of chromosomes of 2 chromatids each. Then this is the anaphase of meiosis 1.
5. In the two phases 5 and 6 the chromosomes are lined up to form the equatorial plate, but they are pairs in 6 while they are single in 5.
6. 1, 6, 3, 5, 2, 4.
7. 4: $Q/2$ since they are $n = 2$ chromosomes of 1 chromatid each.
5: Q since they are $n = 2$ chromosomes of 2 chromatids each.
6: $2Q$ since they are $2n = 4$ chromosomes of 2 chromatids each.



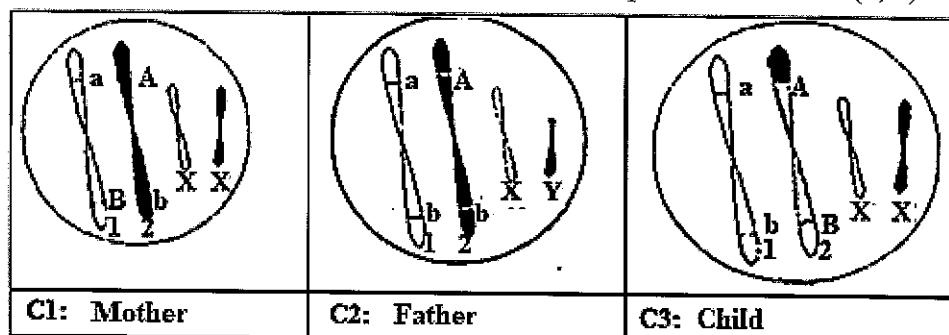
Document 2

EXERCISE 4 Meiosis and gametes

From parents to their descendants, sexual reproduction ensures genetic recombination through meiosis and fertilization. The cells C1, C2 and C3 in document 1 belong respectively to a mother, a father and one of their children.

Only one pair of autosomes (1,2) and the pair of sex chromosomes were considered; the chromosomes of paternal origin are represented in black and that of maternal origin in white.

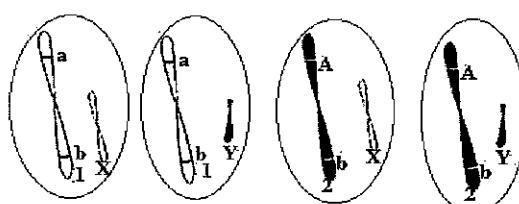
(A, a) and (B, b) are respective alleles of 2 genes located on the pair of autosomes (1, 2).



1. Represent, based on the behavior of chromosomes during meiosis:
 - 1.1 Two types of gametes that can be produced by the father (without crossing over).
 - 1.2 Two types of gametes that can be produced by the mother (without crossing over).
2. Schematize the gamete given by the father to the child.

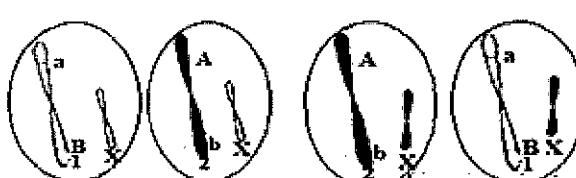
Solution:

1.1.



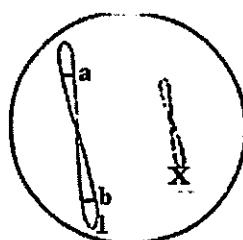
The different types of gametes produced by the father (two gametes are enough)

1.2.



The different types of gametes produced by the mother (two gametes are enough)

2. Gamete given by the father to the child.



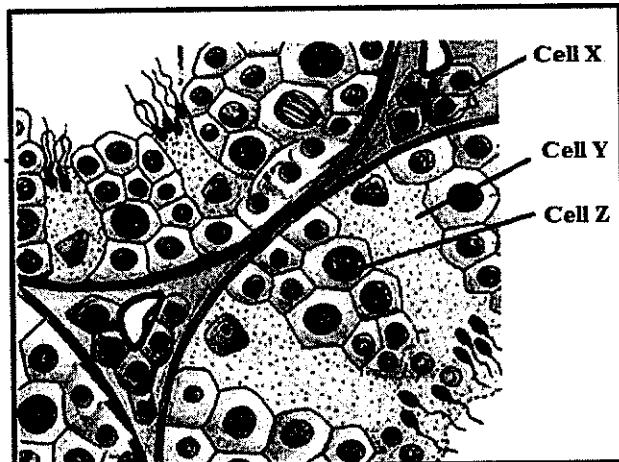
EXERCISE 5 The seminiferous tubules

The diagram in document 1 shows a cross section of some seminiferous tubules of a fertile man.

1. Specify cells X, Y and Z in document 1.
2. Draw the anaphases of meiosis for a cell with $2n = 6$.
3. Distinguish, based on the acquired knowledge, the chromosomal content of spermatocyte I with that of the spermatozoon.

One of the cells shown by document 1 is in anaphase.

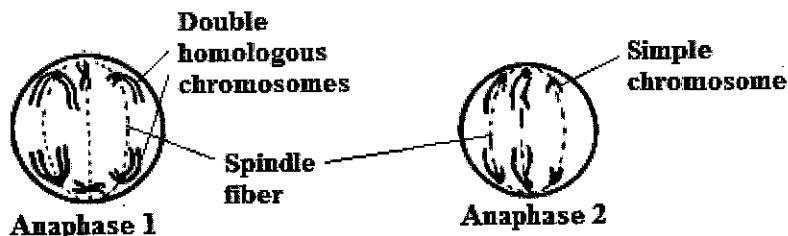
4. Determine the cell division to which this anaphase corresponds.



Document 1

Solution:

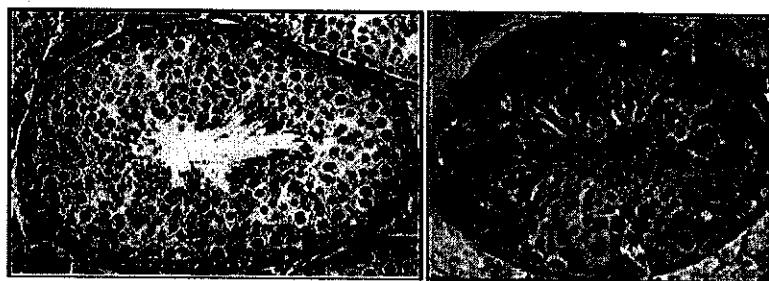
1. The cell X is a Leydig cell since it is located outside the seminiferous tubule between the various tubules.
The cell Y is a Sertoli cell since it is a large cell in contact with all the germ cells.
The cell Z is a spermatocyte I since it is a large cell i.e. it has undergone cellular growth and is close to the basal lamina.
2. Diagram showing the anaphases of meiosis for a cell $2n = 6$



3. Spermatocyte I and spermatozoon each have 23 chromosome types from 1 to 22, these chromosomes are in pairs of homologous chromosomes (same size and bands) each having two chromatids in spermatocyte I whereas they are singular chromosomes for one spermatozoon each with a single chromatid. Spermatocyte I has two sex chromosomes X and Y with two chromatids each while the sperm has a single sex chromosome X or Y with only one chromatid each.
4. It is a single cell that undergoes anaphase and it is large and also very close to the basement membrane so it is the spermatocyte I that does the anaphase. So, this is the anaphase of the first meiotic division.

EXERCISE 6 An abnormality of seminiferous tubules

Microscopic observation was performed for a seminiferous tubule of a normal and sterile man. The photomicrographs obtained are shown in documents 1 and 2 respectively.



Document 1

Document 2

1. Explain, based on acquired knowledge, the variation in the size of the germ cell as it passes towards the center of the seminiferous tubule shown in document 1.
2. Compare the abnormal seminiferous tubule in document 2 to the normal seminiferous tubule.
3. Formulate a hypothesis that explains the sterility of this man.

The table in document 3 shows the variation of the amount of DNA per cell with time during a part of the phenomenon illustrated by document 1.

Time in hours	3	7	7.1	9	9.1
DNA quantity / cell in a.u.	12	12.1	6	6	3

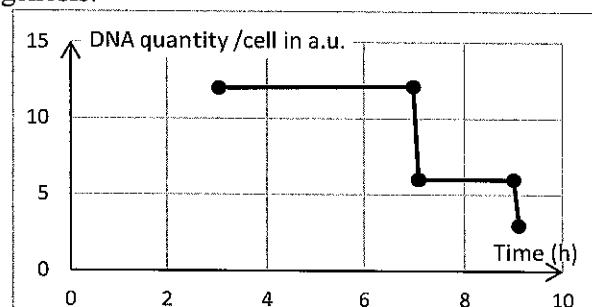
Document 3

4. Represent in a graph the variation in the amount of DNA as a function of time.
5. Specify the cells that correspond to the variations shown by the table in Document 3.

Solution:

1. During the migration of the cells towards the center of the seminiferous tube, they undergo first an increase of size of the spermatogonia so that they become spermatocytes I due to growth phase, then the spermatocytes I undergo meiosis during which each divides into two spermatocytes II and then four spermatids causing a very rapid decrease in cell size as shown in Document 1 in the center of the seminiferous tube where the cells (sperm cells) are very small.
2. Both normal and abnormal seminiferous tubules have the same layers of germ cells, spermatogonia and cells derived from them, with the exception of spermatozoa that appear in the center (lumen) of the normal seminiferous tubule while spermatozoa are absent in the center of the abnormal seminiferous tube that has no lumen.
3. Hypothesis: this man has blocked spermiogenesis.

4. Graph representing the variation of the DNA quantity per cell as a function of time.
(Scale $\boxed{2 \text{ u.a.}}$)



5. Spermatocyte I is the cell that enters meiosis I and gives spermatocyte II, so it is the cell that corresponds to the first drop in the amount of DNA from 12.1 ua to 6 ua between 7 and 7.1 h. Then the spermatocyte II is the cell that enters into meiosis II to give spermatids so it corresponds to the second drop in the amount of DNA between 9 -9.1 h.

EXERCISE 7 Female gametes

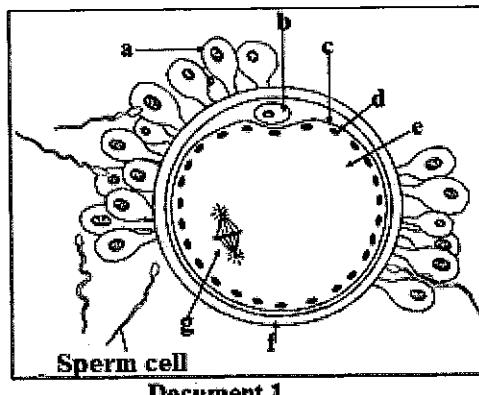
The production of gametes in women and men does not follow the same rules:

- Unlike other mammals, women ovulate spontaneously, cyclically, regardless of the season and for only 30 years.
- In men, spermatozoa are not produced cyclically, but are produced at a continuous rate from puberty and throughout life.

In women, the stock of oocytes or female gametes is not inexhaustible, but limited. A woman has about 400 menstrual cycles during her life.

Document 1 shows a photograph of a female cell.

1. Pick out from the text the expressions that show that the woman reaches menopause.
2. Show that there is a correspondence between the 400 cycles and the thirty years mentioned in the text, knowing that the menstrual cycle of the woman lasts on average 28 days.
3. Label the structures in document 1.
4. Identify this female cell.
5. Locate the cell in document 1 in the female reproductive tract.

**Solution:**

1. The expressions are:
 - In women, the stock of oocytes or female gametes is not inexhaustible, but limited.
 - A woman has about 400 menstrual cycles during her life for thirty years only.
2. The duration of a menstrual cycle in women is, on average, 28 days, i.e. 1 month. Since each oocyte is released during 1 cycle then each year there are approximately 12 cycles ie. oocytes released. The 400 oocytes released corresponding to 400 cycles persist approximately 33 years ($400/12 = 33.33$ years). So, the 400 cycles correspond to thirty years.
3. a: follicular cells, b: 1st polar body, c: oocyte membrane II, d: cortical granules, e: cytoplasm of oocyte II, f: zona pellucida, g: chromosomes blocked in metaphase 2.
4. Since this cell, with pedunculated follicular cells, is surrounded by spermatozoa and is in metaphase with the presence of cortical granules and a single polar body which indicate that no spermatozoa have passed through the membrane and that they have not yet realized meiosis II. These characteristics concern the blocked oocyte II in metaphase 2 whereas this cell corresponds to the oocyte II.
5. The cell in document 1 is located in the fallopian tube.

Reproduction

Solved exercises

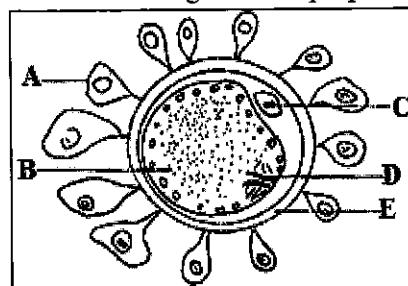
EXERCISE 1 Female gamete and fertilization

Document 1 represents a female gamete taken from the ovary of a woman. This gamete is prepared to be fertilized in vitro.

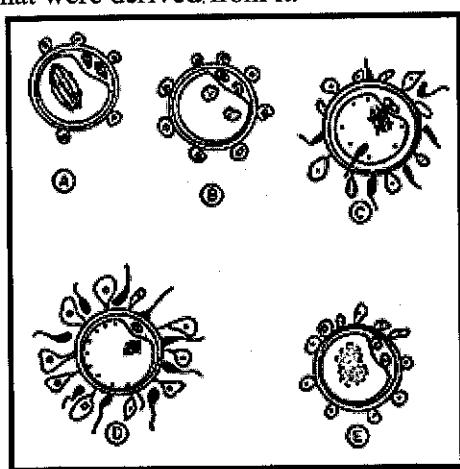
1. Label this document.
2. Show that this scheme is scientifically incorrect.

This gamete is placed in a container with a big number of human sperm cells where fertilization occurs. Document 2 represents some steps of this fertilization observed under optical microscope.

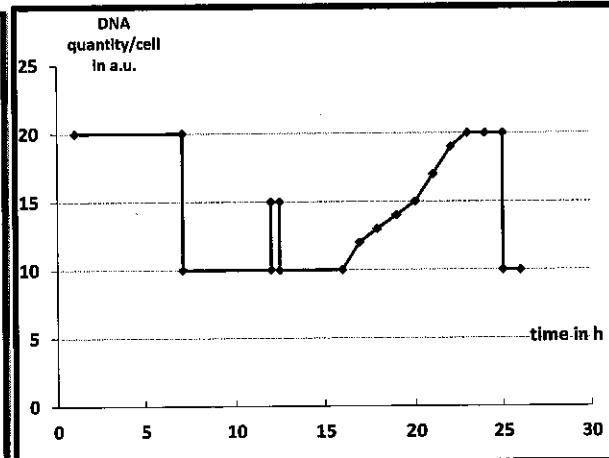
The graph of the document 2 shows the variation of the DNA quantity in the cell that was submitted to fertilization and in the cells that were derived from it.



Document 1



Document 2



Document 3

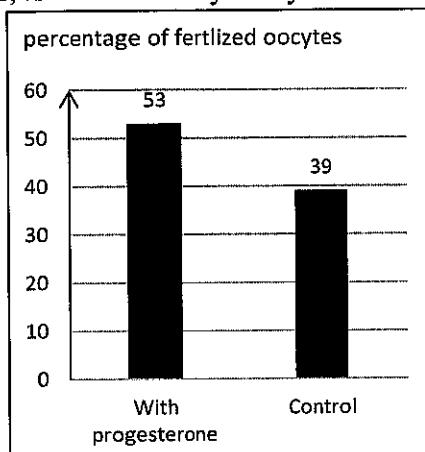
3. Specify for the cells in document 2, those that had accomplished meiosis I and those that had accomplished meiosis II.
4. Show that the increase in the amount of DNA from 10 to 15 a.u. mentioned in document 2, corresponds to the entry of the sperm cell.

Mammalian spermatozoa have to undergo a series of controlled molecular processes in the female reproductive tract. Capacitation, as a complex biological process, is influenced by many molecular factors, among which the hypothetical role of progesterone female hormone.

5. Justify the choice of the hypothesis mentioned above.

An experiment carried out to verify this hypothesis; spermatozoa are incubated in a progesterone medium with progesterone concentration of 20 ng/ml similar to that observed during ovulation period. The percentage of fertilized oocytes is evaluated and compared with that of the control spermatozoa. The results are shown in Document 4.

6. Show that the results obtained validate the hypothesis proposed above.



Document 4

EXERCISE 2 Masculine infertility

A varicocele is an enlargement of the veins within the loose bag of skin that holds the testicles (scrotum). A varicocele is similar to a varicose vein that might be seen in the leg.

Varicocele is the most common factor identified as the cause of male infertility. It is found in 15% of the general population, but is present in men with primary infertility up to 35% and up to 80% of men showing secondary infertility.

Varicoceles are a common cause of low sperm production and decreased sperm quality, which can cause infertility.

Document 1 shows the anatomy the male reproductive system in a man having varicoceles.

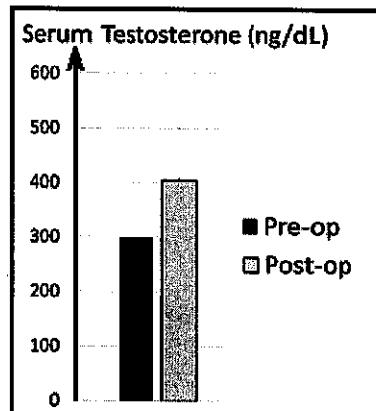
- 1 Label Document 1.

Testosterone is necessary for the stimulation of the spermatogenesis process. On the other hand, this process can't be activated at normal body temperature. Recent researches try to determine how do varicoceles lead to infertility. A series of data was collected concerning the temperature of the testicles and the levels of testosterone in blood.

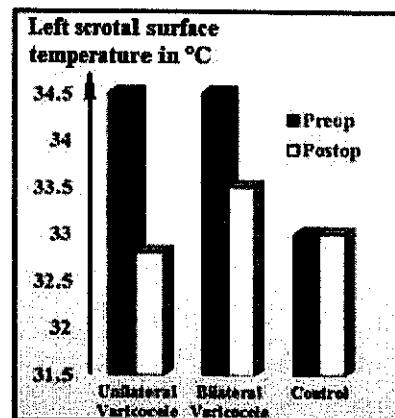
- 2.1 Name the cells that secrete testosterone.
- 2.2 Indicate their localization in the testicles.
3. Pose the studied problem by the recent researches on male infertility.
4. Draw out the two hypotheses that these researches try to test.

In order to test the two hypotheses mentioned above, the plasmatic level of testosterone as well as the scrotum surface temperature are measured in some persons having varicoceles before (preop) and after (postop) an operation of varicocelectomy (surgery of removal of varicoceles). The results are shown in the documents 2 and 3.

- 5.1 Compare the serum testosterone level before and after the operation as well as the scrotal surface temperature in these two situations.
- 5.2 Conclude the effect of varicoceles on the levels of testosterone and on the scrotal temperature.



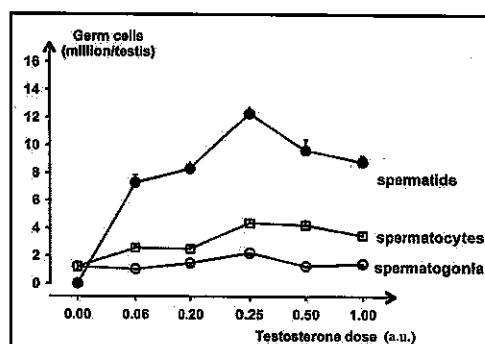
Document 2



Document 3

Document 4 shows the variation of the number of some types of germ cells with the increasing concentrations of injected testosterone to immature mice.

6. Determine the phases of spermatogenesis controlled by testosterone.
7. Explain based on all what preceded the effect of varicoceles on the spermatogenesis.



Document 4

EXERCISE 3 Role of testosterone

In humans, the amount of gonadotropins (hormones secreted by the anterior pituitary gland) in the blood has been measured. The results obtained are collated in the table in document 1.

1. Determine a factor that induces puberty in boys and girls.

Age	Quantity of gonadotropins (a.u.)	
	Boy	Girl
7 years old	9.1	8.4
15 years old	16.7	13.2 à 52

Document 1

To confirm the intervention of this factor, other experiments were made in the monkey and the results are grouped in the table of the document 2.

Experimental conditions	Prepubertal monkey + injection of gonadotropins	Prepubertal monkey without injection
Traits of the testes and the ovaries	Development et functioning	No development neither functioning

Document 2

2. Do these results confirm the decision reached in part 1? Justify.

Other experiments are done in humans. The results of these experiments are shown in Document 3.

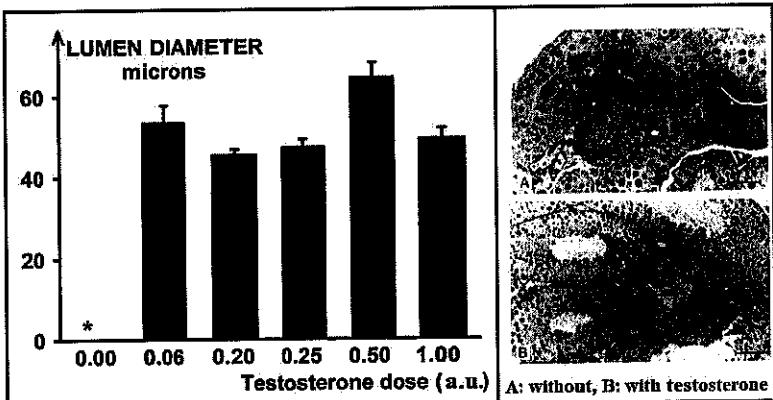
Secondary sexual characteristics		Blood concentration (mg/100ml)			
		Estrogen		Testosterone	
		10	65 to 710	6.9	260 to 400
Girl	Girl	No remarkable traits	Development of breasts	-	-
	Boy	-	-	No remarkable traits	Development of muscles

Document 3

- 3.1 Analyze the results given by document 3.
3.2 Conclude the role of the estrogen and testosterone.

Testosterone is essential for the functioning of the genital tract of man and is secreted regularly from puberty by Leydig cells under the action of gonadotropins. Its blood level is relatively constant. In order to determine the action of testosterone on the histology of the testes, some micrographs are made for the testes of immature mice injected or not with daily doses of testosterone for 35 days. Other measurements are made for the same mice. Document 4 shows the obtained results.

4. What can you deduce starting from the results in document 4?
5. Explain, based on all the above, the role of testosterone in humans.



Document 4

Reproduction

Solved exercises solutions

Exercise 1 Gametes and fertilization

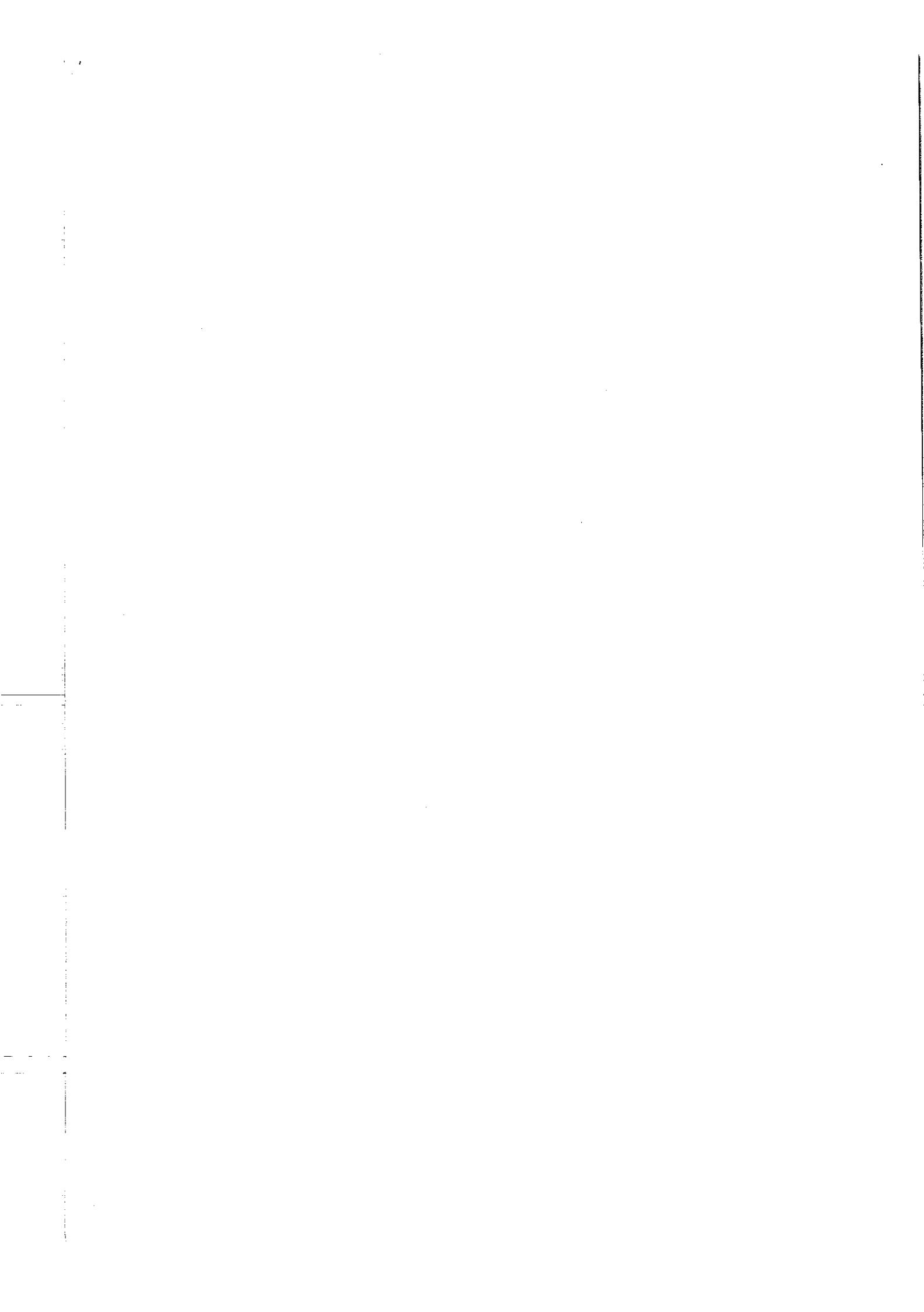
1. A-pedunculated follicular cell, B-oocyte II, C- 1st polar body, D-chromosomes in anaphase II, E-zona pellucida.
2. Since this cell is not already fertilized, then it must be blocked in metaphase II and not in anaphase II because it is the fertilization that releases the cell from the second block.
3. The number of polar bodies is 1 next to oocytes C and D, since at D, before the entry of the spermatozoon, the cell is still blocked in MII of meiosis, it has accomplished only the meiosis I responsible for the expulsion of the first polar body. In C, despite the entry of spermatozoa, but this cell has not yet started meiosis II.
For oocytes A, B and E, a meiosis II has occurred after stimulation of the oocyte by the spermatozoon, resulting in the expulsion of the second polar body, so we observe 2.
4. Oocyte II contains chromosomes with 2 chromatids each corresponding to a quantity of DNA of 10 a.u. The spermatozoon contains half this amount of DNA because it is with n single chromatid chromosomes, this quantity corresponds to 5 a.u. that makes with the 10 a.u. contained in oocyte II, 15 a.u.
5. This hypothesis is done because spermatozoa are exposed to a concentration of progesterone in the female genital tract.
6. The percentage of fertilized oocytes is 53% with progesterone greater than in the absence of progesterone which is 39%, this indicates that progesterone amplifies the sperm fertility rate which validates the hypothesis formulated above.

Exercise 2 Male infertility

1. a. Vas deferens. b. Urethra. c. Epididymis. d. Testis.
- 2.1. Leydig cells.
- 2.2. In the interstitial tissue, between the seminiferous tubules.
3. How does the varicoceles lead to infertility?
4. Hypotheses: The varicoceles increase the temperature of the testes.
The varicoceles inhibit the secretion of testosterone.
- 5.1. The level of serum testosterone before the operation is of 300 ng / dl, less than after the operation which is 400ng / dl.
On the other hand, the temperature of the surface is 34.5 ° C in the case of a single or bilateral varicocele, this temperature is higher than that after the operation, where it is in the case of unilateral varicocele 32.75°C and in the case of bilateral varicoceles 33.5°C.
- 5.2. We can conclude that varicoceles inhibit the secretion of testosterone and increase the temperature of the testes.
6. The number of spermatogonia is constant and equal to about 1 million per testis with increasing testosterone levels from 0 to 1 a.u. which indicates that testosterone does not influence the multiplication phase. On the other hand, the number of spermatocytes increased from 1.5 million per testis to 4 million per testis with increasing testosterone concentration from 0 to 0.5 a.u., which indicates that testosterone boosts the phase of growth.
As the number of spermatids increased from 0 to 12 million with increasing testosterone concentration from 0 to 0.25 units, then testosterone stimulates the maturation phase and this stimulation is amplified with the increase in testosterone concentration then the spermatogenesis phases stimulated by testosterone are increase and maturation.
7. Varicoceles inhibit the secretion of testosterone necessary for the spermatogenesis process and the control of germ cell growth and maturation, while the inhibition of testosterone secretion inhibits the sperm production process by spermatogenesis. On the other hand, spermatogenesis cannot be activated at normal body temperature and, since varicoceles amplify the temperature of the testes, they inhibit the process of spermatogenesis and reduce the production of spermatozoa. Both effects of varicoceles on testosterone and testicular temperature lead to male infertility.

Exercise 3 Role de testosterone

1. Since the amount of gonadotropins after puberty (15 years) is 16.7 years in boys and 13.2 years in girls approximately twice that before puberty (7 years) 9.1 years and 8 years, 4 years respectively, so the rise of pituitary hormones are factors triggering puberty in boys and girls.
2. Yes, because the injection of gonadotropins into a monkey before puberty led to a work and to the development and functioning of the ovaries and testes that normally functions after puberty.
- 3.1. In the girl, no secondary sexual characteristics marked with a blood estrogen concentration of 10mg / 100ml. On the other hand, the breasts develop with a concentration of 65 to 710 mg / 100ml (6 to 70 times greater). Similarly, in the boy no secondary sex characteristics marked with blood testosterone concentration 6.9 mg / 100ml. On the other hand, the muscles develop with a concentration of 260 to 400 mg / 100ml (37 to 57 times greater).
- 3.2. Estrogen and testosterone are responsible for the appearance of secondary sex characteristics in girls and boys at a determined dose.
4. Document 4 shows that the diameter of the lumen of the seminiferous tubes is 0 μm without testosterone, this diameter fluctuates between 40 and 60 μm with the increase of the testosterone concentration from 0.06 to 1 ua, these lights appear on the microphotographs with testosterone and its absent without testosterone. We can conclude that testosterone is essential for the formation of seminiferous tube lumens.
5. Testosterone, the secretion of which is stimulated by gonadotrophins, is indispensable for the appearance of secondary sexual characteristics in boys and the formation of seminiferous tube lumens, which allows the formation of spermatozoa, for which it is indispensable for puberty.

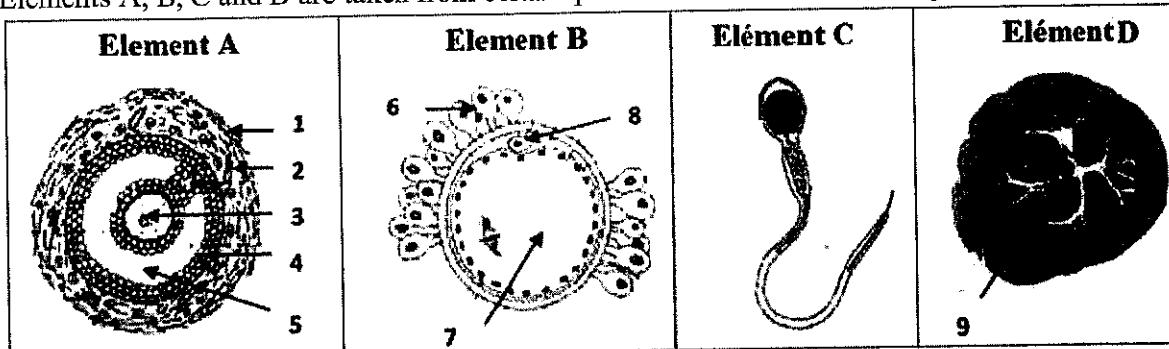


Reproduction

Non-solved exercises

EXERCISE I The gametes and the fertilization

Elements A, B, C and D are taken from certain parts of the male and female genitals.

**Document 1**

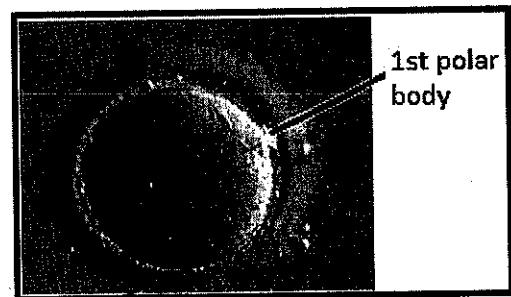
- 1.1.** Name the elements A, B, C and D
- 1.2.** Label the elements A, B and D
- 1.3.** Indicate the place of sampling of each.
- 2.** By choosing $2n = 6$ including a sexual pair, represent the karyotypes of cells 7, 8 of element B and the karyotype of element C.

Oogenesis is the process of production of the female gametes that starts in the ovaries and ends in the fallopian tube.

In contrast to that observed in the male where the production of sperm cells is continuous and begins at puberty and lasting till death, oogenesis begins at the embryonic life, and at puberty, the hormones have high concentrations allowing for the oocytes to be released by ovulation.

Unlike spermatogonia, there is an active multiplication of the oogonia before birth to form a stock that will never be renewed.

Document 2 shows a photograph of a human oocyte.

**Document 2**

- 3.** Pick out starting from the text the statement that explains:
 - 3.1.** The menopause in a woman.
 - 3.2.** That the cell released by the ovary is an oocyte and not an ovum.
- 4.** Show that the oocyte of the document 1 is an oocyte released by the ovary.

The table in document 2 shows the variation of the quantity of the DNA in the oocyte of the document 2 during its passage in the fallopian tube.

Time in hours	3	7	7.1	9	140	144
DNA quantity/ Lot of chromosomes in a. u.	12	12.1	6	6.1	6	8

Document 3

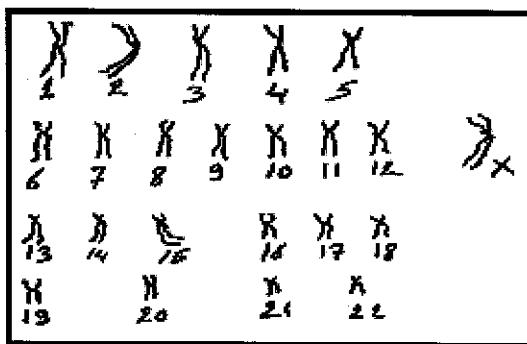
Starting from the table in document 2 and by referring to the acquired knowledge:

- 5.** Show that the fallopian tube that has received the oocyte contains sperm cells.
- 6.** Explain the variation of the DNA quantity shown by the table till 9 hours.
- 7.** Show, by observing the DNA quantity at 144 h, that a problem has occurred in the oocyte after its fertilization by the sperm cells.

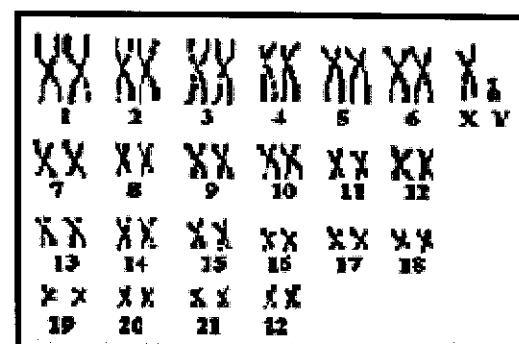
EXERCISE 2 The karyotypes of germ cells

The karyotype is the arranged set of chromosomes of a cell. It can be obtained by blocking the cell at the metaphase of the cell division where the chromosomes are most condensed and appear clearly under microscope. This blockage is made by the addition of colchicine, a substance that blocks the formation of the spindle fibers and the chromosomes will not be able to occupy the equatorial plane. Due to the inability of the sperm cell to make cell division, its karyotype (document 1) cannot be obtained unless during the last cell division made by the germ cell leading to its formation; actually, this karyotype is not a sperm cell karyotype.

Document 2 shows another karyotype made for another germ cell.



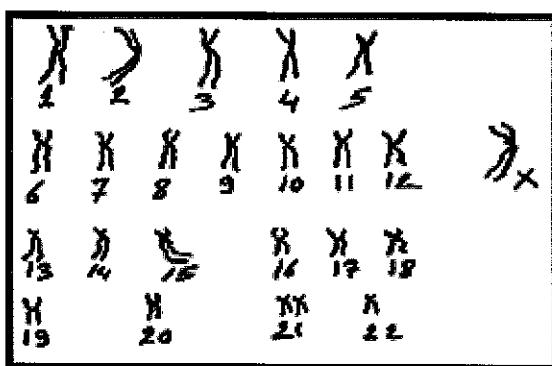
Document 1



Document 2

- 1.1.** Pick out from the above text the statement that shows the mode of action of colchicine.
- 1.2.** Identify starting from the above text and by referring to the acquired knowledge, the cell that provided the karyotype of the document 1.
- 2.** Compare the karyotypes in documents 1 and 2.
- 3.** Explain why the karyotype in document 1 cannot be that of a sperm cell.
- 4.** Draw a graph that shows the variation of the DNA quantity during the passage of the cell from the state of the document 2 to that of the document 1.

In another case, a karyotype is realized during the same cell division indicated in the above text. The karyotype of the manipulated cell is represented by the document 3.



Document 3

- 5.1.** Schematize the cell division that lead to the formation of the cell showing the karyotype in document 3 using only the chromosomes 21 and the gonosomes.
- 5.2.** Name the anomaly from which the child to be born will suffer due to the gamete produced by the cell in document 3. Justify the answer.



Ch. 2 Genetic recombination



Genetic recombination

Course abstract

1. Genes and traits

Each trait exists as several aspects (eye color: blue, green, brown ...).

The traits of an individual are the expression of the genes he carries in the chromosomes inside the nuclei of the cells. Each gene exists in several forms called alleles, each one determines a specific aspect of that trait. For example, the eye color gene determines the color of the eye, the hair color gene determines the color of the hair, each allele of that gene determines a specific color (the allele of the dark color of the hair determines the dark color....)

1.1. Some terms of heredity

Genotype: writing usually consisting of two letters that represent the two alleles of a given gene carried by an individual.

Phenotype: the appearance of a particular trait noticed in an individual.

Homozygote: An individual who has two identical alleles of a gene.

Heterozygote: an individual who has two different alleles of a gene.

Pure race: it is the set of individuals who keep in their descendants the same aspect of a given character, they are obligatorily homozygous.

1.2. Relation between alleles

- Dominant alleles: it is the allele that expresses itself in the homozygote state and still in the heterozygote state. **It is designated by capital letter.**
- Recessive allele: it is the allele that expresses itself in the homozygous state but is hidden by the dominant allele in the heterozygous state. **It is designated by small letter.**
- **Non-dominant alleles: two alleles that together determine the phenotype, they can be:**
 - **Codominant:** if the phenotype determined by the two expresses two phenotypes at the same time, each corresponds to one of the two alleles (striped, dotted, multicolored).
 - **With intermediate dominance:** if the phenotype determined by the two expresses a new intermediate phenotype between the two phenotypes determined by each alone: gray (intermediate between black and white), orange (intermediate between red and white).

N.B: The two alleles are always designated by capital letters.

2. The studies of heredity

2.1. Crosses study the transmission of characters from one generation to another.

Types of crosses:

- **Hybridization:** It is the crossing of two different pure races. The generation obtained from the hybridization is called the first generation (F1).
- **Self-fertilization or self-cross:** The cross of two individuals of the first generation is called self-fertilization, giving the second generation (F2).
- **Test-cross:** It is a cross of a dominant phenotype individual with a recessive individual. This crossing makes it possible to determine the real genotype of an individual of dominant phenotype.

2.2. The cases of the study of the heredity

Monohybrid cross is the study of the transmission of one hereditary trait.

Dihybrid cross is the study, at the same time, of the transmission of two hereditary traits.

Monohybrid cross

- Case of dominance
- Case of non-dominance (Codominance and incomplete dominance or intermediate)

Dihybrid cross

- Independent genes (Interchromosomal recombination)
- Linked genes
 - Absolute linkage
 - Partial linkage. (intrachromosomal recombination).

2.3. Transmission of lethal alleles

A lethal allele is an allele that, in the homozygous state, causes the death of the individual before the age of sexual maturity. When death occurs before birth, there is a change in the proportions of the different phenotypes of the offspring.

3. Genetic assortment

The genetic assortment can be observed in the case of dihybrid cross, it can be:

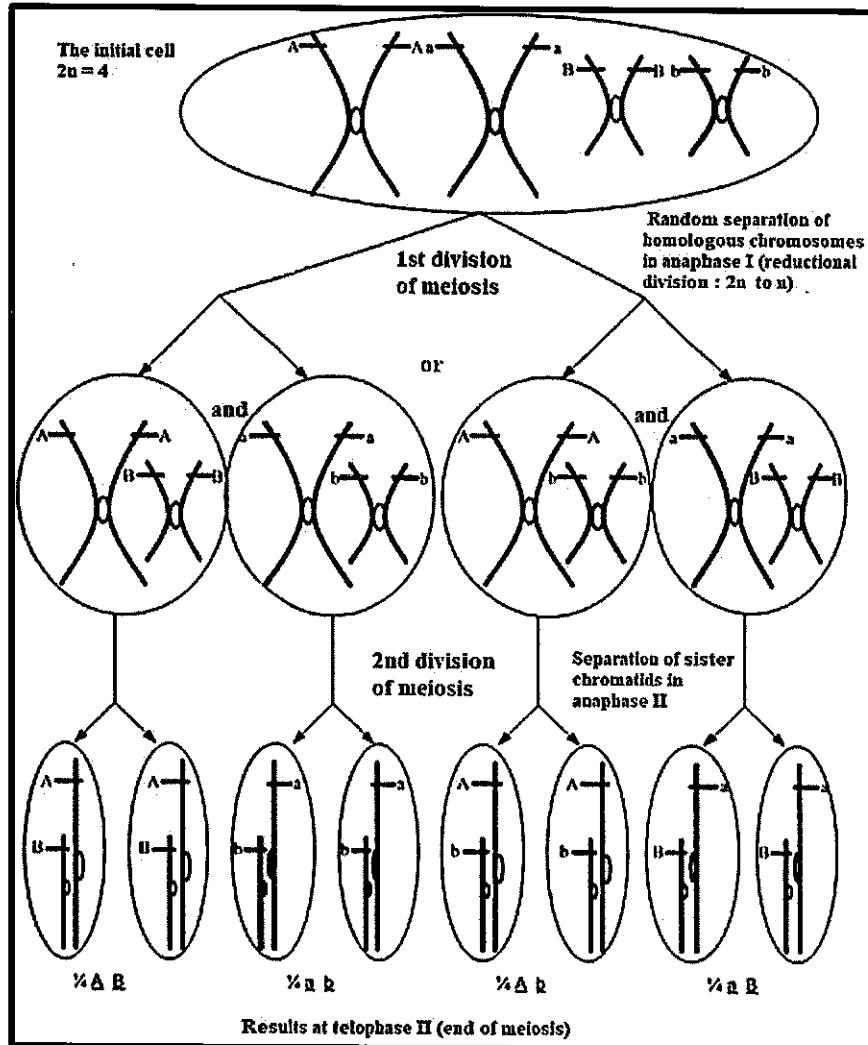
- **Interchromosomal** if the two studied genes are independent where each one of them is situated on a different chromosome.
- **Intrachromosomal** if the two genes are linked on the same chromosome by a partial linkage.

3.1. Interchromosomal assortment

Two genes are said to be "independent" if they are carried by two different pairs of chromosomes. In this case the heterozygous individual will be able to achieve an "independent segregation" of the alleles during the formation of gametes; we speak of "interchromosomal assortment" in anaphase I of meiosis by producing 2^n different types of gametes.

This assortment leads to four phenotypes of equal proportions as results of the test cross and for the results 9, 3, 3, 1/16 in the case of a self-cross.

The following scheme represents the behavior of the chromosomes during the interchromosomal assortment

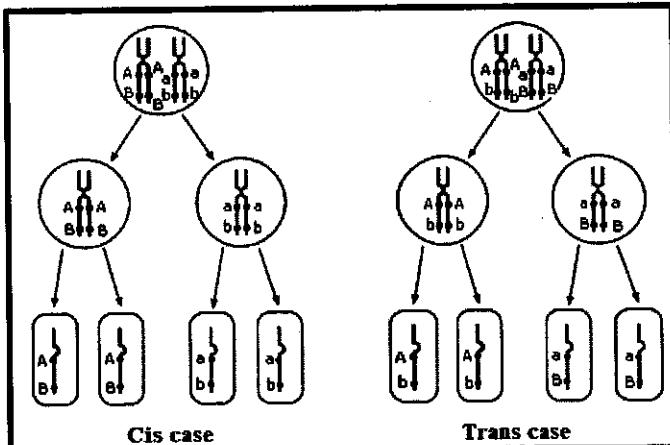


3.2. Intrachromosomal recombination

Two genes are said to be "linked" if they are carried by the same pair of homologous chromosomes.

There are two possible cases: The two genes are linked by an absolute linkage or by a partial linkage.

- Absolute linkage: in this case the two genes on a chromosome are unable to separate during meiosis because the distance between them is very small, for that the heterozygote individual will produce two gametes of equal percentages, in this case we can't observe a genetic assortment during meiosis.



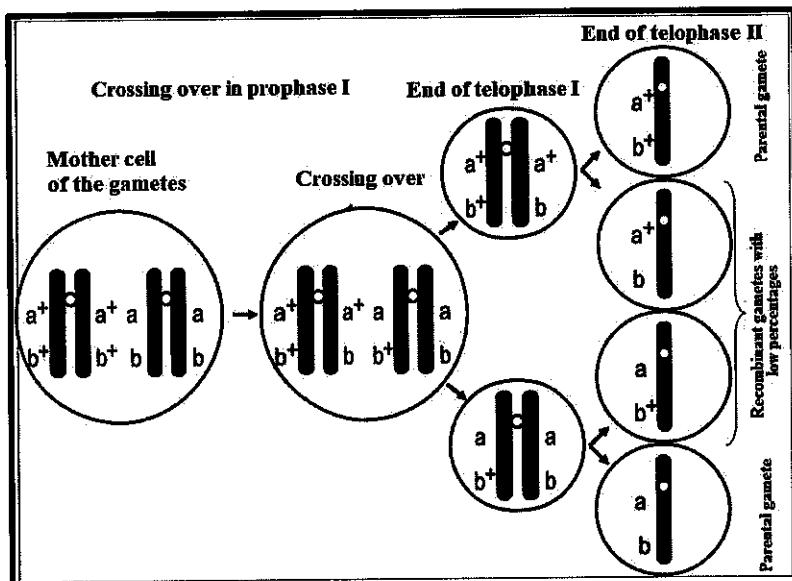
N.B.: in the cis case, the two dominant alleles are located on the same chromosome and the two recessive alleles on the other. In the trans case, a dominant allele and a recessive allele are located on each of the two homologous chromosomes.

- Partial linkage: in this case the two genes studied are able to separate during meiosis, for this the heterozygous individual will produce four types of gametes of unequal percentages with two equal major percentages and two equal minor percentages.

N. B: The genes can be linked in **cis position** (parental phenotype formed of two dominant characters (a +, b +) and two recessive ones (a, b))

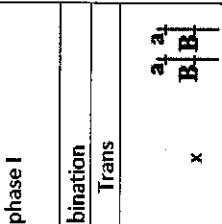
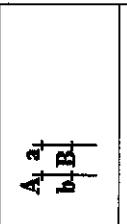
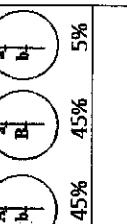
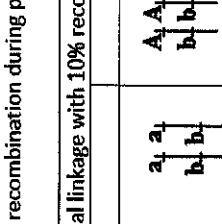
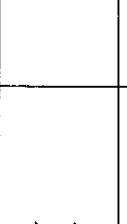
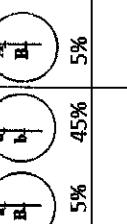
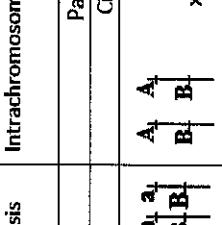
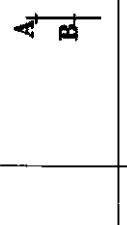
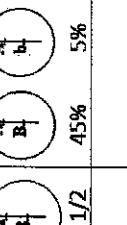
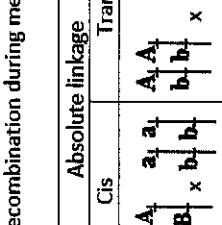
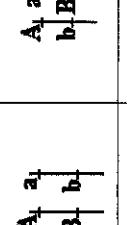
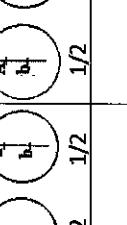
Genes can be linked in the **trans position** (parental phenotype consisting of dominant traits with recessive (a +, b) and recessive with dominant (a, b +))

The crossing over will be done more as the distance between the two genes studied increases, so more recombinant gametes will be produced. This distance expressed in centimorgan corresponds to the sum of percentages of recombinant gametes.



Summary

The types of genetic recombination

Type of recombination	Interchromosomal recombination during anaphase I	No recombination during meiosis	Intrachromosomal recombination during prophase I
Relative position of genes	Independent genes	Absolute linkage	Partial linkage with 10% recombination
Relative position of alleles		Cis Trans	Cis Trans
Hybridization			
Parents genotypes			
F1 (hybrid) genotype			
F1 gametes			
	1/4 1/4 1/4 1/4	1/2 1/2 1/2 1/2	45% 5% 45% 5%
Results of Test cross AaBb x aabb	[A,B] 1/4, [a,b] 1/4	[A,B] 1/2, [a,b] 1/2	[A,B] 45%, [a,b] 45%, [A,B] 5%, [a,b] 5%
They are similar to the gametes given by F1		1/2 1/2	45% 5%
Results of F2: F1xF1 or self cross AaBb x AaBb	[A,B] 9/16, [a,b] 3/16, [a,B] 1/16	[A,B] 3/4, [a,b] 1/4	If one of the parents makes crossing over [A,B] 72.5%, [A,b] 2.5%, [a,B] 22.5%, [a,b] 2.5%

APPLICATIONS:

EXERCISE 1 A cross of rabbits

We cross two true breeding lines of rabbits the first with smooth hair and the other with rough hair. We obtain in the first generation 100 % rabbits with smooth hair.

1. Specify the dominant allele and the recessive one.
 2. Designate these two alleles by symbols.
 3. Indicate the genotypes of the parents and that of the F1.

Two rabbits of the first generation are crossed, we obtain 25 % of rabbits with rough hair.

4. Make the factorial analysis to verify the phenotypic percentage of the offspring with rough hair obtained by this cross.

We cross a rabbit of the F1 with a rabbit of rough hair.

5. Make the factorial analysis that permits us to determine the phenotypic percentages of their offspring

Solution:

- The smooth allele is the dominant allele and the rough allele is the recessive allele because in the first generation all the rabbits are with smooth hair.
 - Let (L) be the symbol of the allele that determines the smooth hair which is dominant in relation with the allele which determines the rough appearance of the hair.
Let (r) be the symbol of the allele that determines the rough aspect of the hair which is recessive in relation with the allele which determines the smooth appearance of the hairs.

③ Genotypes of parents: SS rr
Genotype of F1: Sr

4. Phenotypes of parents:		Smooth	x		Smooth			
Genotypes of parents		Sr	x		Lr			
Gametes:		S 50 %		r 50 %		S 50 %		r 50 %

	S 50 %	r 50 %
S 50 %	SS 25 %	Sr 25 %
r 50 %	Sr 25 %	rr 25 %

Phenotypes of the offspring:

Smooth: 75 %

Rough: 25 %

Thus, the percentage of the phenotype rough is 25 %, it is verified.

- | | | | | | | |
|------------------------|--|-----------|---|--------|-------|---------|
| 5. Parents phenotypes: | | F1 smooth | x | | rough | |
| Parents genotypes | | Sr | x | | rr | |
| Gametes: | | S 50 % | | r 50 % | | r 100 % |

Table of cross:

	S 50 %	r 50 %
	r 100 %	Lr 50 %

Offspring phenotypic percentages:

Smooth: 50 %, rough: 50 %

Case of codominance:

EXERCISE 2 Transmission of the color in guinea pigs

Two guinea pigs, the first of white color and the second of yellow color are crossed. All the descendants of these two guinea pigs are yellow-spotted white.

1. Justify that the two crossed guinea pigs are of pure races.
 2. Name the generation obtained from the cross of the two guinea pigs.
 3. Indicate if this is the case of dominance or codominance. Justify.
 4. Designate the alleles of the guinea pig color gene by symbols.

We cross two yellow spotted white guinea pigs of the offspring obtained above, we obtain the following results:

12 yellow spotted white guinea pigs.

6 white guinea pigs.

6 yellow guinea pigs.

- E.** Calculate the percentages of the results obtained.
 - F.** Perform the factorial analysis to verify the phenotypic percentages of the offspring of this last cross.

Solution:

Table of cross:

	W 50 %	Y 50 %
W 50 %	WW 25 %	WY 25 %
Y 50 %	WY 25 %	YY 25 %

Phenotypes of the offspring: White 25 %

Yellow 25 %

Yellow spotted white 50 %

Then the phenotypic percentages are verified.



Genetic recombination

Training exercises

EXERCISE 1 Genetic recombination

Two drosophilae are crossed, the first is of Gray body and Long wings, and the other is of black body and cut wings. In the first generation we obtain 100 % of drosophila having gray body and long wings.

1. Draw out two conclusions starting from this given.

One female of the obtained drosophila having gray body and long wings is crossed with a male drosophila having black body and cut wings we obtain the following results.

225 Drosophilae with **gray body and long wings**;

220 Drosophilae with **gray body and cut wings**;

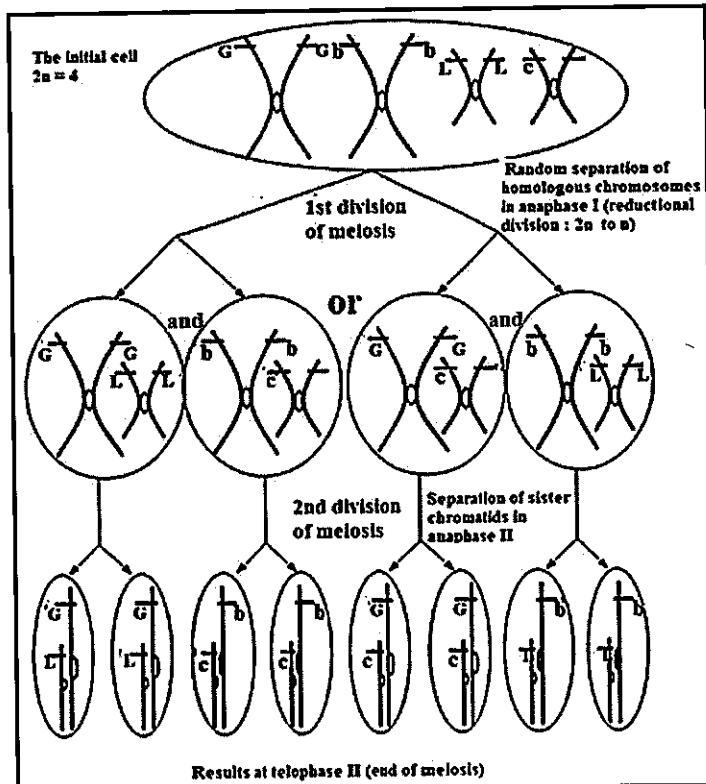
223 Drosophilae with **black body and long wings**;

226 Drosophilae with **black body and cut wings**.

2. Name this cross
3. Explain the last obtained results.
4. Schematize the genetic recombination at the origin of the obtained results in this latter cross.

Solution:

1. The two crossed Drosophilae are of pure races.
The traits gray body and long wings are respectively dominant in comparison to the traits black body and cut wings. (Gray: G, black: n, Long: L, Cut: d)
2. Test cross.
3. The crossing of a hybrid female with a birecessive male gives offspring with four equiprobable phenotypes because the two studied genes are independent, the F1 female produces four types of gametes with equal proportions by random separation of the homologous chromosomes during the anaphase I of meiosis. And since the male produces only one type of gametes, then the results will be determined by the gametes of the female.
4. Interchromosomal recombination at the origin of the results obtained in this cross.



EXERCISE 1 continued

- E.** Make the chromosomal analysis in order to determine the phenotypic proportions of a cross of a female hybrid drosophila with a pure drosophila having black body and long wings such that it is heterozygous for the form of the wings.
- G.** Make the necessary factorial analysis to determine the phenotypic proportions of the results of the cross of two hybrid drosophilae of gray body and long wings.

Solution:

- E.** Phenotypes: F1 Gray body, long wings \times Black body, cut wings
 Genotypes:

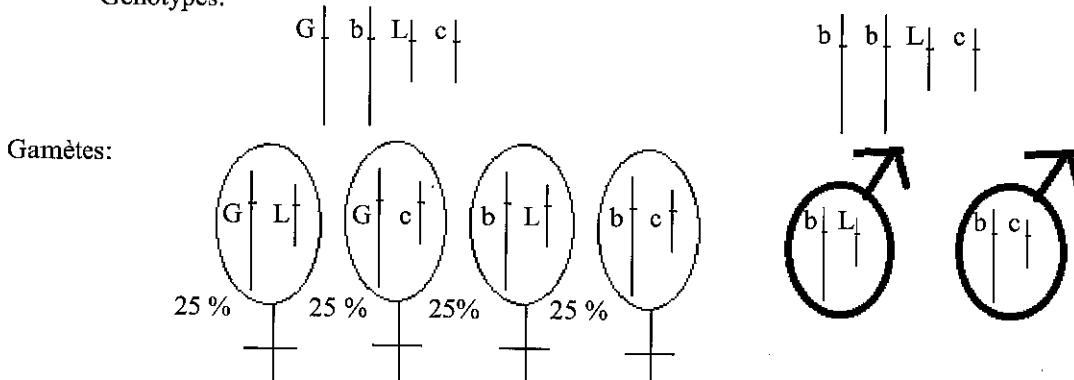


Table of cross:

		Female	G L	G c	b L	b c
Male		25%	25%	25%	25%	25%
b L	50%	G b L L	G b L c	b b L L	b b L c	
b c	50%	G b L c	G b c c	b b L c	b b c c	

Phenotypes: 37.5 % [G,L] 12.5% [G,c] 37.5% [b,L] [b,c] 12.5 %

G.

- Phenotypes: Gray body, long wings \times Gray body, long wings

Génotypes: G//b L//c

Gamètes: GL $\frac{1}{4}$ Gc $\frac{1}{4}$ bL $\frac{1}{4}$ bc $\frac{1}{4}$

Tableau de croisement :

	GL $\frac{1}{4}$	Gc $\frac{1}{4}$	bL $\frac{1}{4}$	bc $\frac{1}{4}$
GL $\frac{1}{4}$	G//G L//L $\frac{1}{16}$	G//G L//c $\frac{1}{16}$	G//n L//L $\frac{1}{16}$	G//b c//c $\frac{1}{16}$
Gc $\frac{1}{4}$	G//G L//c $\frac{1}{16}$	G//G c//c $\frac{1}{16}$	G//n L//c $\frac{1}{16}$	G//b c//c $\frac{1}{16}$
bL $\frac{1}{4}$	G//b L//L $\frac{1}{16}$	G//b L//c $\frac{1}{16}$	b//b L//L $\frac{1}{16}$	b//b L//c $\frac{1}{16}$
bc $\frac{1}{4}$	G//b L//c $\frac{1}{16}$	G//n c//c $\frac{1}{16}$	b//b L//c $\frac{1}{16}$	b//b c//c $\frac{1}{16}$

Phenotypic results: 9/16 Gray body, long wings

3/16 Gray body, cut wings

3/16 Black body, long wings

1/16 Black body, cut wings

EXERCISE 2 Two relative positions of the genes in drosophilae

In Drosophilae, grey body (**G**) dominates black body (**b**); long wing (**L**) dominates cut wing (**t**); and star eye (**S**) dominates normal eye (**n**).

The cross between a drosophila having gray body and star eyes with a drosophila having black body and normal eyes gives:

- 324 Drosophilae with **grey body and star eyes**;
- 320 Drosophilae with **grey body and normal eyes**;
- 332 Drosophilae with **black body and star eyes**;
- 315 Drosophilae with **black body and normal eyes**

- 1.** Indicate in the chromosomal form the genotypes of the two crossed drosophilae.

Another cross between drosophila having long wings and star eyes, with a drosophila having cut wings and normal eyes gives:

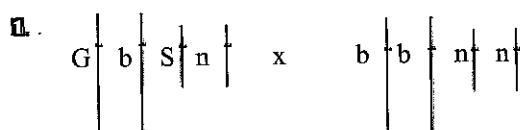
- 50% Drosophilae with **long wings and star eyes**;
- 50% Drosophilae with **cut wings and normal eyes**;

- 2.** Indicate if the two genes (shape of the wings and shape of the eyes) are linked or independent.
3. Make the necessary factorial analysis verifying the percentages of the experimental given.

We try now to make a cross between hybrids drosophilae with gray body and long wings and another one with black body and cut wings.

- 4.** Determine whether the two studied genes are independent or linked.
5. Localize the three studied genes on the chromosomes of a hybrid Drosophilae for the three traits above.

Solution:



- 2.** The two genes of the shape of the wings and the shape of the eyes are linked.

- 3.** Phenotypes: Long wings and star eyes x Cut wings and normal eyes

Genotypes: LS//tn x

Gametes: LS 50 % tn 50 % tn//tn
 tn 100 %

Table of cross:

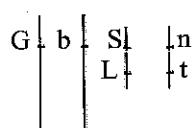
	LS 50 %	tn 50 %
tn 50 %	LS//tn 50 %	tn//tn 50 %

Phenotypic results: 50 % [L,S] , 50 % [t,n]

Then, the results are verified.

- 4.** Since the two genes of body color and eye shape are independent and the two genes of wing shape and eye shape are linked, then the two genes of body color and wing shape are independent.

- 5.**



EXERCISE 3 Genetic recombination

We realize, in the drosophila, many crosses in order to study the respective positions of 3 genes. The cross of a hybrid drosophila having red eyes and smooth antennae with a birecessive male having brown eyes and rough antennae gives the following results:

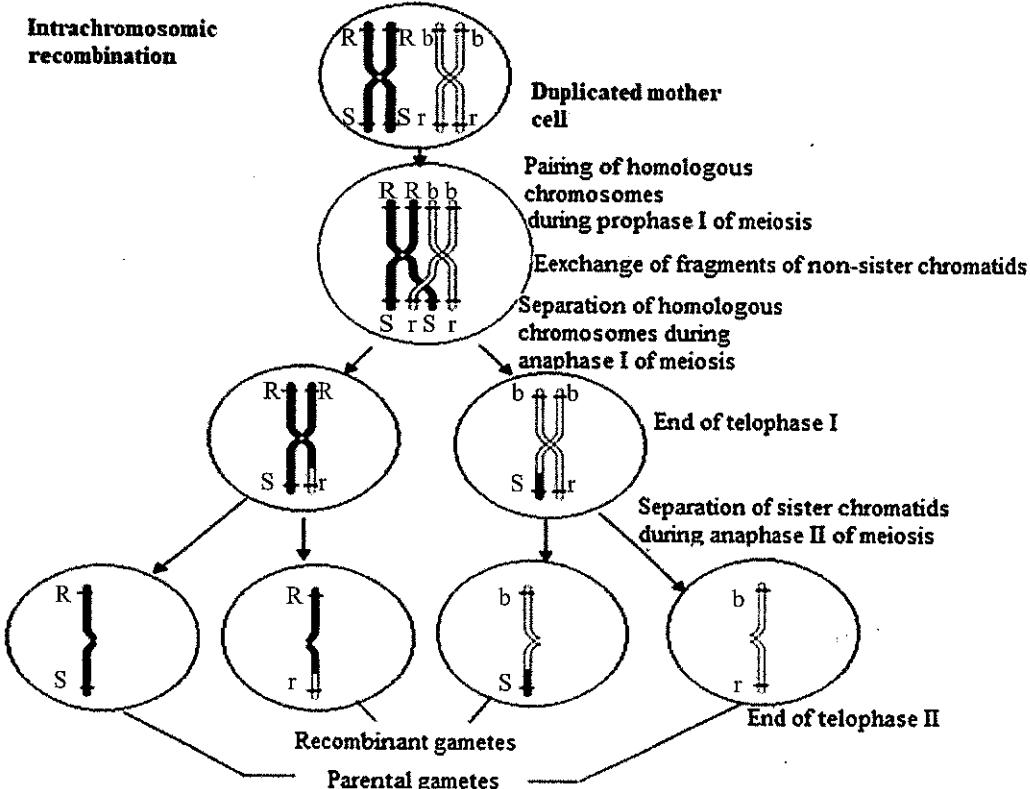
- 415 drosophilae having red eyes and smooth antennae
- 412 drosophilae having brown eyes and rough antennae
- 44 drosophilae having brown eyes and smooth antennae
- 48 drosophilae having red eyes and rough antennae

1. Identify the type of this cross.
2. How can we explain the obtained results in this cross?
3. Schematize the genetic assortment at the origin of the results obtained in this cross.

Solution:

1. In this cross, a hybrid Drosophila with red eyes and smooth antennae is crossed with another birecessive male drosophila, and as in a test cross, a hybrid individual is crossed with a birecessive individual then this cross corresponds to a test cross.
2. This cross of a hybrid female drosophila (red, smooth) with a birecessive male gives four phenotypes with non-equal proportions (two equal majors and two equal minors) because these two genes are linked by a partial linkage where a crossing over took place during prophase I of meiosis and resulting in four types of gametes in the hybrid female with two major equal parental gametes and two minor equal recombinant gametes that are at the origin of the results obtained because the birecessive male only gives a single type of gametes. The birecessive and bidominant phenotypes are major because the alleles of the two genes studied are in the cis position, the two dominant alleles are originally on the same chromosome and the two recessive alleles on its homologue.

3.



EXERCISE 3 continued

4. Make a factorial analysis to confirm the obtained results.
 5. Make a factorial analysis to determine the phenotypic percentages of the offspring of a cross of two drosophilae of the F1.

Solution :

2. Frequency of recombination = $\sum \text{recombinant} \times 100 / \text{total} = (44 + 48) \times 100 / 919 = 10\%$
 So each recombinant gamete has a percentage of $10 / 2 = 5\%$
 Each parental gamete has a percentage of $(100 - 10) / 2 = 45\%$

Phenotypes: Red eyes / Smooth antennas	x	Brown eyes / rough antennas
Genotypes: RS//br	x	br // br
Gametes: RS Rr bS br		br
45% 5% 5% 45%		100%

Table of cross:

	RS 45%	Rr 5%	bS 5%	br 45%
br 100%	45% RS//br	Rr//br 5%	bS//br 5%	br//br 45%

Phenotypic results: [R,S] 45%, [R,r] 5 %, [b,S] 5 %, [b,r] 45 %

Then, the results are verified.

5. Phenotypes: Red eyes / smooth antennas x Red eyes / smooth antennas
 Genotypes: RS//br x RS//br

Gametes :	RS	Rr	bS	br		RS	br
	45%	5%	5%	45%		50%	50%

Table of cross:

 	RS 45%	Rr 5%	bS 5%	br 45%
RS 50%	RS//RS 22.5%	RS//Rr 2.5%	RS//bS 2.5%	RS//br 22.5%
br 50%	RS//br 22.5%	Rr//br 2.5%	bS//br 2.5%	br//br 22.5%

Phenotypic results: [R,S] 72.5%, [R,r] 2.5 %, [b,S] 2.5 %, [b,r] 22.5%

EXERCISE 3 continued

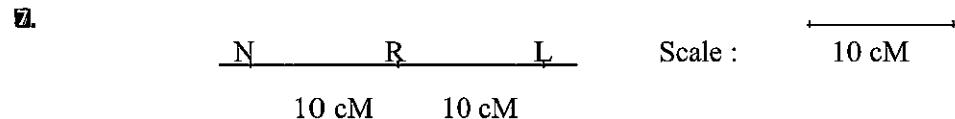
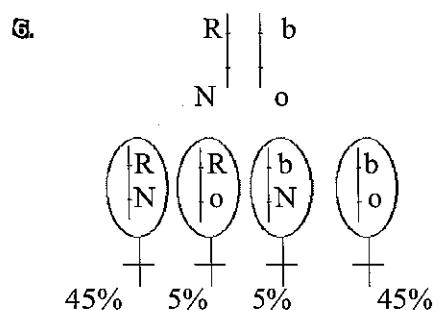
Another cross is made between an F1 drosophila having red eyes (R) and normal eyes (N) with a male drosophila having birecessive phenotype brown eyes (b) and oblique eyes (o). This cross leads to 45% of birecessive phenotypes.

- Q. Write, in the chromosomal forms, the gametes given by the F1 and their percentages.

Knowing that the gene determining the color of the eyes is situated between the two other genes, and basing on the results of the two crosses given above:

- 1. Draw the factorial map of the three given genes.
- 2. Conclude from what preceded the relative position of the two genes of antennas and form of eyes.
- 3. What will be the relative position of the two genes of antennas and the shape of eyes if the gene of the color of eyes is situated on the same chromosome but not between the two other genes.

Solution:



- 3. The two genes of eh antenna and the shape of eyes are linked with a gene distance of 20 cM.
- 4. If the gene of the color of eyes is situated out of the two other genes of antenna and shape of eyes, these two genes will be linked by a complete linkage (absolute).

EXERCISE 4 Two linked genes in drosophilae

We make the two series of crosses represented by the table below:

	Case 1				Case 2			
Cross 1	Pure race Gray body (G) red eyes (R)	X	pure race black body (b) purple eyes (p)	Pure race Gray body purple eyes	X	pure race black body red eyes		
F1: Gray body Red eyes				F1: Gray body Red eyes				
Cross 2	F1 X birecessive 160 gray body, red eyes 159 black body, purple eyes 40 black body, red eyes 41 gray body, purple eyes			F1 X birecessive 39 gray body, red eyes 41 black body, purple eyes 158 black body, red eyes 161 gray body, purple eyes				

1. Indicate the importance of the two crosses 1 and 2.

2. Explain the results of the second cross of case 2.

Two F₁ drosophilae of case 2 were crossed.

- E. make the necessary chromosomal analysis that permits to determine the percentages of the offspring's phenotypes.

Solution:

- 1 Cross 1 permits to obtain hybrid individuals.

Cross 2 permits to determine the types and percentages of the gametes given by the hybrid.

2. This cross of a hybrid female drosophila (gray, red) with a birecessive male gives four phenotypes with unequal proportions (two equal majors and two equal minors) because these two genes are linked by a partial linkage where a crossing over took place during prophase I of meiosis and resulting in four types of gametes in the hybrid female with two major equal parental gametes and two minor equal recombinant gametes that are at the origin of the obtained results because the birecessive male only gives a single type of gametes. The birecessive and bidominant phenotypes are minor because the alleles of the two studied genes are in the trans position, each of the two dominant alleles is originally located on one of the two homologous chromosomes as well as each of the two recessive alleles.

- $$3. \text{ Frequency of recombination} = \sum \text{recombinant} \times 100 / \text{total} = (39 + 41) \times 100 / 399 = 20\%$$

So, each recombinant gamete has a percentage of $20 / 2 = 10\%$

Each parental gamete has a percentage of $(100-20) / 2 = 40\%$

Phenotypes: Red eyes / gray body x Purple eyes / black body

Genotypes: Rb//pG x Rb//pG

Gametes: Rb RG pb pG Rb pG

40% 10% 10% 40% 50% 50%

of cross

	Pt. 40%	Pt. 10%	Pt. 10%

	Rb 40%	RG 10%	Pb 10%
O ⁺			

Table of cross

 	Rb 40%	RG 10%	Pb 10%	pG 40 %
Rb 50 %	Rb//Rb 20 %	RG//Rb 5 %	Rb//pb 5%	Rb//pG 20%
pG 50%	Rb//pG 20 %	RG//pG 5 %	pG//pb 5 %	pG//pG 20 %

Phenotypic results: [R,G] 50%, [R,b] 25 %, [p,G] 25 %

Genetic recombination

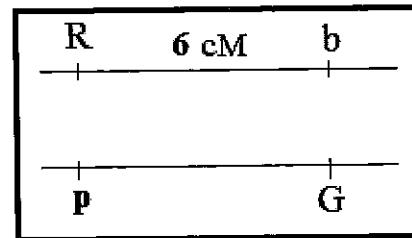
Solved exercises

EXERCISE I The factorial map

In order to study the relative positions of some genes in drosophila, we consider the gene of the color of the body, Gray (G) is dominant over black (b); and the gene of the color of eyes, red (R) dominates purple (p).

Document 1 shows the positions of these two couples of alleles in a female drosophila and a male drosophila, both hybrid, with the relative distance that separates the two studied genes.

1. Specify the relative position (cis or trans) of the alleles of the two genes shown in document 1.
2. Represent under the chromosomal forms the genotypes of gametes of the female and the male hybrid drosophilae while indicating their percentages.
3. Schematize in the hybrid female drosophila the genetic assortment that lead to its gametes.



Document 1

A cross is made between two drosophilae, the first is a hybrid with gray body and normal eyes, and the second is birecessive for the two traits. In the offspring of this cross we find:

- 4.5 % of drosophilae with gray body and normal eyes.
- 4.5 % of drosophilae with black body and cinnabar eyes.
- 45.5 % of drosophilae with gray body and cinnabar eyes.
- 45.5 % of drosophilae with black body and normal eyes.

4. Draw under the light of these results the two possible factorial maps for the three genes indicated above.
5. Indicate the genotypes of the drosophilae that should be crossed in order to determine the correct factorial map of the two maps drawn in the part (4).

A student searches some cross results on the internet in order to determine the correct factorial map of the three given genes. Document 2 shows some of the found results.

6. How can we explain the obtained results in the crosses represented by document 2?
7. Show that the results found on the web are not compatible with the data given above.

Drosophilae of pure line	X	Drosophilae of pure line
Red and normal eyes		Purple and cinnabar eyes
100 % Red and normal eyes		
Female F1	X	birecessive male
A generation comprising 40 % of birecessive drosophilae		

Document 2

EXERCISE 2 Relative positions of many genes

In order to study the relative positions of many genes in drosophila; two crosses were made starting from F1 female drosophila and birecessive males. These two crosses are represented in documents 1 and 2.

1. Write the genotypes of the two crossed drosophilae in the first cross shown in document 1.
2. Represent the results given by document 1 in a histogram.
3. Make the chromosomal analysis necessary to verify the results obtained in the first cross.
4. Schematize the recombination at the origin of the results of the first cross.

First cross:	
F1 female drosophila	Birecessive male
Normal eyes (N)	x Star eyes (n)
Gray body (G)	Stripe body (g)
↓	
325 Normal eyes, gray body	
322 Star eyes, stripe body	
320 Normal eyes, stripe body	
319 Star eyes, gray body	

Document 1

5. Indicate the relative position of the two genes studied by the second cross.
6. Justify that in the second cross, no recombination takes place.
7. Make the factorial analysis necessary to verify the results obtained in the second cross.

Second cross:	
F1 female drosophila	Birecessive male
Normal Bristles (B)	x Spineless bristles (b)
Normal body (T)	Trithorax body (t)
↓	
470 Normal bristles, normal body	
468 Spineless bristles, trithorax body	

Document 2

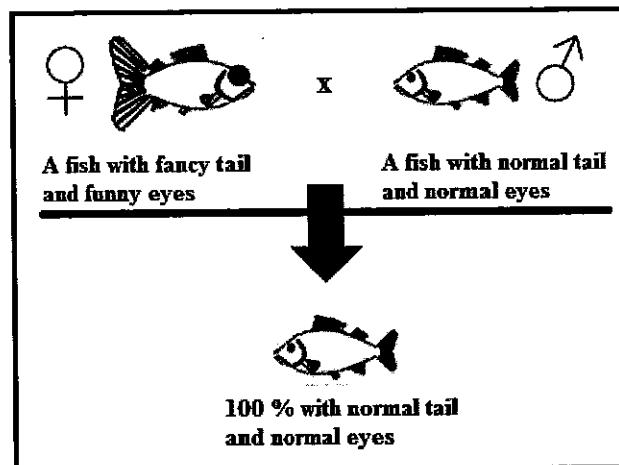
A study showed that the gene of the body color (gray or stripe) is linked with that of the body shape (normal or trithorax).

8. Indicate the relative position of:
 - 8.1. The gene of the body color (gray or stripe) with that of the state of the bristles (normal or spineless).
 - 8.2. The gene of the shape of eyes (normal or star) with that of the body shape (normal or trithorax).

EXERCISE 3 A cross made in fish

A female fish with fancy tail and funny eyes is crossed with a male fish with normal tail and normal eyes, the two genes that are responsible for the traits of the shape of the tail and that of the eyes are independent. The result of this cross is represented in the document 1.

1. Pick out from the document 1 an information that confirms that the two crossed fish are of pure races.
2. Justify that the alleles responsible for normal tail and normal eyes are dominant with respect to the alleles responsible for fancy tail and funny eyes respectively.
3. Write the genotypes of the two crossed parents as well as the fish resulted from this cross.



Document 1

A male and a female fish of the F1 generation are crossed to obtain an F2 generation. The results of this second cross are:

- 2800 fish with normal tails and normal eyes
- 960 fish with normal tails and funny eyes
- 920 fish with fancy tails and normal eyes
- 312 fish with fancy tails and funny eyes

1. Specify the genotype of the obtained fish with fancy tails and funny eyes.
2. Calculate the proportions of the different phenotypes of fish obtained in F2.
3. Make the necessary factorial analysis to confirm the obtained proportions in F2.
4. Indicate the results of the F2 generation in the case where the allele of normal tail is lethal.

EXERCISE 4 The genetic recombination

In a mosquito, certain crosses such as the back cross could help looking for the relative position of the genes responsible for the color of the body and that of eyes.

Given the following document 1, that shows the done experiments.

Experiments	Description	Results
1 st set of experiments	Wild type mosquitoes of grey body and brown eyes are crossed with mosquitoes of black body and clear eyes	In F ₁ all mosquitoes are of wild type
2 nd set of experiments	Females F ₁ crossed with males of black body and clear eyes	35.5% of mosquitoes of gray body and brown eyes 35.5% of mosquitoes of black body and clear eyes 14.5% of mosquitoes of gray body and clear eyes 14.5% of mosquitoes of black body and brown eyes

Document 1

1. Specify the dominant alleles and the recessive ones in the done experiments.
2. Write the genotypes of the two crossed mosquitoes in the first set of experiments. Justify.
3. Indicate the relative position of the two genes studied in this cross.
4. Name the phenomenon done by the chromosomes during meiosis and leading for the results of the second set of experiments.

The following document 2 explains a scientific research done on a disease infecting humans and mosquitos.

Malaria is one of Earth's oldest and deadliest diseases caused by the plasmodium, and despite our fiercest efforts at treatment and eradication — bed nets, quinine pills, pesticides, vaccines — the illness persists in killing us, to the tune of roughly half a million deaths per year. So, scientists are trying a different tactic: Instead of treating the humans, why not treat the mosquitoes that transfer the plasmodium to humans?

The breakthrough relies on a controversial new gene-editing technique called CRISPR-Cas9, a sort of molecular cut-and-paste that allows scientists to snip out segments of a creature's DNA and insert new ones. With CRISPR, the California researchers added a set of genes conferring malaria resistance to their mutant mosquitoes. They coupled the malaria resistance gene with one that makes mosquitoes' eyes red, so they could measure whether the resistance gene was passed on.

The Washington post, By Sarah Kaplan November 24, 2015

Document 2

5. Pick out, from document 2, the statement that indicates that the gene of resistance to malaria and the gene of the eyes color of mosquito are linked.
6. Explain why the linkage between the two given genes in the document 2 should be absolute in order to give the results desired by the scientists.

The gene of the resistance to malaria is dominant with respect to the gene of non-resistance. The gene of red eyes is dominant with respect to the gene of clear eyes.

We consider that the two genes mentioned in document 2 are linked by absolute linkage.

7. Make the factorial analysis to find the phenotypic percentages of the offspring of a hybrid drosophila and a birecessive one for the two genes mentioned in document 2.

EXERCISES The genetic recombination

In Drosophilae, gray body (**G**) dominates black body (**b**) trait; long wing (**L**) dominates cut wing (**c**) trait; and star eye (**S**) dominates normal eye (**n**) trait.

A- Another cross between dihybrid drosophila having long wings and star eyes, with a drosophila having cut wings and normal eyes gives:

- 98 Drosophilae with **long** wings and **star** eyes;
- 102 Drosophilae with **cut** wings and **normal** eyes;
- 10 Drosophilae with **long** wings and **normal** eyes;
- 12 Drosophilae with **cut** wings and **star** eyes.

B- The cross between a drosophila having gray body and star eyes with a drosophila having black body and normal eyes gives:

- 324 Drosophilae with **grey** body and **star** eyes;
- 320 Drosophilae with **grey** body and **normal** eyes;
- 332 Drosophilae with **black** body and **star** eyes;
- 315 Drosophilae with **black** body and **normal** eyes

- 1.** How can we explain the result obtained in each of the two given crosses?
 - 2.** Indicate under the chromosomal form the genotypes of the crossed dihybrid drosophilae of the two given crosses as well as their corresponding gametes.
- The obtained drosophilae from the cross B that has black body and star eyes is crossed with a birecessive one.
- 3.** Determine, by using a punnet square, the phenotypic percentages of the offspring.
 - 4.** Name the type of recombination made in the F1 drosophilae in cross A.

C- We try now to make a cross between hybrids drosophilae with grey body and long wings and another one with black body and cut wings.

- 5.** Indicate the phenotypic results of this cross. Justify.

- D- A cross let us to determine the distance between the gene of the shape of the eye (star/S, normal/n) and the gene of the silk of the legs (smooth/M, rough/r), the distance is of 4 cM.
- 6.** Draw the two possible factorial maps of the three linked genes given in this exercise.
 - 7.** Indicate the genotypes of the drosophilae that should be crossed in order to determine the real factorial map of the three given genes.

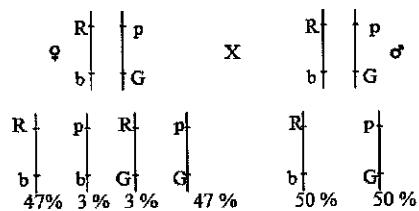
Genetic recombination

Solved exercises solutions

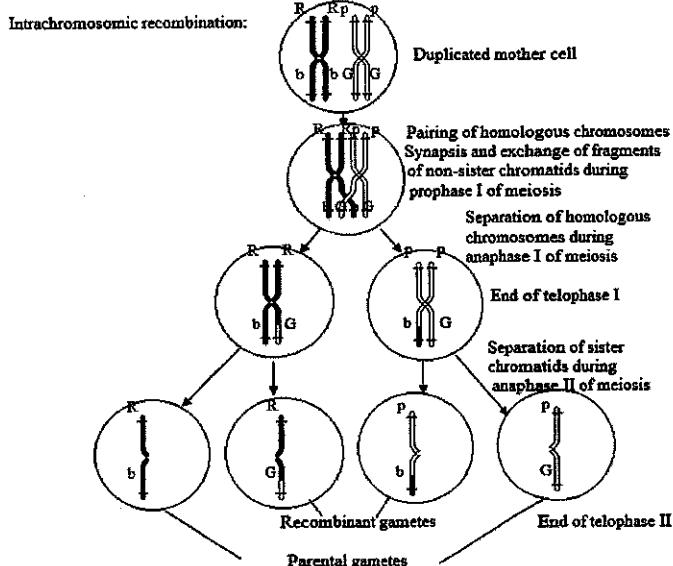
Exercise 1 The factorial map

1. The relative position of the alleles of the two genes is trans since the dominant allele R of the gene of the color of the eyes is associated with the recessive allele b of the gene of the color of the body on one chromosome, and the dominant allele G of the gene of the color of the body is associated with the recessive allele p of the gene of the color of eyes on the other homologous chromosome.

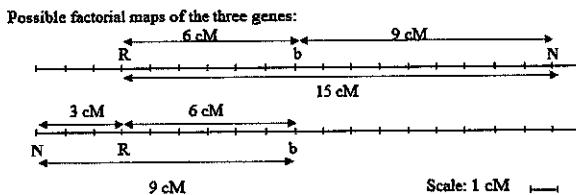
2.



3.



4.



5. RpNc x ppcc

6. The cross of the two drosophilae of pure lines of red normal eyes and of purple cinnabar eyes gives in the first generation 100 % of drosophilae of red and normal eyes since the two alleles normal and red are dominant respectively over cinnabar and purple and the parents are pure, the drosophilae obtained had taken a gamete carrying the two alleles red and normal from one of the two parents which make them of red and normal eyes.

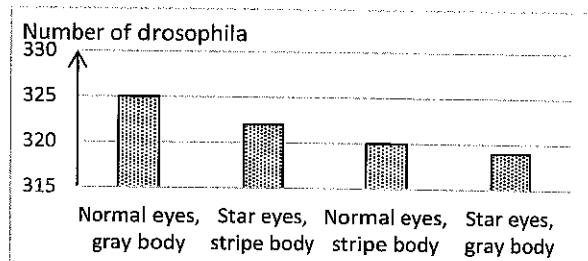
The cross of a female F1 with a male of birecessive phenotype leads to a generation comprising 40 % of birecessive drosophilae since the two genes are linked by a partial linkage where crossing over takes place at the prophase I of meiosis, this is the intrachromosomal recombination that leads to the female F1 to give four gametes of unequal percentages with two equal majors and two equal minors that are responsible for the appearance of 40 % of birecessive offspring that is from the major phenotypes.

The obtained birecessive phenotypes are of major percentages since the relative position of the alleles of the two genes is cis, the two recessive alleles are originally located on the same chromosome then the gamete carrying these two alleles at the same time will be parental of major percentage.

7. The results obtained on the web are not compatible with the results given above since in the results of the web the distance between the two genes of the color of eyes and the shape of eyes will be of 20 cM ($100 - 40 \times 2$) but according to the factorial maps made in the part (4) this distance should be either of 3 cM or of 15 cM.

Exercise 2 Relative positions of genes

- $NnGg \times nngg$. (0.25 pt)
- Histogram showing the results obtained by the cross of a female F1 with normal eyes and gray body with a birecessive one having star eyes and stripe body.



3.

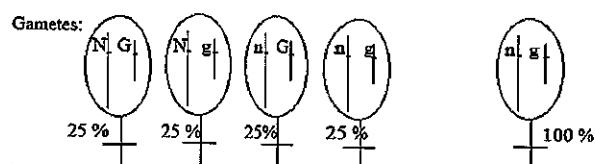
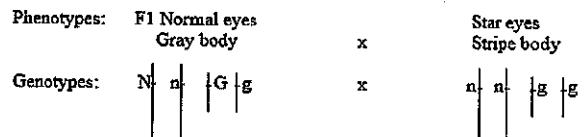


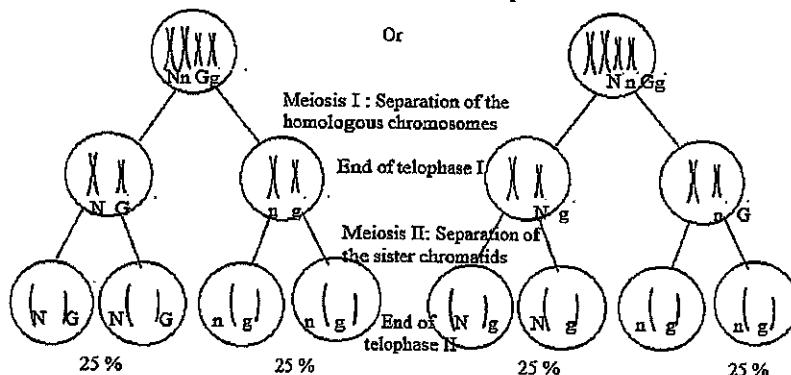
Table of cross:

	$N\ \ G$ 25%	$N\ \ g$ 25%	$n\ \ G$ 25%	$n\ \ g$ 25%
$n\ \ g$ 100%	$N\ \ n$ 25%	$N\ \ n$ 25%	$n\ \ n$ 25%	$n\ \ n$ 25%

Phenotypes: 25% [N,G] 25% [N,g] 25% [n,G] 25% [n,g]. The results are verified.

4.

Interchromosomal recombination done in the female drosophila of F1.



- These two genes are linked.
- In the second cross, no recombination takes place, since the allele of normal bristles is always linked to that of normal body, it is not linked during meiosis with the allele of spineless bristles, the same for the allele of spineless bristles that is linked always to the allele of trithorax body.

7.

Phenotypes: Normal bristles
Normal body \times Spineless bristles
Trithorax bodyGenotypes: BbTt \times bbtt

Gametes: 50% BT 50% bt

Table of cross:

	50% BT	50% bt
100% bt	BbTt 50 %	Bbtt 50 %

Phenotypes: 50 % [B,T] 50 % [b,t]

Then, the results are verified.

8.1. Linked.

8.2. Independent.

Exercise 3 A cross made in fish

- The result is 100% with normal tail and normal eyes.
- Since we obtain in the offspring all the fish with normal tail and normal eyes so the two alleles of fancy tail and funny eyes are hidden in the offspring so they are recessive, then the alleles of normal tail and normal eyes are dominant over the alleles of fancy tail and funny eyes respectively.

3. Symbols:

Let F be the symbol of the allele that determines the normal tail dominant with respect to the allele that determines the fancy tail that is recessive symbolized by f.

Let E be the symbol of the allele that determines the normal eyes dominant with respect to the allele that determines the funny eyes that is recessive symbolized by e.

Genotypes of the parents: FFEE x ffee

Genotype of the resulted fish: FfEe

- ffee, since the recessive alleles are expressed only in homozygous state.

- Normal tail, normal eyes: $2800/312 = 8.97 = 9$

Normal tail, funny eyes: $960/312 = 3.07 = 3$

Fancy tail, normal eyes: $920/312 = 2.94 = 3$

Fancy tail, funny eyes: $312/312 = 1$

Total $+ 9 + 3 + 3 + 1 = 16$

The proportions are:

Normal tail, normal eyes: 9/16

Normal tail, funny eyes: 3/16

Fancy tail, normal eyes: 3/16

Fancy tail, funny eyes: 1/16

- Phenotypes: Normal tail, normal eyes x Normal tail, normal eyes

Genotypes: FfEe

Gametes:	FE							
	1/4	1/4	1/4	1/4	1/4	1/4	1/4	1/4

Table of cross:

	FE 1/4	Fe 1/4	fE 1/4	fe 1/4
FE 1/4	F//F E//E 1/16	F//F E//e 1/16	F//f E//E 1/16	F//f E//e 1/16
Fe 1/4	F//F E//e 1/16	F//Fe//e 1/16	F//f E//d 1/16	F//f e//e 1/16
fE 1/4	F//f E//E 1/16	F//f E//d 1/16	f//f E//E 1/16	f//f E//e 1/16
fe 1/4	F//f E//e 1/16	F//f e//e 1/16	f//f E//e 1/16	F//f e//e 1/16

Phenotypes: [F,E] 9/16, [F,e] 3/16, [f,E] 3/16 [f,e] 1/16

Then the results are verified.

- 6/12[F,E], 2/12 [F,e], 3/12 [f,E], 1/12 [f,e].

Exercise 4 The genetic recombination

1. The dominant alleles are the alleles responsible for the gray color of the body and the brown color of the eyes car in F1 all mosquitoes are wild type. Therefore recessive alleles are the alleles responsible for the black color of the body and the clear color of the eyes.
Symbols: Gray: G, black: b, Brown:B, clear: r.
 2. GGBB x bbrr, because in F1 all the mosquitoes are with gray body color and brown eye color meaning that the parents are of true breeding lines.
 3. Partial linkage.
 4. Crossing over.
 5. They coupled the malaria resistance gene with one that makes mosquitoes' eyes red, so they could measure whether the resistance gene was passed on.
 6. Researchers have coupled the red eye color gene with the malaria resistance gene to distinguish resistant mosquitoes from non-resistant mosquitoes, so if a crossing over has occurred, red-eyed mosquitoes will either be resistant or non-resistant then the distinction desired by the researchers will no longer be reached.
 7. Let "R" be the symbol of the allele coding for red eyes that is dominant with respect to the allele coding for clear eyes that is symbolized by "c".
Let "S" be the symbol of the allele coding for the resistance to malaria that is dominant with

respect to the allele coding for the non-resistance that is symbolized by R .

Phenotypes: Red, resistant X clear, no resistance

50 % DS 50 % 100 %

Table of cross:

	RS 50 %	cn 50 %
cn 100 %	RS//cn 50 %	cn//cn 50 %

Phtenotypes: 50 % [RS] 50 % [cn]

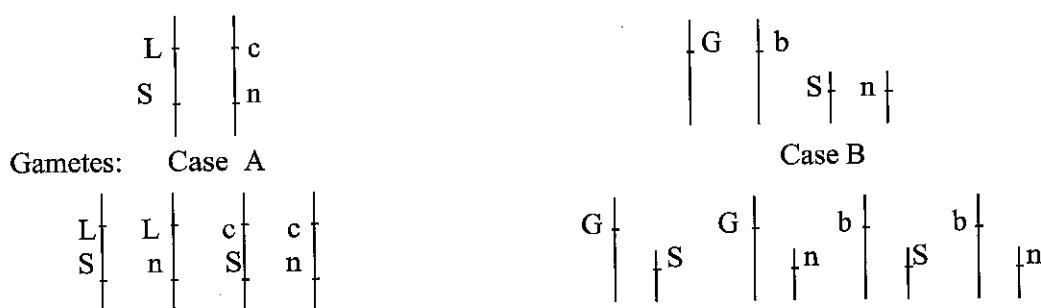
Exercise 5 Genetic recombination

1. A: In this case the two genes are linked in a partial linkage where crossing over takes place leading for the female F1 to give 4 types of gametes with unequal percentages with two parental majors and two recombinant minors that are responsible for the obtained results since the birecessive male gives only one type of gametes.

The two alleles are with cis relative position, the two dominant alleles on a side and the two recessive alleles on the other side are located on one of the two homologous chromosomes leading for bidominants and birecessive offspring of major percentages.

B: In this case the two genes are independent where the female F1 had given 4 types of gametes with equal proportions by separation of the homologous chromosomes during the anaphase I of meiosis I, and since the male gives only one type of gametes the phenotypic results of this cross will be determined by the gametes of the female F1 and will be 4 types of equal phenotypes.

2. Genotypes:



3. Genotypes bbSn X
 gametes bS 50% bn 50% bbn
 table of cross:

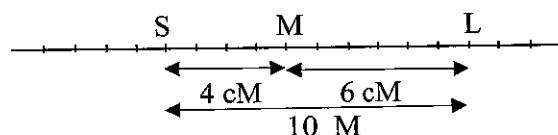
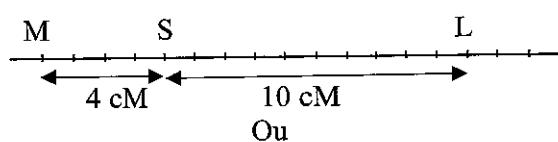
	bS 50 %	bn 50 %
bn 100 %	bbSn 50 %	bbnn 50 %

Phenotypes:

50 % black star 50 % black normal

4. the type of the recombination is intrachromosomal (scheme: see the copy book).
5. The results will be 4 equal phenotypes: [GL] 25%, [Gc] 25%, [bL] 25%, [bc] 25%. Since the two genes of the color of the body and the form of the eyes are independent and the two genes of the form of the wings and the form of the eyes are linked then the two genes of the form of the wings and the color of the body are independent.

6. Scale: 1 cM →



7. S'L//rc x rc//rc

Genetic recombination

Non-solved exercises

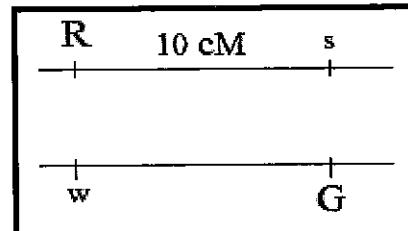
EXERCISE 1 The factorial map

Three traits are studied in the drosophilae; the first concerns the color of the eyes, red (R) is dominant over white (w); the second concerns the silk of the antennas, rough (G) dominates smooth (s); the third concerns the shape of the eyes, round (D) dominates bar (b).

The document 1 shows the factorial map of the two first couples of alleles with the relative distance that separate the two studied genes.

Two hybrid drosophilae for the first two traits are crossed together.

1. Show under the chromosomal forms the genotypes of the two crossed drosophilae and their gametes while determining the percentages of these gametes.
2. Determine, based on the answer of the part (1) and on a table of cross, the phenotypic percentages of the second generation concerning the two studied traits.

**Document 1**

A cross is made between two drosophilae, the first is hybrid with red and round eyes, and the second is birecessive for the two traits. In the offspring of this cross we find 6 % of recombinant drosophilae comprising among them drosophilae with red and round eyes.

3. Draw under the light of these results the two possible factorial maps for the three genes indicated above.
4. Indicate the genotypes of the drosophilae that should be crossed in order to determine the correct factorial map of the two maps drawn in the part (3).

Document 2 shows another series of crossings realized on other drosophilae.

5. How can we explain the obtained results in the crosses represented by the document 2?
6. Show starting from Document 2 the relative position of the two genes studied by this document as well as the gene distance that permits us to precise the correct factorial map of the maps done in the part (3).

Drosophilae of pure line		Drosophilae of pure line
round eyes	X	bar eyes
smooth silk		rough silk
	F1 100 % rounds eyes	
	rough silk	
Female F1	X	birecessive male
		A generation comprising 8 %
		of birecessive drosophilae

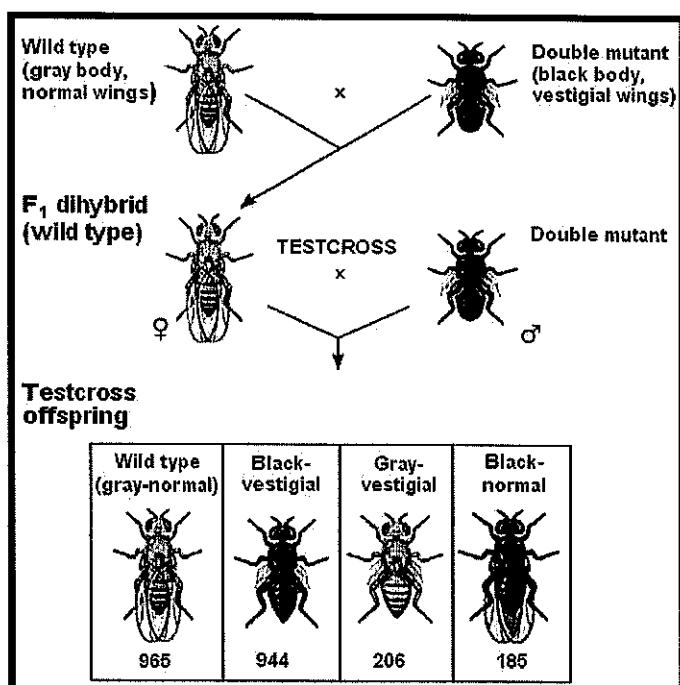
Document 2

EXERCISE 2 A cross of drosophilae

Document 1 shows a cross made between two drosophilae of pure lines, the first of wild type (gray G and normal N) and the second double mutant (black b and vestigial v).

An F1 generation of drosophila all of wild type is obtained. The cross of a female F1 drosophila of this generation with a birecessive male is done, the results are given by the document 1.

1. Justify that the alleles of gray body and normal wings are dominant respectively over the allele of black body and vestigial wings.
2. Determine the genotype of the obtained F1 dihybrid female.
3. Calculate the frequency of recombination of the two studied genes.
4. Make the necessary factorial analysis to verify the obtained results.

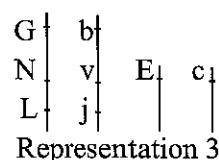
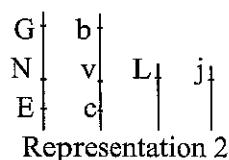
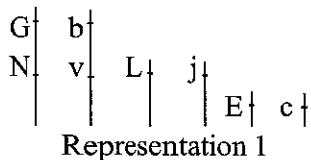


Document 1

Another female hybrid drosophila having loose wings (L) and normal eyes (E) is crossed with a birecessive male having jammed wings (j) and cardinal eyes (c), this cross leads for four phenotypes of equal proportions.

5. Schematize the genetic assortment at the origin of the obtained results in this cross.

In order to show the possible relative positions of the four studied genes, they are represented on the chromosomes of a diploid cell heterozygous for all of them. This representation can be one of the three representations shown by Document 2.



6. Write the genotypes of the drosophila that should be crossed in order to determine the correct representation.

A cross of a hybrid drosophila having loose wings and gray body with a birecessive one gives 7 % of recombinant phenotypes.

7. Show that the correct representation among the three representations given above is the representation 3.
8. Draw the two possible factorial maps for the three linked genes of the four given genes.

EXERCISE 3 Study of the relative positions of many genes in drosophila

The genes that are responsible for the traits of the individual are located on its chromosomes; some genes are located on two different chromosomes, others are located on the same chromosome.

In order to study the relative positions of some genes in drosophila; two crosses were made starting from F1 female drosophila and birecessive males. These two crosses are represented in the documents 1 and 2.

1. Compare the results of these two documents 1 and 2. Explain the difference in the obtained results between these two crosses.
2. Write the genotypes of the crossed drosophila in the two crosses 1 and 2 in chromosomal forms.
3. Make for the cross in document 2 the factorial map of the two given genes.
4. Name the type of the genetic assortment made in each of the two cases 1 and 2.

F1 female drosophila	x	birecessive male
Normal eyes	x	star eyes
Ribbed wings		non ribbed wings
327 Normal eyes, ribbed wings		
332 Star eyes, ribbed wings		
330 Normal eyes, non ribbed wings		
329 Star eyes, non ribbed wings		

Document 1

F1 female drosophila	x	birecessive male
Normal eyes	x	star eyes
Gray body		Black body
310 Normal eyes, gray body		
43 Star eyes, gray body		
41 Normal eyes, black body		
308 Star eyes, black body		

Document 2

We decide to determine the position of a fourth gene with respect to the genes studied in the documents 1 and 2. In this purpose, we cross an F1 female drosophila having ribbed wings and red eyes with a birecessive male having non-ribbed wings and white eyes. The results of this cross show 4 phenotypes of equal percentages.

5. Illustrate the genetic assortment made by the female F1 with ribbed wings and red eyes by an explanatory drawing.
6. Determine the different possible positions of the gene of the color of eyes regarding the three other studied genes.
7. Write the genotypes of the drosophila that we should cross in order to determine the real position of the gene of the color of eyes with respect to the other three studied genes.

Ch. 3 Human genetics



Human genetics

Course abstract

Human genetics is the study of the transmission of the hereditary traits in organisms as the study of the transmission of some hereditary diseases and chromosomal abnormalities. These chromosomal abnormalities affect the karyotypes of the individuals due to errors during meiosis or due to abnormalities that affect the structures in chromosomes.

1. Transmission of hereditary traits

We are interested in the study of the traits that are governed only by one gene having only two alleles (except the gene of blood types of ABO system that has three alleles).

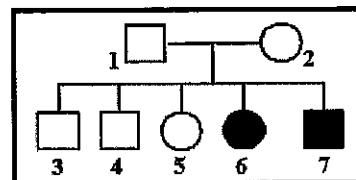
The studies in human genetics concerning the gene carried either by an autosome or by a sex chromosome. When this gene has a mutant allele, it leads usually to a disease. This mutant allele can be recessive or dominant, or it can be codominant or of intermediate dominance with the normal one.

In all the pedigrees below, the circle represents a female, the square represents a male, the black individuals represent the mutant phenotypes.

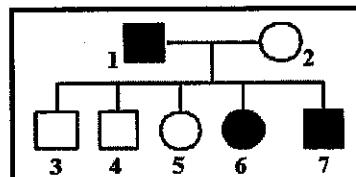
1.1. Autosomal gene

1.1.1. How to prove that the autosomal mutant allele is recessive?

Case 1: According to the adjacent pedigree, an individual of mutant phenotype has two parents of normal phenotype, this means that the mutant allele was hidden in the two parents (since we know that the gene is autosomal) then it is recessive.

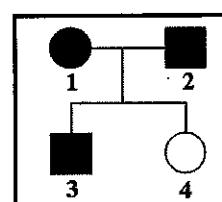


Case 2: In the adjacent pedigree, if the parent with mutant phenotype is homozygote (by given), this parent had given surely one mutant allele to the child that is normal, so the mutant allele is hidden in the child, then it is recessive.

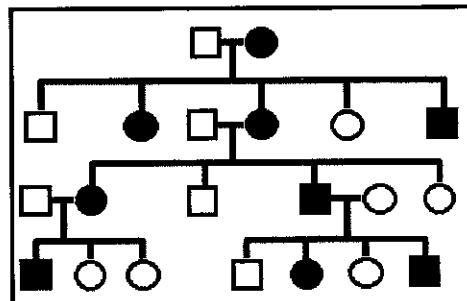


1.1.2. How to prove that the autosomal mutant allele is dominant?

Case 1: A mutant allele is dominant when two parents having the mutant phenotype have normal children, in that case the normal allele is hidden in the two parents (since we know that the gene is autosomal) so it is recessive, then the mutant allele is dominant.



Case 2: When each mutant parent has at least one mutant child, and the mutant phenotype is rare, it is impossible for all the normal parents to be heterozygous in order to consider the mutant allele recessive, so the mutant allele is considered to be dominant.



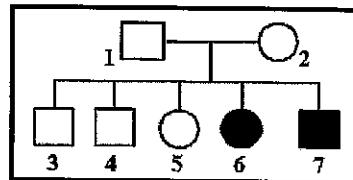
Case 3: Refer to the case 2 of recessive mutant allele, if the normal parent is homozygote, the affected children take surely the normal allele that is hidden, then the mutant allele is dominant.

1.1.3. How to prove that a gene is localized on an autosome?

We have to negate the localization of the gene on:

- The non-homologous segment of the chromosome Y, starting from a father and a son of different phenotypes.
- The non-homologous segment of the chromosome X, starting from a father with dominant phenotype having a daughter of recessive phenotype.
- The homologous segment of the chromosomes X and Y, starting from a father of dominant phenotype having a daughter and a son of recessive phenotype.

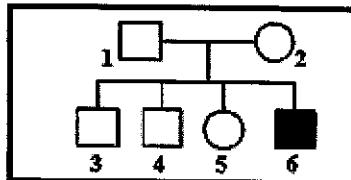
Then we affirm the localization on an autosome.



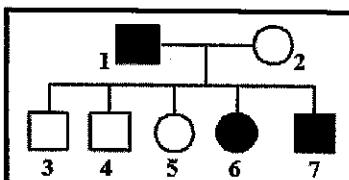
1.2. Gene carried on the non-homologous segment of chromosome X

1.2.1. How to prove that the mutant X linked allele is recessive?

Case 1: According to the opposite pedigree, a boy of mutant phenotype has two parents of normal phenotype, this means that the mutant allele was hidden in the mother (since we know that the gene is on the non-homologous segment of X) then it is recessive.

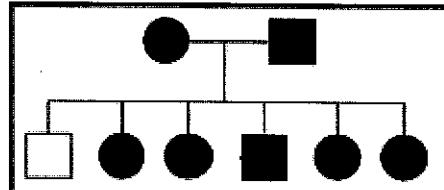


Case 2: In the opposite pedigree, the father had given surely one mutant allele on X to the normal girl (5), so the mutant allele is hidden in this girl, then it is recessive.

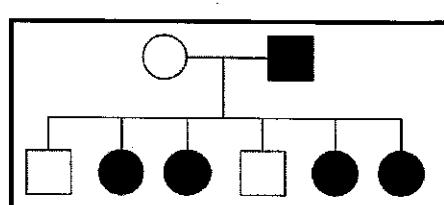


1.2.2. How to prove that the mutant X linked allele is dominant?

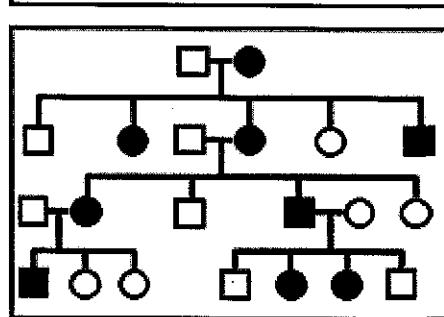
Case 1: A mutant allele is dominant when two parents having the mutant phenotype have normal boys, in that case the normal allele is hidden in the mother (since we know that the gene is located on the non-homologous segment of chromosome X) so it is recessive, then the mutant allele is dominant.



Case 2: When the mother is not carrier and the mutant father has one or more girls having mutant phenotype, the mutant allele carried on the chromosome X, given by the father to the girls, is expressed in presence of the normal allele given by the mother, then the mutant allele is dominant.



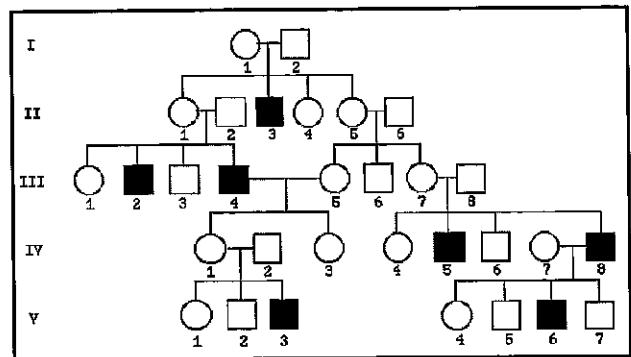
Case 3: In the opposite pedigree, the affected mothers transmit the mutant allele to girls that had taken the normal allele from the father that is hidden, then the mutant allele is dominant.



1.2.3. How to prove a gene located on the non-homologous segment of chromosome X?

Recessive mutant allele

Case 1: As we can see in the pedigree besides, all the affected persons are of male sex, this certifies that the gene responsible for the studied trait is sex linked and it is either located on the non-homologous segment of X or Y. One affected boy with normal father indicates that the gene is not located on the non-homologous segment of Y. Thus, the gene is located on the non-homologous segment of X.

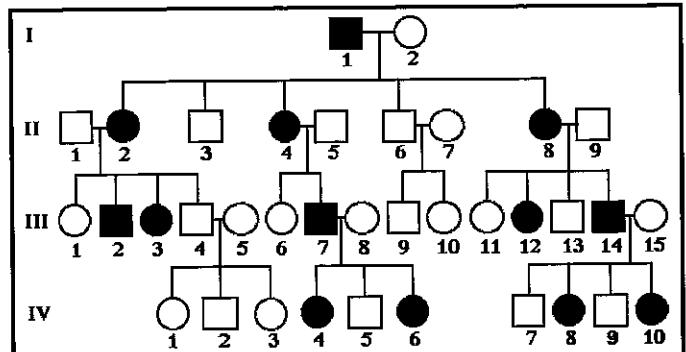


Case 2: In the same pedigree, if it is given that the father I-2 is not carrier of the mutant allele, then only one allele taken from the mother is responsible for the boy II-3 to be affected, this allele is taken surely on X chromosome, otherwise, the boy will inherit another normal allele from the father and becomes normal, this is not the case.

Case 3: In the case of a lethal mutant allele, the presence of a mutant boy indicates that this boy has only one allele of the gene that is expressed in the phenotype, it is then located either on the non-homologous segment of X or Y. From the discussion of the genotypes of a father and an affected son, we can certify that the gene is located on the non-homologous segment of X.

Dominant mutant allele

In this case the father with mutant phenotype will have all the girls affected, the data of the pedigree is not enough statistically, it should be completed by other information taken from texts or other documents, in that case, the father is always transmitting the disease only to girls and never to boys, it means that the gene is located on the chromosome X that is the sole chromosome that the father cannot transmit it to the boys.



1.3.Determination of the risk for an individual to be of mutant phenotype

Case of recessive mutant autosomal allele

In this case the risk for a child to be mutant is equal to:

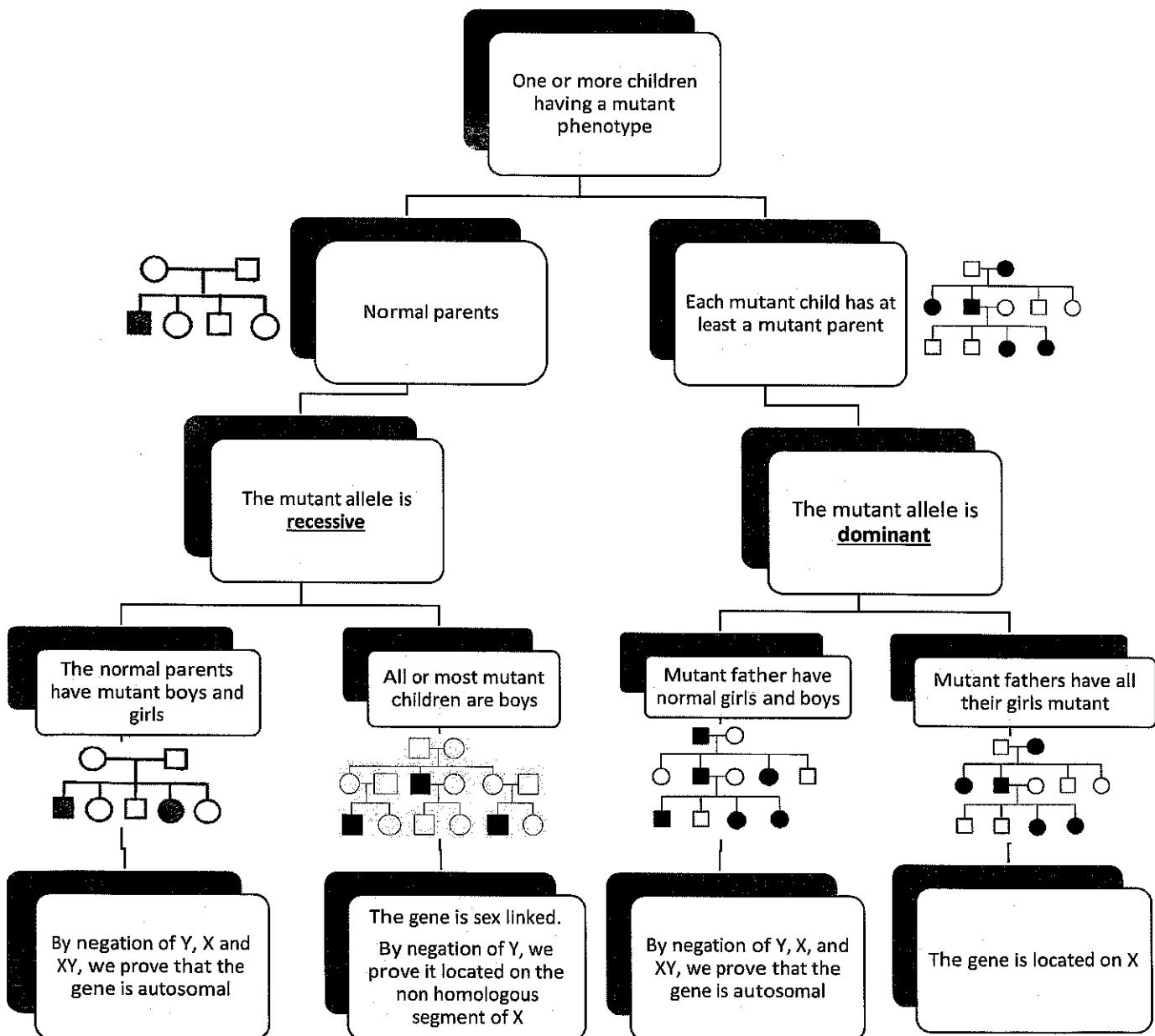
$$\text{Risk of mother to be heterozygous} \times \text{risk of the father to be heterozygous} = \text{risk for the child to be mutant if the parents are heterozygous}$$

Case of dominant mutant autosomal allele

In this case the risk for a child to be mutant is equal to:

(The sum of the risks of the different possible mutant children) or
1 - (chance for it to be normal)

1.4.Schema showing the different cases of the transmission of the mutant allele



2. Chromosomal abnormalities

2.1.Trisomy

It consists of the presence in the karyotype of three copies of a given chromosome instead of two, it can be:

- Free, when the extra chromosome is free, it is due to abnormal disjunction of chromosomes during meiosis I or II. It is the case of Klinefelter syndrome XXY.
- Linked or translocated, when the extra chromosome is linked to another chromosome, it is due to a translocation.

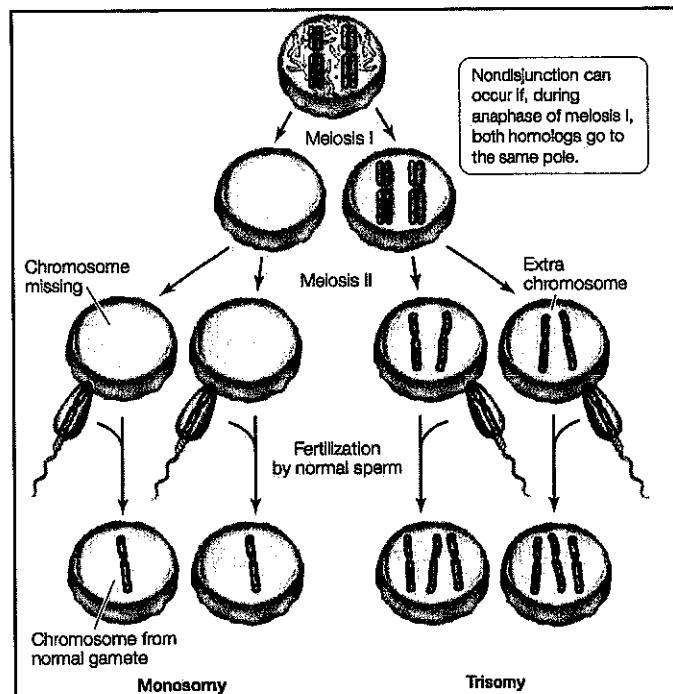
2.2. Monosomy

It consists of the presence of a chromosome having only one copy in the karyotype instead of two, and it is due to either an abnormal meiosis or a translocation in one of the parents. It is the case of Turner syndrome XO.

2.3. Examples of abnormal meiosis leading to monosomy and trisomy

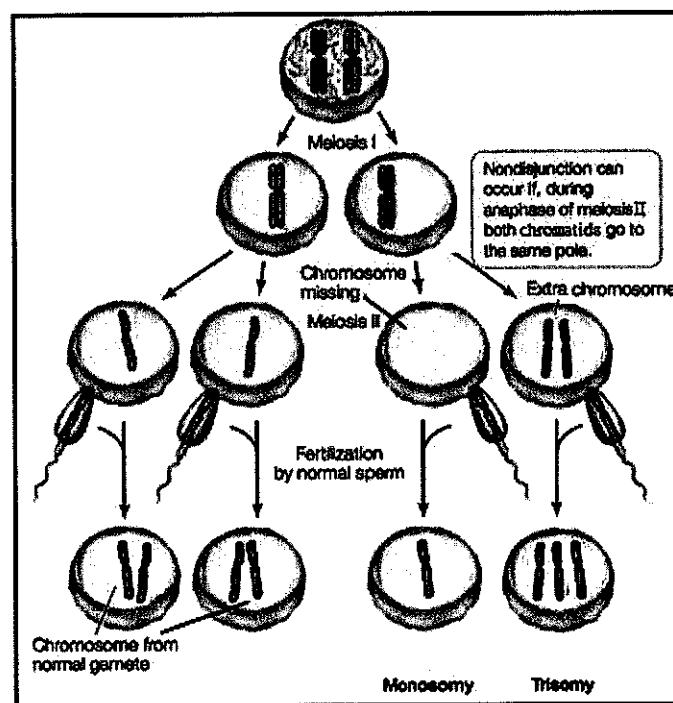
2.3.1. Abnormality during meiosis I.

In this example we can see nondisjunction of homologous chromosomes during meiosis I.



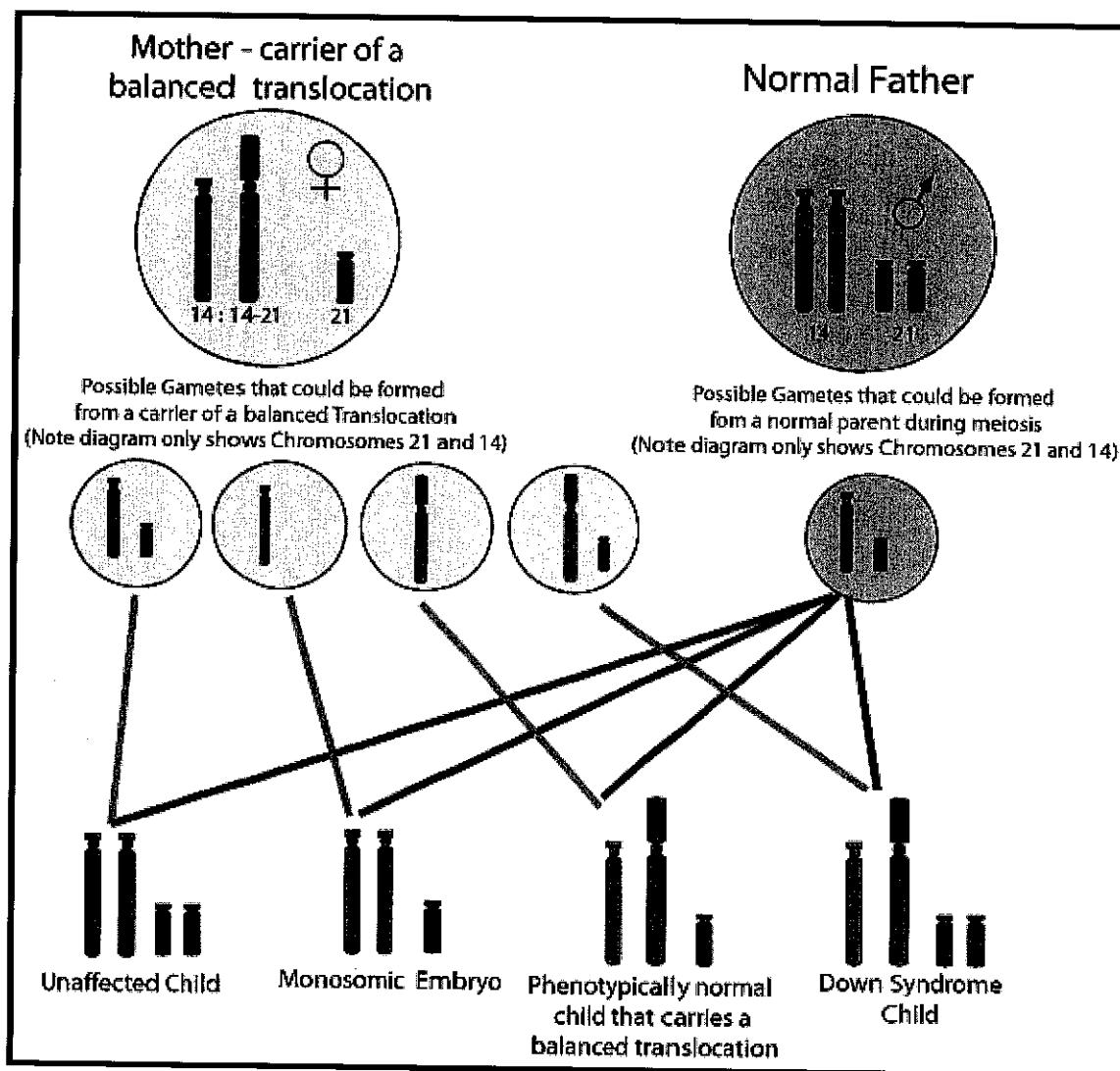
2.3.2. Abnormality during meiosis II.

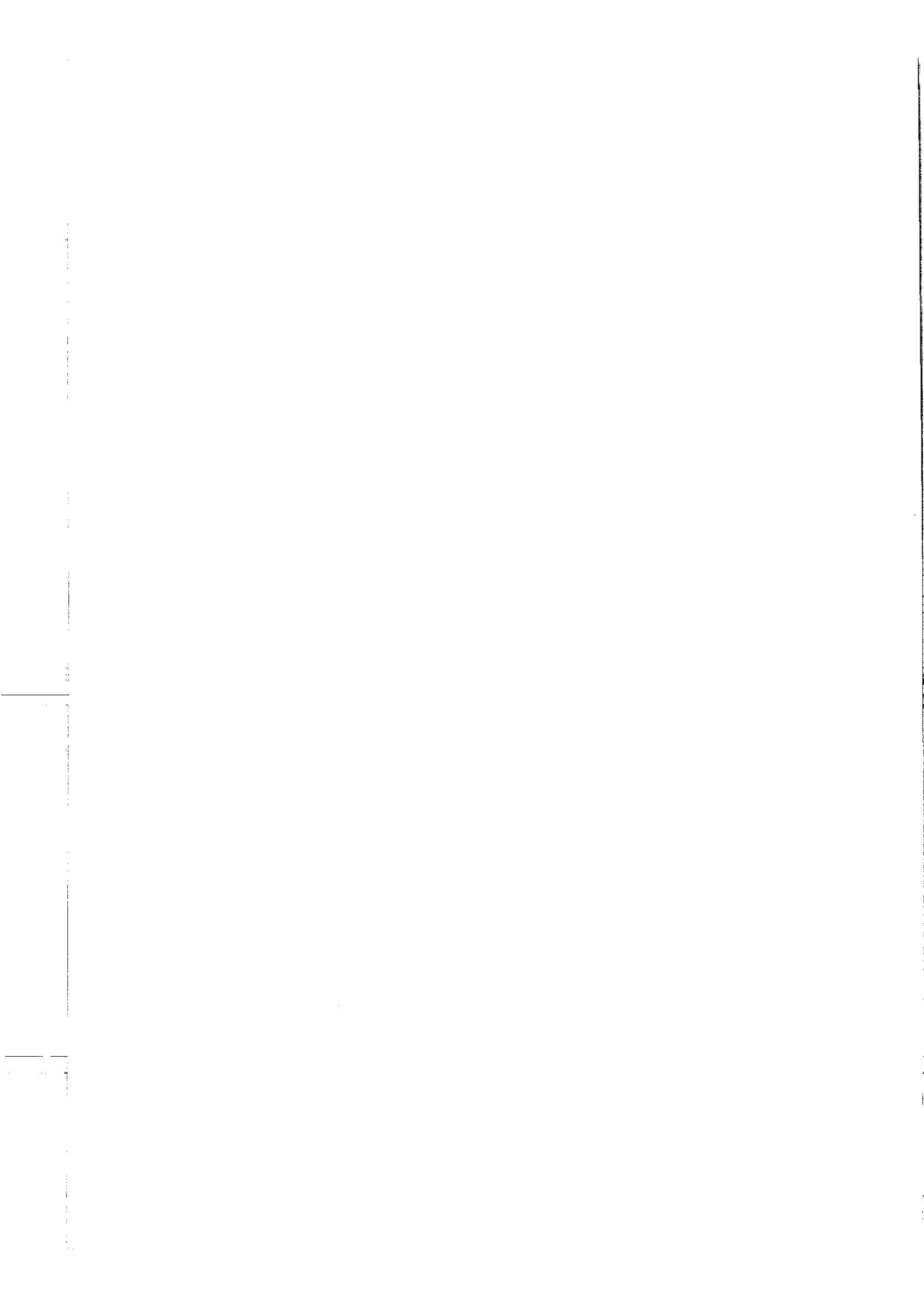
Nondisjunction of sister chromatids during meiosis II.



2.3.3. Translocation in the parents leading to trisomy and monosomy

A chromosome 21 translocated on the chromosome 14 in one of the parents can lead to cases of trisomy 21 and monosomy 21 in the children.





Human genetics

Training exercises

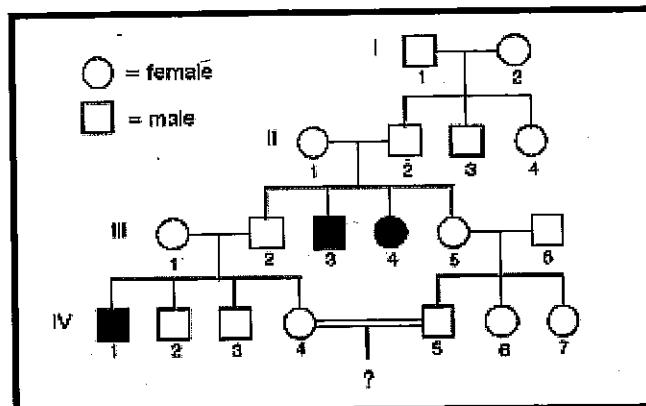
EXERCISE I Localisation of the gene of cystic fibrosis

The following pedigree shows the transmission of cystic fibrosis in a family. The persons colored black are the affected ones.

1. Indicate if the allele responsible of this disease dominant or recessive. Justify.
2. Show that the gene responsible for this disease is autosomal.
3. Specify the genotypes of the individuals: II1, IV1 and IV2.

One of the two individuals II1 and II2 is surely heterozygous for the gene of the cystic fibrosis.

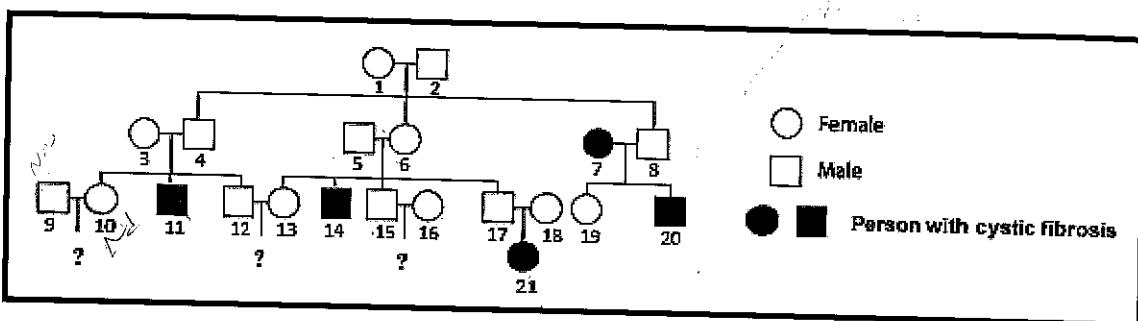
4. Justify this affirmation.
5. Explain why the risk for having an affected child by cystic fibrosis is high for the couple IV4-IV5.

**Solution:**

1. The allele responsible for this disease is recessive since the two parents II1 and II2 are normal having an affected child so the allele of the disease is hidden at least in one of them. Let N be the symbol of the normal allele that is dominant and d be the symbol of the mutant allele that is recessive.
2. If the gene of the disease is carried on the non-homologous segment of the chromosome Y, the disease would be transmitted from father to son, but the son III3 is affected, he would have the genotype X^d/Y^d , and would have taken the allele of the disease from the father II2 that would be of genotype X^d/Y^d and would be affected but he is normal. Then, the gene is not carried on the non-homologous segment of the chromosome Y.
If the gene is carried by the non-homologous segment of the chromosome X, the affected girl III4 must be homozygous of genotype X^d/X^d , she should have inherited the mutant allele from her father II2 who should be affected of genotype X^d/Y . But her father is normal. Then, the gene is not carried by non-homologous segment of X.
If the gene is carried by the homologous segments of X and Y, affected girl III4 of genotype X^d/X^d should have inherited X^d from her father II2; the affected boy III3 of genotype X^d/Y^d should have inherited Y^d from his father II2. Father II2 should be affected of genotype X^d/Y^d which is not the case since he is normal. Then, the gene is not carried by the homologous segments of X and Y.
Therefore, the gene is carried by an autosome.
3. II1 has the genotype Nd since she is normal having the allele N and she has affected children III3 and III4 to which she had given the allele d.
IV1 has the genotype dd, since he is affected and the mutant allele is recessive expressed only in homozygous.
IV2 is of genotype NN or Nd, since he is normal and the normal allele is dominant expressed in the case of homozygous and heterozygous state.
4. The Individual II2 is surely heterozygous since he has affected children III3 and III4, he had taken the allele d from one of his parents, then at least one of them is of genotype Nd.
5. The two persons IV4 and IV5 are cousins, and they have a family history for the disease, the risk of each one of them to be carrier for the allele of the disease is relatively high, then they have a high risk to give birth to a child affected by cystic fibrosis.

EXERCISE 2 Risk to be affected by cystic fibrosis

The following pedigree shows the transmission of cystic fibrosis in a family. This disease is recessive. It has the gene located on the chromosome number 7. The proportion of heterozygous persons in the population to which this family belongs is 1/25.



1. Pick out from the text the statement that indicates that cystic fibrosis is an autosomal disease.
2. Evaluate, based on a punnet square the risk for the individuals 17 and 18 of the pedigree to have another child affected by cystic fibrosis.
3. Indicate the risk for the couple (7-8) to have another affected child by cystic fibrosis.
4. Determine the risk for the couple (15-16) to have an affected child by this disease.

Knowing that the individual 9 is from another population where there is no recording of cases of cystic fibrosis.

5. Specify the risk for the couple (9-10) to have an affected child.

Solution:

1. It has the gene located on the chromosome number 7.
2. Let N be the symbol of the normal allele and d be the symbol of the allele of cystic fibrosis. The two individuals 17 and 18 have already an affected child, they are heterozygous of genotypes Nd. Starting from the opposite punnet square the risk for this couple to have another affected child is 1/4.

	N 1/2	d 1/2
N 1/2	NN 1/4	Nd 1/4
d 1/2	Nd 1/4	dd 1/4

3. 1/2.
4. In order to have an affected child, the two parents should be of genotype Nd, and they should both give the allele d to the child to be of genotype d/d and to be affected.

The male 15 has two heterozygous parents since his brother is affected of genotype dd and had taken an allele d from each of the parents, then this male 15 has a risk of 2/3 to be heterozygous N//d (1/4 to be N//N, 1/2 to be N//d, and 1/4 to be d//d that is rejected since he is normal one).

The risk for the female 16 to be heterozygous is equal to the proportion of heterozygous persons in the population since she has no family history. Then her risk to be heterozygous is 1/25.

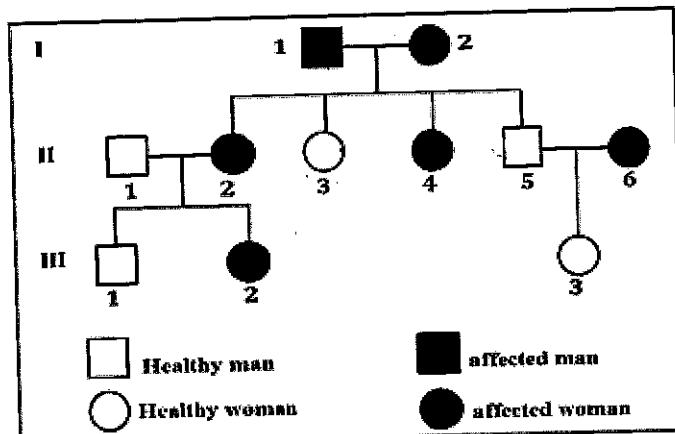
If the parents are heterozygous, each has a risk of 1/2 to give the allele d to the child. The risk for the child to be affected is $2/3 \times 1/2 \times 1/25 \times 1/2 = 1/150$.

5. The risk for the couple (9-10) to have an affected child is null since the male 9 has no risk to be heterozygous since the proportion of the heterozygous in its population is null knowing that there are no affected persons in it, then he will give surely an allele N to each of its children and make them all normal.

EXERCISE 3 Transmission of a dominant autosomal disease

Document 1 shows the pedigree of a family whose members are affected by a hereditary disease.

1. Justify that the allele of the disease is dominant.
2. Show that this disease is transmitted in an autosomal mode.
3. Indicate the genotypes of the individuals I1, II3, II4 and III2. Justify the answer of each.
4. Determine the risk for the couple (II5-II6) to have an affected child.

**Solution:**

1. The two parents II1 and II2 are affected having normal children II3 and II5, then the normal allele is hidden at least in one of the two parents indicating that the normal allele is recessive, so the allele of the disease is dominant.
2. If the gene of the disease is carried on the non-homologous segment of the chromosome Y, the disease would be transmitted from father to son, but the son II5 is normal, he would have the genotype $X^n//Y^n$, and would have taken the normal allele from the father II1 that would be of genotype $X^n//Y^n$ and would be normal, but he is affected. Then, the gene is not carried on the non-homologous segment of the chromosome Y.
If the gene is carried by the non-homologous segment of the chromosome X, the healthy girl II3 must be homozygous of genotype $X^n//X^n$; she should have inherited the normal allele from her father II1 who should be healthy of genotype $X^n//Y^n$. But her father is affected. Then, the gene is not carried by non-homologous segment of X.
If the gene is carried by the homologous segments of X and Y, healthy girl II3 of genotype $X^n//X^n$ should have inherited X^n from her father II1; the healthy boy II5 of genotype $X^n//Y^n$ should have inherited Y^n from his father II1. Father II1 should be healthy of genotype $X^n//Y^n$ which is not the case since he is affected. Thus, the gene is not carried by the homologous segments of X and Y.
Therefore, the gene is carried by an autosome.
3. Let D be the symbol of the allele of the disease that is dominant, and n be the symbol of the normal allele that is recessive.
II1: D//n, since he is affected having the allele D and he has normal girl of genotype n/n to which he had given an allele n.
II3: n/n since she is normal homozygous knowing that the normal allele is recessive cannot be expressed unless in the homozygous state.
II4: D/D or D//n since she is affected and the allele of the disease is dominant can be expressed in homozygous and heterozygous state.
III2: D//n since she is affected having the allele D and having taken the allele n from the normal father II1 of genotype n/n.
4. The genotype of II5 is n/n since he is normal, and the normal allele is recessive expressed only in homozygous.
The genotype of II6 is D//n since she is affected having an allele D and she has a normal girl of genotype n/n to which she had given an allele n.
In order to be affected, a child should take an allele D from the mother, this child will take surely an allele n from the father.
The risk for the child to take an allele D from the father is 1/2.
Then the risk for the child to be affected is 1/2.

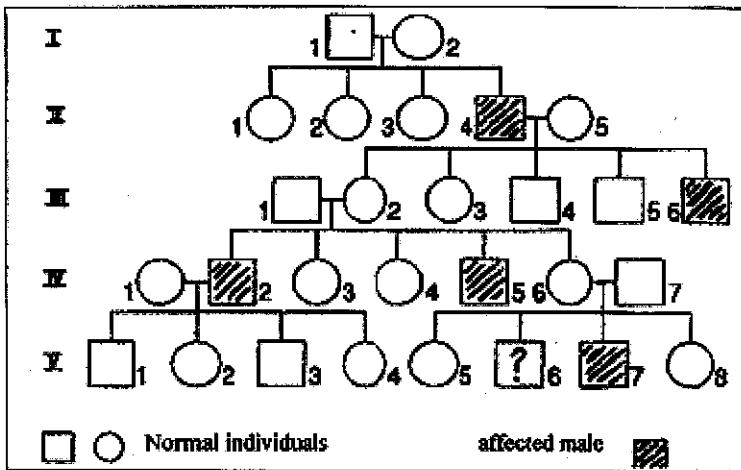
EXERCISE 4 The transmission of hemophilia in humans

Hemophilia A is a hereditary recessive disease characterized by an anomaly in the mechanism of blood coagulation and is due to absence or anomalies of a protein implied in the coagulation, the factor VIII. The pedigree of the document 1 shows the transmission of this illness in a family.

1. Justify that the allele responsible for the hemophilia is recessive.
2. Determine, starting from the pedigree of the document 1, the localization of the gene of hemophilia.
3. Indicate the genotypes of the individuals: III₃, III₄, IV₅ and V₅. Justify.

Knowing that the phenotype of the individual V₆ is unknown.

4. Indicate the risk for it to be hemophilic.



Document 1

Solution:

1. The two parents I₁ and I₂ are normal having an affected child II₄, then the allele of the disease is hidden at least in one of the two parents so it is recessive. Let N be the symbol of the normal allele and d be the symbol of the mutant one.
2. In this pedigree, the affected individuals are all boys, this means that the disease is sex linked, located on the non-homologous segment of X or Y, then it is not located neither on an autosome nor on the homologous segment of X and Y. It is located either on the non-homologous segment of X or on the non-homologous segment of Y.
 If it is located on the non-homologous segment of Y, the boy II₄ will be of genotype X/Y^d, he would have taken Y^d from his father that would be of genotype X/Y^d and would be affected but he is normal, so this is not the case. Then the gene of the disease is located on the non-homologous segment of chromosome X.
3. III₃: X^N//X^d since she is normal having surely an allele N and she inherited X^d from her affected father of genotype X^d/Y.
 III₄: X^NY since he is normal having an allele N carried on the chromosome X.
 IV₅: X^d//Y since he is affected by hemophilia, having the allele of the disease on his chromosome X.
 V₅: X^NX^N or X^N//X^d since she is normal and the normal allele is dominant can be expressed in homozygous and heterozygous.
4. 1/2.

EXERCISE 5 Dominant hereditary disease

Document 1 shows the pedigree of a family where some members are affected by a rare hereditary disease.

1. Knowing that the female II-1 is homozygote show that this disease cannot be recessive.

The children of the other couples that have the same phenotypes as the couple (II-1, II-2) are always similar to the children obtained by this couple.

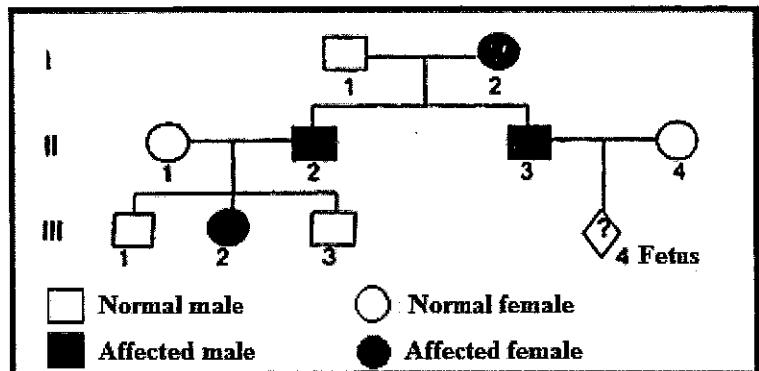
2. Show that the allele of this disease is located on the non-homologous segment of X.
3. Determine, based on a punnet square, the risk for the fetus to be affected by this disease.

Solution:

1. The female II1 is normal homozygous, then she has two normal alleles (whatever the localization of the gene of the disease) this means that she would have given a normal allele to her daughter III2, this allele is hidden in the daughter since the daughter is affected then the normal allele is recessive, therefore the allele of the disease is dominant.
2. All the affected fathers that got married from normal females have affected daughters and normal sons, so the allele of the disease is transmitted from the father only the girls, never to the boys. So, the allele is located on the non-homologous segment of chromosome X that is transmitted from the father only to the girls.
3. The genotypes of the parents are $X^D Y$ and $X^n X^n$ since the father is affected and the mother is normal homozygous.

	$X^D 1/2$	$Y 1/2$
$X^n 1/1$	$X^D X^n 1/2$	$X^n Y 1/2$

The risk for a girl to be affected is 1, the risk for a boy to be affected is null.



Document 1

EXERCISE 6 Detection of a gene mutation

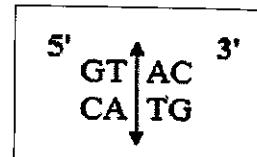
Hemochromatosis is a recessive disease linked to a gene located on chromosome 6. This gene has a normal allele and a mutated allele causing an abnormality characterized by the accumulation of iron in the cells.

Document 1 presents the partial sequence of the non-transcribed strand of the two alleles, the normal and the mutated ones. Document 2 presents the restriction site of a restriction enzyme RsaI. Document 3 shows the genetic code table.

Number of the nucleotide	1	240	250	270	278	387
Normal HFE Allele		↓	↓	↓	↓	↓
Mutated HFE Allele			↓	↓	↓	↓

5' GCTGTACCCCC....ACGTGCCAG...C 3'

..... GCTGTACCCCC....ACGTACCCAG...C

**Document 1**

1. Compare the sequence of the normal HFE allele to the mutated one.
2. Indicate the type of mutation that lead to the formation of the mutated allele.
3. Determine, by referring to document 3, the amino acid sequence translated from the two alleles of HFE gene.
4. Explain how the modification in the nucleotide sequence of the allele leads to the appearance of the corresponding disease.
5. Determine for each allele, the number and the length of the restriction fragments obtained after cutting by RsaI enzyme.
6. Justify that the enzyme RsaI is able to detect the genetic polymorphism of this gene.

Solution:

1. All the nucleotides of the normal and the mutated are the same except in the nucleotide 274 which is a guanosine in the normal allele but it is an adenosine in the mutated one.
2. The mutation leading for the mutated allele is a mutation by substitution.
3. For the normal allele: DNA sequence: .G,CTG,TAC,CCC,C..... A,CGT,GCC,AG.....C.
mRNA sequence: .G,CUG,UAC,CCC,C...A,CGU,GCC,AG.....C.
since the mRNA is similar the non-transcribed strand except U instead of T.
Amino acid sequence: ...Leu,Tyr,pro,... Arg,Thr.
- For the mutated allele: DNA sequence:G,CTG,TAC,CCC,C... A,CGT,GCC,AG.....C.
mRNA sequence: .G,CUG,UAC,CCC,C.....A,CGU,GCC,AG.....C.
Amino acid sequence: ...Leu,Tyr,pro,... Arg(Ala).
4. The substitution mutation in this case is a missense mutation that leads to the change of the amino acid threonine by another one which is alanine, this change leads to the change of the 3D shape of the protein that causes the change in its function, this protein becomes with another effect leading for the accumulation of iron in the cells that will lead for the hemochromatosis.
5. The number of fragments in the normal allele is 2 since the enzyme cuts in one restriction site after the nucleotide 244, the length of these two fragments are 244 bp and 387-244 = 143 bp.
In the mutated allele, the number of fragments is 3 since the enzyme cuts in two restriction sites, the first after the nucleotide 244 and the second after the nucleotide 273, the length of these fragments are 244 bp, 273-244 = 29 bp and 387-273=114 bp.
6. This enzyme is able to detect the genetic polymorphism of this gene since it leads to different number and length of fragment in the two alleles (normal and mutated) that can be separated and detected by electrophoresis.

Nucleotides position 2:							
		U	C	A	G		
		UUU UUC UUA UUG	UCU UCC UCA UCG	UAU UAC UAA UAG	UGU UGC UGA UGG		
Nucleotides position 1	U	CUU CUC CUA CUG	CCU CCC CCA CCG	CAU CAC CAA CAG	CGU CCG CGA CGG	UC CA GA AG	
	C	I leucine	P proline	H histidine	R arginine		
	A	AUU AUC AUU AUG	AAC ACC ACA ACG	T threonine	Q asparagine	U serine	
	G	M methionine	GCU GCC GCA GGC	S alanine	K lysine	R arginine	
		V valine		E glutamic acid	D aspartic acid	G glycine	
				GAA GAG	GAC GAT	GGG	
				A	A		

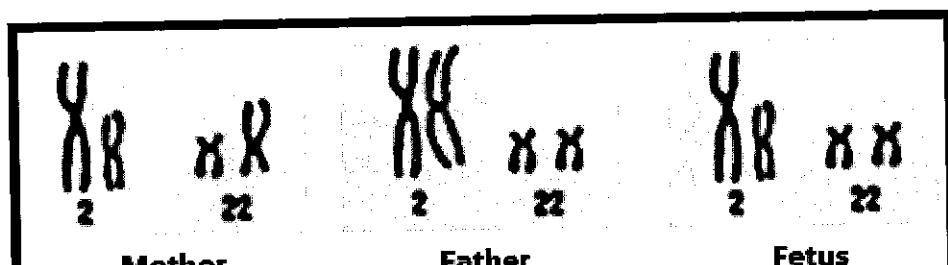
A: Adenine U: Uracil G: Guanine C: Cytosine

Document 3

EXERCISE 7 Repeated abortions

A mother is facing a problem of repeated abortions. In order to diagnose the possible origin of these abortions, the doctor decides to prepare the karyotype of the mother, the father and the aborted fetus. One of them has a normal karyotype.

The partial karyotypes obtained are shown in the document 1.

**Document 1**

1. Draw out from the above text the hypothesis of the doctor.
2. Identify the normal partial karyotype among those in document 1.
3. Compare the karyotypes of the mother and the fetus to that of the father.
4. Explain why the fetus died while the mother has a normal phenotype.

Solution:

1. Hypothesis: The origin of the abortions of the mother is a chromosomal abnormality.
2. In the normal karyotype the autosomes are found in homologous pairs where each two chromosomes of the same pair are of same size. In these karyotypes we notice that the homologous chromosomes of the pair 2 are of same size, the same for the chromosomes of the pair 22 in the father while the mother has the two chromosomes of pair 2 on a hand and the two chromosomes of pair 22 on the other hand having different sizes, the same for the fetus in the pair 2, thus only the father has normal karyotype.
 - 3. The number of chromosomes in the partial karyotypes of the father, the mother and the fetus is the same equal to 2 in each of the chromosomes 2 and 22, all of two chromatids. On the other hand, the two chromosomes 2 of the father are of same size as one of the chromosomes 22 of the mother and the fetus, but the other chromosome 2 of the mother and the fetus are smaller and of same sizes compared together. Moreover, the two chromosomes 22 of the father are of the same sizes as both chromosomes 22 of the fetus and as one of the chromosomes 22 of the mother but the other one is longer.
3. The mother has one chromosome 22 taller than the normal one and one chromosome 2 smaller than the normal one, so she has a translocation of one part of the chromosome 2 on the chromosome 22, thus she has a complete genetic makeup with no loss or gains. Thus, her phenotype is normal. While the fetus died since it has one chromosome 2 smaller from the normal one and two normal chromosomes 22, thus it has a loss in its genetic material, which led to the death of the fetus.

Human genetics

Solved exercises

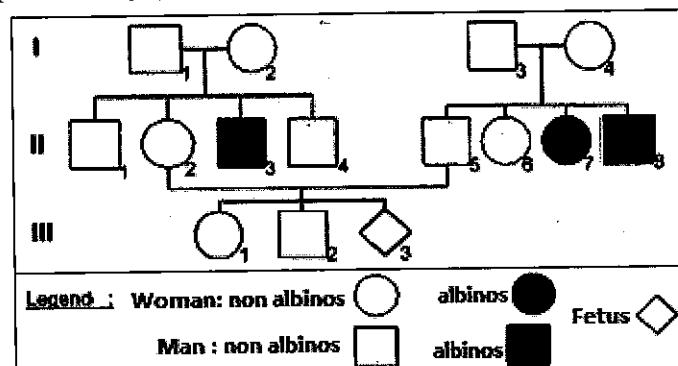
EXERCISE 1 Genetic polymorphism of the gene of albinism

In the human, the albinism is a rare genetic particularity (1 case over 20000 birth).

The albinism is due to the absence of melanin, a brown pigment responsible for the color of the skin and the hair.

In the family in document 1, we notice three alleles of the gene of albinism:

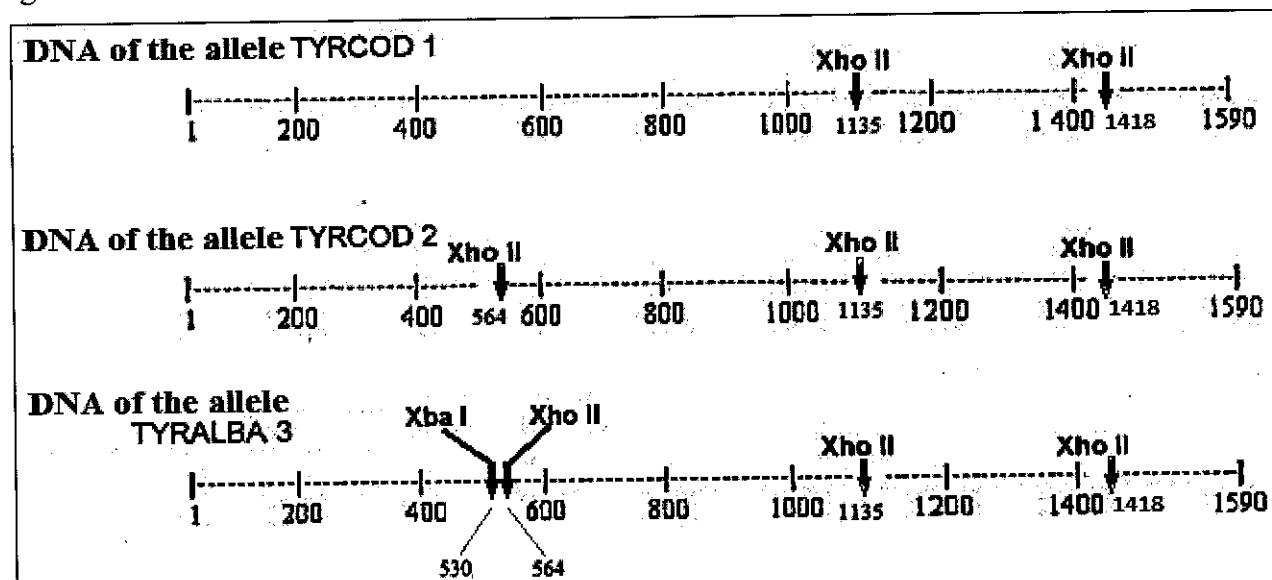
- 2 alleles TYRCOD 1 and TYRCOD 2 that code for a functional tyrosinase, the enzyme responsible for the biosynthesis of melanin.
- 1 allele TYRALBA 3 that codes for a nonfunctional tyrosinase.



Document 1: pedigree of the a family affected by the albinism

1. Specify if the allele of this disease is dominant or recessive.
2. Determine the localization of the gene of albinism.
3. Determine the risk for the fetus III-3 to be affected by this disease.
4. Explain the cause of the increase of the risk of the fetus in comparison with the worldwide rarity of the disease.

The document 2 shows the restriction sites of two restriction enzymes at the level of the alleles of the gene of albinism.



Document 2: representation of the identified alleles and the restriction sites of the enzymes XhoII and XbaI.

5. Show that the mutation that had changed the restriction site of the enzyme Xho II at the level of the nucleotide 564 is a silent mutation.
- 6.1 Compare the action of the enzymes Xba I and Xho II on the three alleles of the gene of albinism.
- 6.2. Identify the enzyme that is able to detect the mutant alleles of the gene of albinism.

EXERCISE 2 The hemophilia B, a recessive disease

Blood clotting is a process known as cascade of blood clotting since a number of ten proteins called clotting factors are successively activated by each other's in order to stop bleeding.

Hemophilia B, also called factor IX (FIX) deficiency or Christmas disease, is a genetic disorder caused by missing or defective factor IX, a clotting protein. Although it is passed down from parents to children, about 1/3 of cases are caused by a spontaneous mutation, a change in a gene.

The main medication to treat hemophilia B is concentrated FIX product, called clotting factor or simply factor. Recombinant factor products, which are developed in a lab through the use of DNA technology, preclude the use of human donor-sourced plasma. And while plasma-derived FIX products are still available, approximately 75% of the hemophilia community takes a recombinant FIX product.

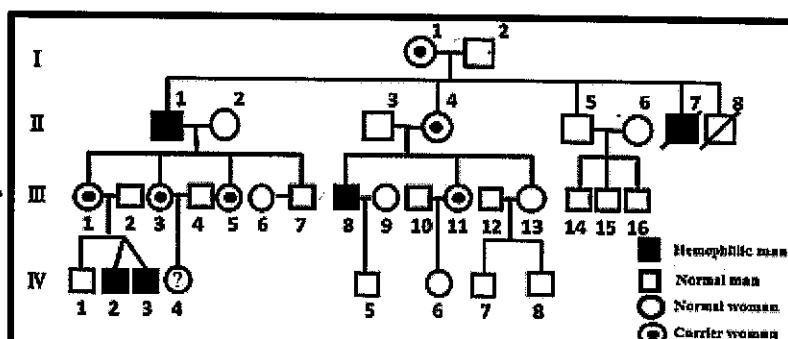
Document 1 shows the transmission of this disease in a family.

- 1.** Pick out from the text:

- 1.1** Two other names of hemophilia B disease.
- 1.2** Two sources of FIX product.
- 2.** Show that the gene of hemophilia is located on the non-homologous segment of the chromosome X.

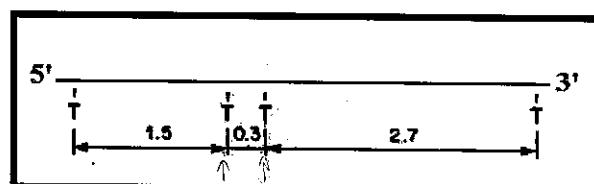
- 3.** Determine the risk to have an affected boy:

- 3.1** For the girl IV-4 knowing that her husband is normal and we don't know if she is carrier.
- 3.2** For the couple III-(6,7).



Document 1

Document 2 shows the restriction sites in a portion of the normal allele of factor IX for the enzyme Taq 1. Document 3 shows the DNA analysis by southern blot technique of some members of the family in document 1.



Document 2

- 4.** Determine, by referring to the documents 2 and 3, the site of mutation that lead to the mutant allele of the factor XI.

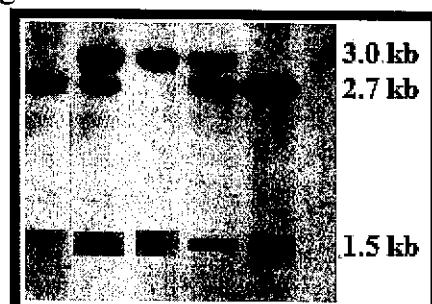
In this electrophoresis, the thick band corresponds to an amount of DNA that is the double of the amount that corresponds for the thin band of the same DNA fragment.

- 5.** Explain the presence of thick bands in some persons and of thin bands in others for certain DNA fragments.

A prenatal diagnosis permits to realize the southern blot technique that reveals an electropherogram for a girl similar to that of her hemophilic father. The doctor supposed under the light of this result that the fetus will suffer from hemophilia and from a chromosomal abnormality.

- 6.1** Specify the abnormality this girl suffers from.

- 6.2** Schematize one possible meiosis at the origin of the state of this girl.

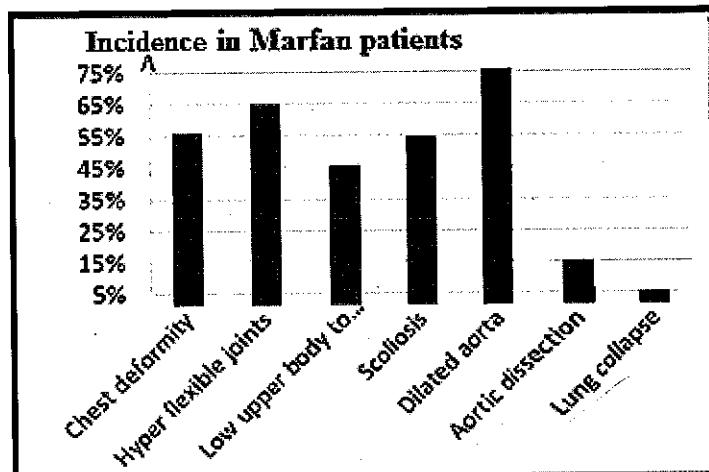


Document 3

EXERCISE 3 Inherited disorders: Marfan syndrome

Marfan syndrome is a rare (1 in 5,000) genetic disorder that is characterized by several symptoms affecting connective tissues. The predominant genetic defect is in the FBN1 gene, which codes for the glycoprotein fibrillin-1 and important component of the extracellular matrix (ECM). Fibrillin-1 is particularly important for the proper formation of elastic fibers, such as those which constitute the walls of large blood vessels, ligaments, and regions of the eye.

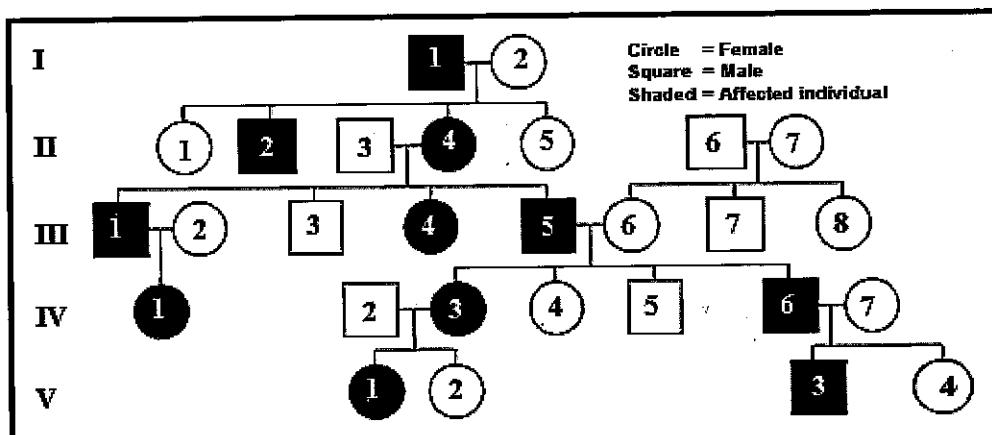
Marfan syndrome is interesting in that while all individuals with the mutated FBN1 genotype will demonstrate distinct symptoms, each individual may have a completely different phenotype. Document 1 shows the frequency of common symptoms of Marfan syndrome among patients with the same mutation. The symptoms associated with Marfan syndrome are easily understood in terms of defects in the physiological roles of fibrillin-1.



1. Draw out, from the text and from document 1, three organs of the body in which fibrillin-1 has a role.
2. Represent the data in document 1 in a table.

Document 1

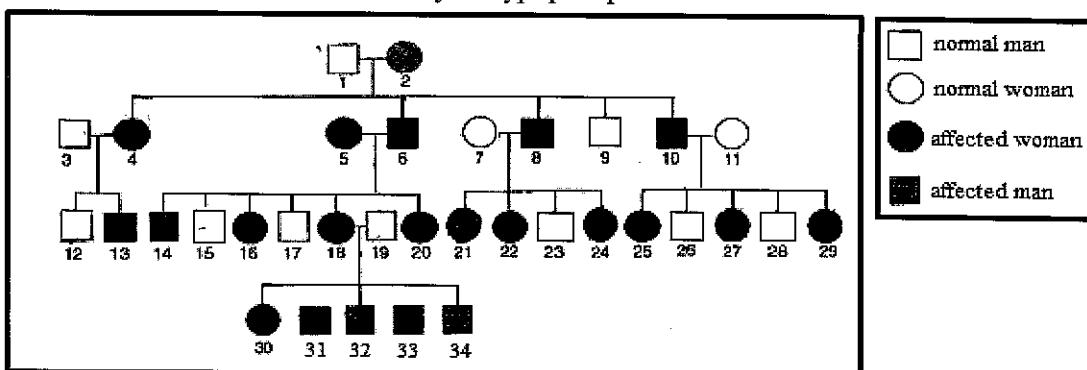
Document 2 shows the transmission of some of the symptoms shown by document 1 in a family.

**Document 2**

3. Show that Marfan disease is dominant.
4. Specify the localization of the gene of fibrillin-1 that leads for Marfan disease when mutated.
5. Determine the risk for the couple IV (2-3) to have another child affected by Marfan syndrome.

EXERCISE 2 The hypophosphatemia, a hereditary disease

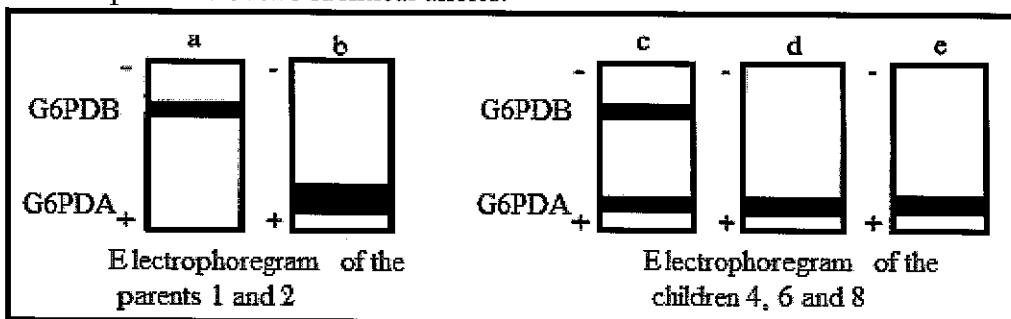
Familial hypophosphatemia is a disorder and the principal cause of rickets in the developed nations. The gene responsible for this disease is located on the non-homologous segment of chromosome X. **Familial hypophosphatemia** is caused by a metabolic defect that leads to the loss of phosphate through the kidneys. The resulting low concentration of phosphate in the blood results in the reduced deposition in the bones of calcium phosphate, which is the substance responsible for the rigidity of bone. The result, in children, is the curved and stunted bones that are the chief symptoms of rickets. Document 1 shows the transmission in a family of hypophosphatemia.



Document 1

1. Justify that the allele responsible for this disease is dominant.
2. Show that the transmission of the disease to some members of the family in document 1 contradict the hypothesis of an autosomal transmission of this disease.
3. Specify the genotypes of the individuals 18, 19 and their children.
4. Determine the chance for individuals 3 and 4 to have a normal child.

We perform tests for an enzyme, the G6PD, on some members of this family; this enzyme has the gene localized on the X chromosome, and has two forms A and B. The performed test is able to determine whether certain individuals will be affected by the disease studied above or not affected. The results of these tests are indicated in the electropherogram in document 2. The thick band corresponds to the presence of two identical alleles.



Document 2

5. Determine the genotypes of the tested individuals concerning the disease and the G6PD enzyme.

Individual 13 has an electropherogram identical to that of the father 1.

6. How can you explain the state of the individual 13?

EXERCISE 5 Two hereditary gonoosomal diseases

Color blindness (inability to recognize colors) and favism (abnormality that triggers hemolysis when the individual ingests beans) are two hereditary diseases.

In order to study the mode of transmission of both genes of both diseases, we use the statistical table in document 1 representing, in several families the offspring of a healthy woman whose father is affected by the favism and colorblindness and being married to a healthy man.

1. Represent the family tree of one of the families concerned by this study; in this tree represent all types of descendants without taking into consideration the proportions.

Sex	Phenotype	Number
Girl	Healthy girls	195
Boys	Healthy	92
	Affected by the favism and daltonism	96
	Affected only by daltonism	7
	Affected only by favism	5

Document 1

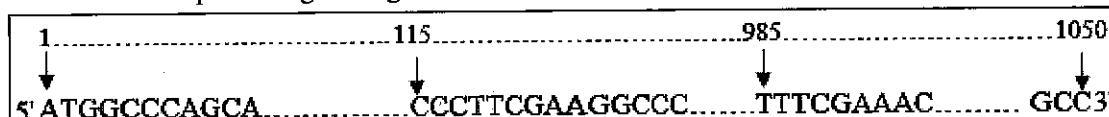
2. Justify By referring to the obtained results that:

2.1. The alleles responsible for both diseases are recessive.

2.2. Both genes are carried by the non-homologous part of chromosome X.

- 3.1. Schematize the type of genetic recombination that is at the origin of the results observed in males of the studied families.

- 3.2. Determine the percentages of gametes obtained at the end of meiosis.



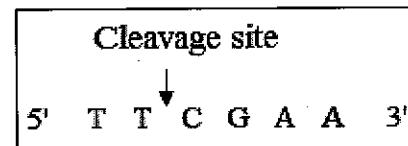
Document 2

Color blindness gene was isolated and sequenced, its sequence is given by the document 2. Four substitution mutations are the cause of the formation of the mutant alleles of this gene, these mutations are shown in the table 3 of the document.

Document 4 shows on the strand 5'-3' the restriction site of a restriction enzyme called BstBI. This enzyme has been used for detecting gene polymorphism of the gene of color blindness.

Site of the Mutation	Nucleotide of the normal allele	Nucleotide of the mutant allele
282	C	A
529	T	C
607	T	C
989	G	A

Document 3



Document 4

Based on the document data 2, 3 and 4.

4. Show, by determining the number and size of the restriction fragments of the normal allele and the mutant allele, that the BstBI enzyme is able to detect the genetic polymorphism of the color blindness gene at position 989.

A mother (Mary) in a similar family of the families discussed above asks if her fetus is affected by both diseases at the same time. Two methods are available: a calculation of the probability for the child to be affected and a DNA analysis. The analysis of DNA using the enzyme BstBI gives for Mary and her father the same position of bands on the electrophoresis gel.

5.1. Calculate the probability for this mother to have a child affected by both diseases.

5.2. Formulate an explanatory hypothesis of the result of the DNA analysis of Mary and her father.

EXERCISE 6 A chromosomal abnormality

The red blood cells of certain persons have an agglutinogen (antigen) for which its synthesis is commanded by a gene « g ». If we transfuse blood from one of these persons to another person having the allele ga of this gene, a slight agglutination occurs.

In the individuals having normal karyotypes, when the father is of phenotype [g] and the mother of phenotype [ga], the sons will be always of phenotype [ga] and the daughters of phenotype [g].

When the mother is of phenotype [g] and the father of phenotype [ga], the sons and the daughters might have one of the two phenotypes depending on the mother is homozygote or heterozygote. These crosses are represented in document 1.

1 st case: father [g]	X	mother [ga]
		sons [ga] and daughters [g]
2 nd case: father [ga]	X	mother [g]
		possibility of having sons and daughters [g] and [ga].

Document 1

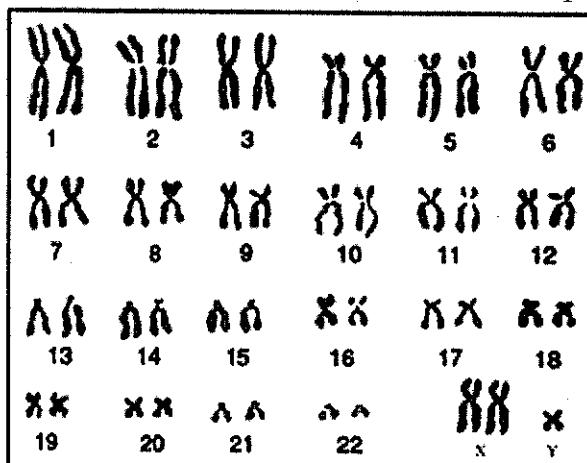
1. Show that this gene is located on the non-homologous segment of the chromosome X.
2. By a logical analysis confirm that the allele g is dominant relative to the allele ga.
3. Make the necessary factorial analyses to verify the experimental results of the second case.

A man has the phenotype [g]. His father has the phenotype [g] and his mother is homozygote and has the phenotype [ga].

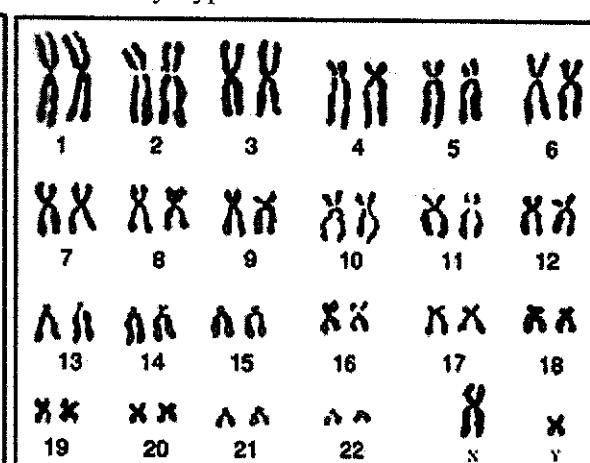
4. Pose the problem posed by the birth of this man.



In order to determine the origin of the state of this man, his karyotype is realized. This karyotype is represented in the document 2. Document 3 represent the karyotype of the father.



Document 2



Document 3

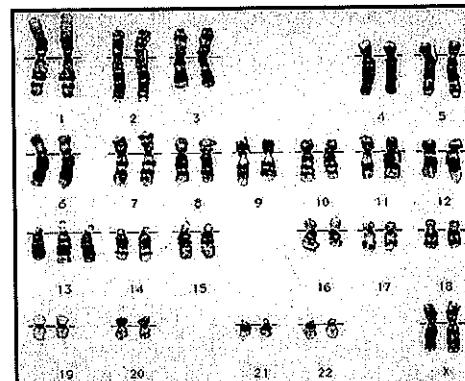
- 5.1. Write the chromosomal formula of this individual.
- 5.2. Compare this karyotype to the normal karyotype of the father (document 2)
- 5.3. Draw out the origin of the phenotype of this man.
6. Schematize the behavior of the chromosomes during the gametogenesis of the individual in which the abnormality took place.

EXERCISE 7 Trisomy 13

Trisomy 13 is a chromosomal abnormality due to the presence of an additional chromosome 13. It is characterized by the association of cerebral malformations (holoprosencephaly), facial dysmorphism, ocular abnormalities, postaxial polydactyly, visceral malformations (cardiopathy) and very severe psychomotor retardation. All of these symptoms are known as Patau's syndrome. This syndrome has a risk of 1/12 000 in the case of free trisomy 13 which is due to an error in the disjunction of chromosomes 13 during one of the meiotic divisions. About half of pregnancies with trisomy 13 result in abortion, 90% of children born with trisomy 13 die in their first year of life.

Document 1 shows a karyotype of an individual with Patau's syndrome.

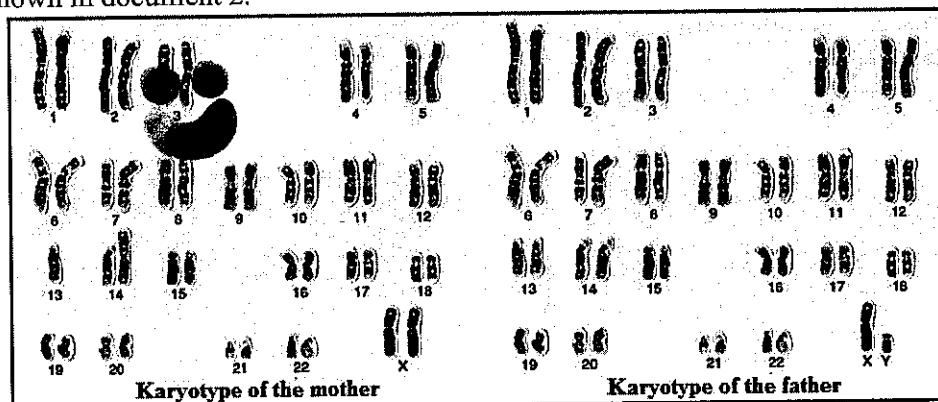
1. Write the chromosomal formula of the karyotype in document 1.
2. Specify whether this trisomy 13 is linked or free.

**Document 1**

One family suffers from multiple abortions and two cases of trisomy 13. A normal woman in this family has had an abortion of a fetus with trisomy 13.

3. Formulate a hypothesis about the origin of trisomy 13 in this family.

In order to determine the cause of the repeated abortions of this woman's family and to clarify the origin of trisomy 13, a doctor decides to realize the karyotype of this woman and her husband. These karyotypes are shown in document 2.

**Document 2**

4. Compare the karyotype of the mother to that of her husband.
5. Explain the normal phenotype of this woman.
6. Schematize, by limiting to chromosomes 13 and 14 in the woman, the anaphase that is at the origin of the gamete leading to abortion.

This woman decides to have another child and she wonders about the chance she has to have a normal child and the risk for this normal child to face the same difficulties of having normal children. Considering that, in the woman, normal chromosome 14 separates from abnormal chromosome 14:

7. Schematize, considering the chromosomes 13 and 14 only, the different gametes produced by this woman while indicating their proportions.
8. Determine:
 - 8.1. The chance for this woman to have a normal child.
 - 8.2. The risk for this woman's normal child to have a problem similar to hers.

EXERCISE 3 A chromosomal translocation

Chromosomal translocation has been linked to several types of human leukemia (blood cancer). Through chromosomal translocation one segment of a chromosome breaks off and is joined to another chromosome. As a result of such an event, two separate genes can be fused. In some cases, the newly created gene leads to tumor development. Such is the case with the so-called Philadelphia chromosome. The Philadelphia chromosome is found in more than 90 percent of patients with chronic leukemia. This well-known example of translocation involves the fusion of a gene called *c-ABL*, which is located on chromosome 9, to a site on chromosome 22 known as region (*BCR*). *BCR* and the *c-ABL* gene produce a hybrid oncogene, *BCR-ABL*, which produces a mutant protein that aberrantly regulates cellular proliferation.

- 1.** Explain starting from the acquired knowledge and the text the origin of the synthesis of a new protein leading to cancer.

Document 1 shows a partial karyotype of Mr. A, a normal man, having a family where certain members are affected by certain types of myeloma. The document 2 shows the karyotype of Mr. B, a normal man having normal family members.



Document 1

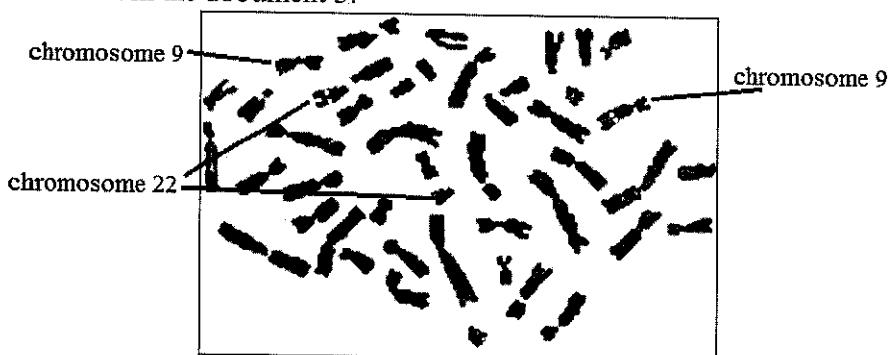


Document 2

- 2.1.** Compare the two karyotypes.

- 2.2.** Conclude the nature of the mutation that exist in the family of Mr. A and the risk that faces him.

In order to validate the risk that faces Mr. A, his doctor decides to practice the FISH technique concerning the gene *c-ABL*, the results of this technique show fluorescent spots on the non-arranged chromosomes as shown in the document 3.



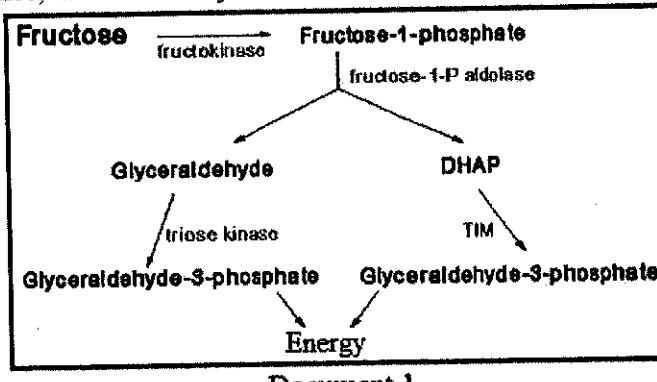
Document 3

- 3.** Starting from the analysis of the results in document 3, validate the risk of Mr. A.
- 4.** Make the factorial analysis concerning the chromosomes in relation with the anomaly in order to determine the risk for the children of Mr. A to be affected by the same anomaly as if his wife has normal karyotype.
- 5.** Show, starting from the given above, how does a screening test of body proteins is able to confirm the state of Mr. A.

EXERCISE 9 Fructosemia

Fructosemia, or hereditary fructose intolerance, was clinically demonstrated in 1956. It is a rare disease that affects 1/10 000 at birth, and it is estimated that the percentage of heterozygotes in the human population is 1/50.

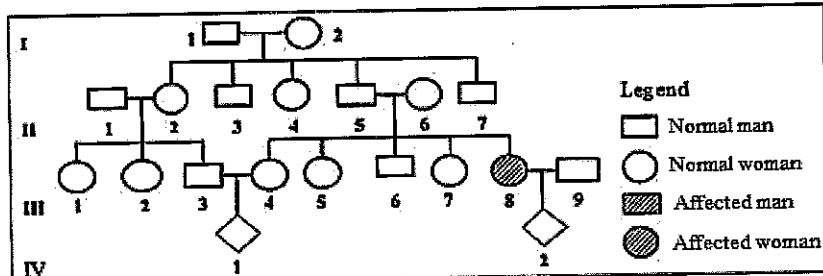
This disease is a congenital/hereditary disease related to a deficiency in fructose-1-P aldolase. This enzyme is located in the liver, small intestine and kidneys. Its action in the metabolism of fructose is given in document 1. On the other hand, the accumulation of fructose-1-phosphate in the liver and kidneys becomes toxic to these organs since this molecule can't follow other metabolic pathways.



Document 1

1. Justify starting from the text that this disease is recessive.
2. Justify, by referring to the text and to document 1, why a mutation at the gene coding for fructose-1-P aldolase results in a metabolic disease.

The study of the transmission of this disease has demonstrated that it is transmitted in an autosomal mode. The genealogical tree in document 2 shows a family in which one of the members is affected by fructosemia.



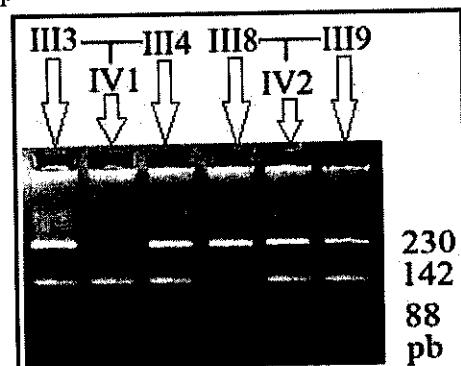
Document 2

3. Determine the risk of the fetus IV-2 to be affected by this disease.
4. Show that the risk for the fetus IV-1 to be affected by this disease is higher than 1 / 10 000.

Mutations that can be detected by DNA electrophoresis with addition of ethidium bromide are the mutations that result either from the creation of a restriction site of a given enzyme, they are indicated by the enzyme name preceded by a (+) sign, or from the disappearance of a restriction site of a given enzyme, they are indicated by the name of the enzyme preceded by a (-) sign.

A DNA fragment of the aldolase gene is tested by RFLP using the BanI enzyme. The results of the electrophoresis are shown by document 3 concerning certain members of the family in document 2.

5. Specify whether the mutation of the aldolase gene in this family is a mutation + Ban I or a mutation - Ban I.
6. Indicate the prenatal diagnosis of the two fetuses IV1 and IV2 of this family.

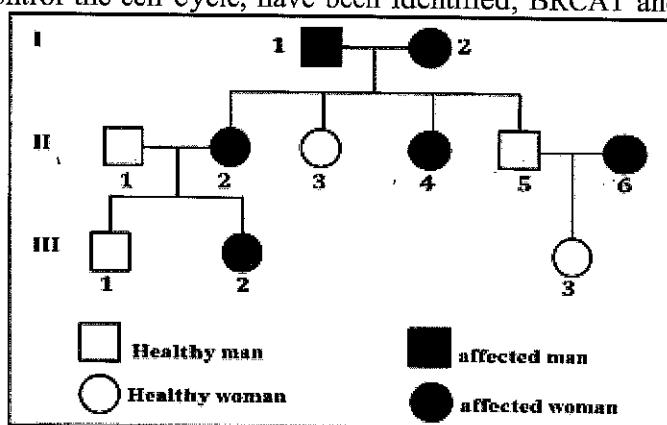


Document 3

EXERCISE 10 Breast cancer

Breast cancer is a rare disease characterized by the presence of a tumor in the mammary glands. Two major genes, suppressors for tumors that control the cell cycle, have been identified; BRCA1 and BRCA2, respectively located on chromosomes 17 and 13, and are the causative genes in families predisposed to breast cancer. Document 1 shows the pedigree of a family whose members are affected by breast cancer.

1. Specify if the allele of the disease is dominant or recessive.
2. Show that this disease is transmitted in an autosomal mode.
3. Determine the risk for the couple (II5-II6) of having an affected child.



Document 1

Document 2 shows a portion of the nucleotide sequences of the non-transcribed strand of two alleles of the BRCA1 gene.

Using the genetic code table

1. Determine the amino acid sequence coded by each of these two alleles (Document 2).
2. Explain how the modification in the nucleotide sequence of the BRCA1 gene leads to the appearance of breast cancer.

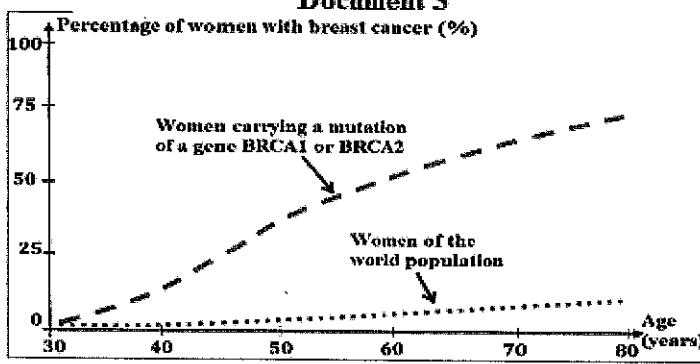
In order to determine the factors that promote the development of breast cancer, we measure the evolution of the percentages of women affected by cancer according to age in two different situations. The results are presented in document 4.

Nº of nucleotide	67
Normal allele	GAGTGTCCCATCTGTCTGGAGTTG
Mutant allele	GTGTCCCATCTGTCTGGAGTTG

Document 2

First letter	U	C	A	G	Second letter	U	C	A	G
U	UUU } Phe UUC UUA UUG	UCU } Ser UCC UCA UCG	UAU } Tyr UAC UAA Stop UAG Stop	UGU } Cys UGC UGA Stop UGG Tri	U	CGU CGC CGA Arg CGG	C	A	G
C	CUU CUC CUA CUG	CCU CCC CCA CCG	CAU } His CAC CAA CAG	CGU CGC CGA Arg CGG	C	U	C	A	G
A	AUU AUC AUU AUG	ACU ACC ACA ACG	AAU } Asn AAC AAA AAG	AGU } Ser AGC AGA Arg AGG	A	U	C	A	G
G	GUU GUC GUA GUG	GCU GCC GCA GCG	GAU } Asp GAC GAA GAG	GGU GGC GGA Gly GGG	G	U	C	A	G

Document 3



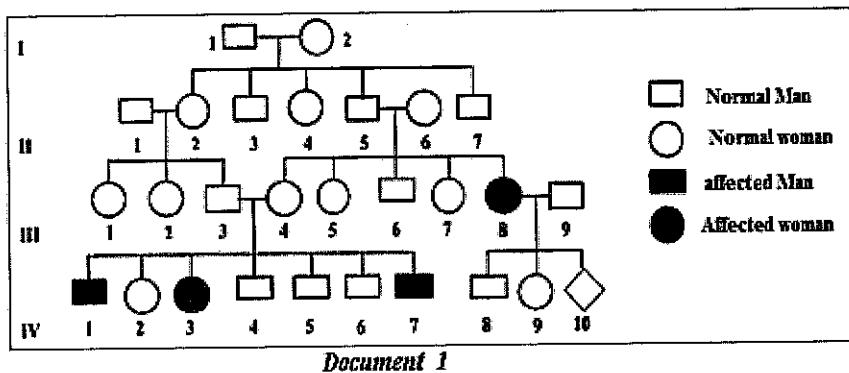
Document 4

EXERCISE II An enzymatic deficiency

Fructose, or hereditary fructose intolerance, was clinically demonstrated in 1956. It is a congenital (hereditary) disease that prevents the absorption of fructose in the form of polymers (It is linked to a deficiency in aldolase B). This enzyme, found in the liver, small intestine and kidneys, cleaves fructose-1-phosphate to DHAP and glyceraldehyde in order to allow further glycolysis for ATP production by the cell resulting in an accumulation of fructose-1-phosphate in the liver and kidneys. This molecule, which cannot follow another metabolic pathway, becomes toxic to these organs in the long term.

It is a rare disease that affects 1 / 20,000 births, and it is estimated that the % of heterozygotes in the human population is 1/5000.

1. Pick out from the text:
 - a. The probable cause of fructose intolerance.
 - b. the consequences of this long-term impairment.



The study of the transmission of this disease in a family made it possible to make the following genealogical tree:

2. Specify according to the pedigree:

2.1. If the allele responsible for this disease is dominant or recessive.

2.2. If the allele responsible for this disease is linked to an autosome or on a gonosome.

Document 2 below shows the DNA-base sequence of a fragment of the gene (non-transcribed strand) coding for normal and mutated aldolase B:

Normal Sequence	AAT	GGA	CTG	GTA	CCT	ATT	GTT	GAA
Abnormal Sequence	AAT	GGA	CCT	ATT	GTT			GAA

Document 2

3. Determine, using the table of the genetic code, the amino acid sequences of each fragment of the aldolase coded by each of two alleles, and draw out the cause of the disease.

The couple III (8, 9) awaits a 3rd child (10) and suspects that this child will be affected as his cousins 1, 3 and 7 (IV).

4. Evaluate the risk of this child being affected.
5. Explain why this risk is much lower compared to his cousins.

	U	C	A	G	
U	UUU phényl-alanine	UCU	UAU tyrosine	UGU cystéine	
	UUC	UCU	UAC	UGC	
	UUA	UCA			
	UUG	UCG			
C	CUU	CCU	CAU histidine	CGU arginine	
	CUC	CCC	CAC	CGC	
	CUA	CCA	CAA glutamine	CGA	
	CUG	CCG	CAG	CGG tryptophane	
A	AUU	ACU	CAU asparagine	AGU sérine	
	AUC	ACC	AAC	AGC	
	AUA	ACA	AAA lysine	AGA arginine	
	AUG	ACG	AAG	AGG	
G	GUU	ACU	AAU thréonine	GGU glycine	
	GUC	ACC	AAA	GGC	
	GUA	ACA	AGA	GGG	
	GUG	ACG	AGG		

Document 3

Human genetics

Solved exercises solutions

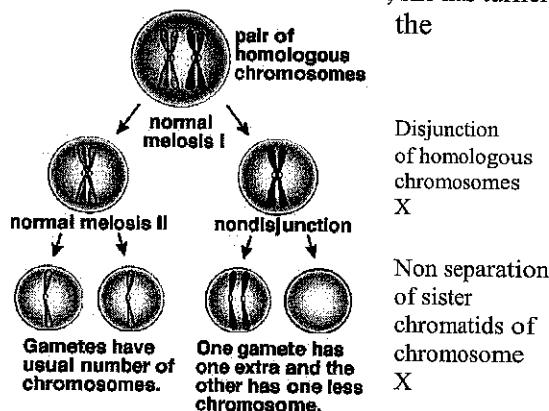
Exercise 1: Genetic polymorphism of the gene of albinism

1. The allele of the disease is recessive since the two parents I1 and I2 are normal having an affected child II-3 so the allele was masked at least in one of the parents.
2. If the gene of the disease is carried on the non-homologous segment of the chromosome Y, the disease would be transmitted from father to son, but the son II8 is affected, he would have the genotype X/Y^d, and would have taken the allele of the disease from the father I3 that would be of genotype X/Y^d and would be affected but he is normal. Thus, the gene is not carried on the non-homologous segment of the chromosome Y.
If the gene is carried by the non-homologous segment of the chromosome X, the affected girl II7 must be homozygous of genotype X^d/X^d; she should have inherited the mutant allele from her father I3 who should be affected of genotype X^d/Y. But her father is normal. Thus the gene is not carried by non-homologous segment of X.
If the gene is carried by the homologous segments of X and Y, affected girl II7 of genotype X^d/X^d should have inherited X^d from her father I3; the affected boy II8 of genotype X^d/Y^d should have inherited Y^d from his father I3. Father I3 should be affected of genotype X^d/Y^d which is not the case since he is normal. Thus the gene is not carried by the homologous segments of X and Y. Therefore, the gene is carried by an autosome.
3. The mother II-2 has two heterozygous parents because her brother II-3 is affected, then II-2 has a risk of 2/3 to be carrier (1/4 to be NN, 2/4 to be Nd and 1/4 to be dd that is rejected because she is normal) the same for the father II-5. If the two parents are heterozygous Nd, the risk for each of them to give d to the fetus is of 1/2, then the risk for the fetus to be affected is 2/3 x 2/3 x 1/2 x 1/2 = 1/9.
4. The risk is higher than the world-wide risk (1/9 > 1/20000) because the child comes from two parents, both having family history for the disease so their risk to be heterozygous is very high compared to the risk of the heterozygous in the population.
5. The change of the restriction site for the enzyme Xho II at the level of the nucleotide 564 did not change the function of the protein coded by this gene since the two alleles TYRCOD 1 and 2 code for normal phenotypes. Then this mutation did not change the three-dimensional structure neither the sequence of the protein, so it is silent.
- 6.1. The enzyme Xho II cuts the allele TYRCOD 1 in two restriction sites, into three fragments at the level of the nucleotides 1135 and 1418, and cuts the allele TYRCOD 2 at the same sites but in addition to another site at the level of the nucleotide 564 into four fragments, two of the same size as those of TYRCOD 1 and two of a sum equal to the third fragment obtained from TYRCOD 1.
The same fragments of TYRCOD 2 are obtained from the allele TYRALBA 3 by the enzyme Xho II. The enzyme Xba I cuts only the allele TYRALBA 3 into two fragments and does not cut any of the two other alleles.
- 6.2. since the enzyme Xba I cuts only the allele TYRALBA 3 into two fragments, then it will give on the gel two bands of DNA while it will give us only one band with the two other alleles that are both normal, then the normal and the affected allele will give different restriction map on the gel by using this enzyme, then it is able to detect the genetic polymorphism of the gene of albinism.

Exercise 2 The hemophilia B, a recessive disease

- 1.1. Factor IX (FIX) deficiency or Christmas disease.
- 1.2. Recombinant factor products, human donor-sourced plasma.
2. As given in the title, the hemophilia B is a recessive disease.
Let h be the symbol of the recessive allele coding for hemophilia B and N be the symbol of the dominant allele coding for the normal phenotype.
Since in this family all the affected persons are boys, then the disease is sex linked, its gene is not located neither on an autosome nor on the homologous segment of X and Y since in that cases both girls and boys can be affected in the same proportions. Then the gene of the disease is located either on the non-homologous segment of X or Y.
If the allele of the disease is located on the non-homologous segment of chromosome Y, the boy III-8 must be of genotype X/Yh, he would have inherited the chromosome Y carrying the allele h from his father II-3 who should be affected of genotype X/Yh, this is not the case since the father II-3 is normal. So the gene of hemophilia B is located on the non-homologous segment of X.
- 3.1. The girl IV-4 had taken X^N from her normal father of genotype X^NY, her mother is carrier of genotype X^N/X^h, the risk for this mother to give X^h to this girl is 1/2.
In order to have an affected boy, this girl has to give an X^h to her child that should take Y from the father. If this girl is carrier, she has a risk of 1/2 to give X^h to her child, this child has a probability of 1/2 to take Y from the father in order to be a boy and to be affected.
Then the risk to have an affected boy is: 1/2 x 1/2 x 1/2 = 1/8.
- 3.2. The female III-6 is not carrier, she has a genotype X^N/X^N, she will give surely a chromosome X^N to each of her children, and since the allele N is dominant so all her children will be normal. Then the risk for the couple III-6, 7 to have an affected child is null.
4. The normal allele shown in the document 2 shows two restriction sites inside it, giving three fragments of lengths 1.5, 0.3 and 2.7 Kb.
The electrophoresis shown in the document 3 shows the two fragments 1.5 and 2.7 Kb corresponding for the normal allele, the fragment 0.3 Kb is not observed.
Although, the electrophoresis shows also a fragment of 3 Kb of length, this fragment corresponds for the sum of the two fragments 2.7 Kb and 0.3 Kb. So the site of the mutation is in the restriction site that separates the fragment 0.3 Kb and 2.7 Kb.
5. This gene is located on the non-homologous segment of chromosome X, it is found in one copy in the male individuals and in two copies in the female individuals. The thick bands observed in the electrophoresis correspond for the two copies of the allele found in a homozygous female, the thin bands correspond for the single alleles found either in a male or in a heterozygous females.
- 6.1. Since this girl has an electropherogram similar to her hemophilic father, then she has thin bands of the mutant allele indicating that she has only one X chromosome carrying the mutant allele that is taken from the father, so, she did not take a chromosome X from her mother, she has turner syndrome.
- 6.2. Abnormal meiosis in mother

Reductional Meiosis I



Equational meiosis II

Separation of sister chromatids of chromosome X

Exercise 3 Inherited disorders: Marfan syndrome

1. Eye, aorta, lung.

2.

Symptoms	Chest Deformity	Hyper flexible joints	Low upper body to ...	Scoliosis	Dilated aorta	Aortic dissection	Lung collapse
Incidence in Marfan patients in %	55	65	45	55	75	15	5

Table showing the incidence of the different symptoms in Marfan patients.

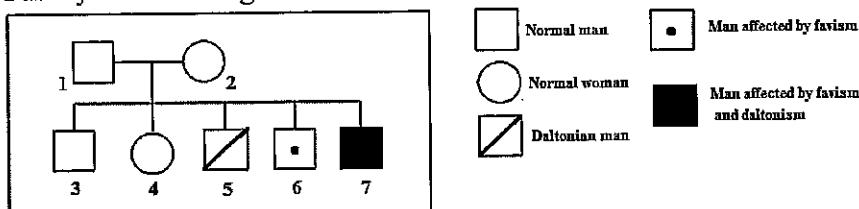
3. In this family, each affected person has at least one of its parents affected, then if the disease is recessive, every normal parent should be heterozygous which is impossible since the disease is rare, so the allele of the disease is dominant. Let D be the symbol of the allele responsible for the disease, and n be the symbol of the allele responsible for the normal phenotype.
4. If the allele is carried on the non-homologous segment of the chromosome Y, the disease would be transmitted from father to son, but the affected son III5 has a healthy father II3. Thus, the gene is not carried on the non-homologous segment of the chromosome Y. If the gene is carried by the non-homologous segment of the chromosome X, the healthy girl IV4 must be homozygous of genotype Xn/Xn; she should have inherited the normal allele from her father III5 who should be healthy of genotype Xn/Y. But her father is affected. So, the gene is not carried by non-homologous segment of X. If the gene is carried by the homologous segments of X and Y, healthy girl IV4 of genotype Xn/Xn should have inherited Xn from her father III5; the healthy boy IV5 of genotype Xn/Yn should have inherited Yn from his father III5. Father III5 should be healthy of genotype Xn/Yn which is not the case (III5 is affected). thus, the gene is not carried by the homologous segments of X and Y. Therefore, the gene is carried by an autosome. The mother IV3 is affected by the disease and is heterozygous since she inherited the allele D from her father and the allele n from her homozygous healthy mother who produces only one type of gametes having the allele n. Thus, she produces two types of gametes of equal probabilities: $\frac{1}{2}$ D and $\frac{1}{2}$ n. Since the affected allele of the disease is dominant; it is sufficient for the child of the couple IV (2,3) to have at least one allele of the disease in order to be affected. This child has a risk of 1/2 to take the allele D from the mother. Thus, the risk to have an affected child is 1/2 of the children.

Exercise 4 The hypophosphatemia, a hereditary disease

1. The gene responsible for this disease is located on the non-homologous segment of the chromosome X, then the father 8 affected by the disease, carries the mutant allele on X, this allele is given to the girls that had taken the normal allele from the homozygous mother, and since these girls are affected, then the mutant allele is expressed in the heterozygous state, thus it is dominant. (or two affected parents having normal child)
2. In the family in document 1 the fathers 8 and 10 that are affected by this disease have only affected girls, no one of their boys is affected. If the gene of the disease is located on an autosome, it will be transmitted in equal proportions to the two sexes of the children, then the children of the fathers 8 and 10 contradict that the gene is autosomal.
3. Let D be the symbol of the mutant allele that is dominant and n be the symbol of the normal allele that is recessive
 18: $X^D//Xn$ or $X^D//X^D$, since she is affected and the allele of the disease is dominant, can be expressed in homozygous and heterozygous states.
 19: $X^n//Y$, since he is normal, having the normal allele on X chromosome.
 30: $X^D//X^n$ since she is affected then she has the allele D and she had taken the chromosome X carrying the normal allele from her father.
 31, 32, 33, 34: $X^D//Y$, since they are affected carrying the mutant allele on X.
4. Individual 3 is of genotype $X^n//Y$ since he is normal, individual 4 is of genotype $X^D//X^n$ since she is affected having taken the normal allele from the father.
 In order to be normal, the child has to take the normal allele from the mother, she has a chance of 1/2 to give it, whatever the child take from the father it will be normal if the normal allele is taken from the mother.
 Thus, the chance for the child to be normal is 1/2 among all the children.
5. Let A be the symbol of the enzyme G6PDA and B the symbol of the enzyme G6PDB. The presence of one band in the electrophoregram corresponds for one allele that refer to a male, while the presence of two bands corresponds for two alleles of the gene that refer for a female.
 The genotypes of the tested individuals are:
 (a) corresponds for the father since there is only one allele of the gene of the enzyme G6PD, this father is normal having the allele n so his genotype is: $x_n^B//y$.
 (b) corresponds for the mother since there is a thick band corresponding for two alleles A of the gene of the enzyme G6PD, this mother is heterozygous for the gene of the disease since she is affected having normal son 9. Thus, her genotype is $x_D^A//x_n^A$
 (c) corresponds for the female 4 having two alleles of the gene of G6PD, she had taken x_n^B from her father and x_D^A from her mother since she is affected, her genotype is: $x_D^A//x_n^B$.
 (d) and (e) correspond for males 6 and 8 that are affected having the enzyme A then the genotypes are: $x_D^A//Y$.
6. The individual 13 has the same electropherogram as the father 1, then his genotype is $x_D^B//y$, his mother has the genotype $x_D^A//x_n^B$, she is not able to give x_D^B to him, a crossing over was made in the mother leading for the exchange of the alleles A and B between the two chromosomes X and allowing the formation of recombinant gametes x_D^B and x_n^A , the boy 13 took the gamete x_D^B from her mother and becomes affected having the enzyme B.

Exercise 5 Two hereditary diseases

1. Family tree showing the studied families.



- 2.1. The father and mother are healthy with children affected by both diseases, then both alleles were hidden at least in one of the parents and are recessive.

Symbols:

Let F and f the respective symbols of the normal allele dominant and recessive mutated gene and the favism D and the symbols of the normal allele dominant and recessive mutated gene of color blindness.

- 2.2. As there are only boys affected by both diseases, so the two diseases are sex linked. The alleles are carried either by the non-homologous segment of the X chromosome or Y chromosome. But the boys with both diseases always have a healthy father which means that the alleles are not carried by the non-homologous part of the chromosome Y because in this case the father will be affected by the two diseases. Then both alleles are carried by the clean side to the X chromosome.

- 3.1. The intrachromosomal recombination.

- 3.2. The girls take the father's X chromosome carrying the two normal alleles. They will always be normal and the phenomenon of crossing over does not affect them, then to determine the percentages of gametes we must use only phenotypic results of boys.

Frequency of recombination = $(7+5) \times 100 / 200 = 6\%$.

Each recombinant gamete: X^{Fd} and X^{Df} has a percentage of 3 %.

Each parental gamete X^{FD} and X^{fd} has a percentage of 47 %.

4. The enzyme BstBI cuts the normal allele color blindness gene at positions 119 and 987 to give three fragments of lengths 119, 987-119 and 868 = 1050-987 = 63 bp, whereas the mutant allele is cut only once at position 119 as the corresponding restriction site at position 987 is affected by the mutation at position 989 that is included in the enzyme restriction site, so the mutant allele gives only two fragments of lengths 119 and 868 + 63 = 931 bp.

By giving different restriction fragments according as the allele is mutant or normal, electrophoresis separates in different ways the fragments to give a different restriction map of the two alleles and hence detect the gene polymorphism of this gene at this position.

- 5.1. As the father is healthy, he will always give an X chromosome carrying the two normal alleles, the girls will all be normal, whatever the alleles taken from the mother. So, if the child is a girl, she will have zero probability of being affected by both diseases.

But if the child is a boy, he will take a Y chromosome from his father, and he will have the phenotype expressed by the alleles taken from the mother, the mother has a percentage of 47% to give an X chromosome carrying the two mutant alleles then the child will have a 47/100 chance of being affected by both diseases.

- 5.2. Hypothesis: The mutation affecting this mother is not at the position 989.

Exercise 6 Transmission of a trait of red blood cells

1. If the mother is of phenotype ga all the sons will be of the same phenotype whereas all the daughters will be of the same phenotype of the father. If the gene is autosomal or carried by the homologous segment of the chromosomes X and Y it will be any difference in the transmission of the gene to the sons and the daughters. If the gene is carried by the non-homologous segment of Y the girls will not have this gene. So, the gene is carried by the non-homologous segment of X.
2. Since in the first case the sons had taken the chromosome X from their mother carrying ga so the mother is homozygote, then the daughters had taken also ga from the mother but they are of phenotype [g] then g is dominant.
3. If the mother is homozygote: If the mother is heterozygote

$$X^{ga}Y \times X^gX^g \quad \text{Genotypes} \quad X^{ga}Y \quad \times \quad X^gX^{ga}$$

X^{ga}	Y	X^g	Gametes	X^{ga}	Y	X^g	X^{ga}
$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{1}$		$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$

	$X^{ga} \frac{1}{2}$	$Y \frac{1}{2}$	Table of cross		$X^{ga} \frac{1}{2}$	$Y \frac{1}{2}$
$X^g 1$	$\frac{1}{2} X^g$	$\frac{1}{2} X^g Y$		$X^g \frac{1}{2}$	$X^g X^{ga} \frac{1}{4}$	$X^g Y \frac{1}{4}$
X^{ga}				$X^{ga} \frac{1}{2}$	$X^{ga} X^{ga} \frac{1}{4}$	$X^{ga} Y \frac{1}{4}$

Phenotypes: $\frac{1}{2}$ girls [g] $\frac{1}{2}$ boys [g]

Phenotypes: $\frac{1}{4}$ girls [g] $\frac{1}{4}$ boys [g]

$\frac{1}{4}$ girls[ga] $\frac{1}{4}$ boys [ga]

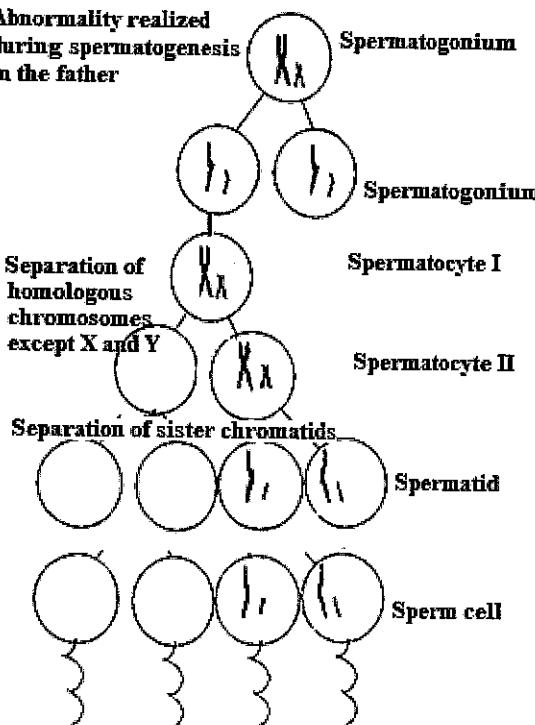
4. how can a boy be of phenotype [g], although he received X^g from his mother?

5.1. 44+XXY or 47, XXY

5.2. This karyotype contains the same number, size and chromatid number of the autosomes as the father; at the level of the gonosomes he has a single Y chromosome as the father with two chromatids and the same size and he has two X chromosomes while the father has a single one with the same size and with two chromatids. This man has an extra X chromosome taken from his father surely allowing him to be of phenotype [g].

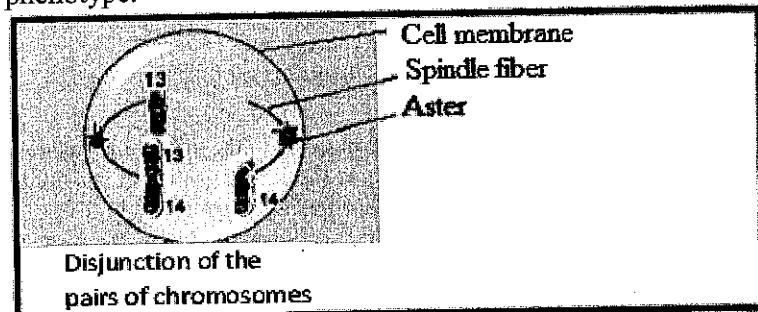
6.

Title: Abnormality realized during spermatogenesis in the father



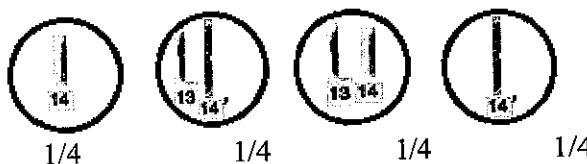
Exercice 7 Trisomy 13

- 1 47, XX, + 13
- 2 This trisomy 13 is free because the extra chromosome 13 is not attached to another chromosome
- 3 Hypothesis: The origin of trisomy 13 in this family is a translocation of one chromosome 13 on another chromosome.
- 4 The karyotype of the woman has 45 chromosomes, a smaller number than the karyotype of the father who has 46 chromosomes. All the chromosomes, except 13, are present in duplicate in the woman and her husband; at chromosome 13 there is only one copy in the woman's karyotype but in the husband's karyotype there are two. On the other hand, one of the two chromosomes 14 of the woman is of the same size as the chromosomes 14 of the husband while the other is longer. All other pairs of chromosomes have the same sizes in the woman as in the husband. The karyotype of the woman shows two X chromosomes while that of the husband shows one X chromosome and another Y chromosome.
5. This woman has complete genetic makeup without excess or loss because the missing chromosome 13 is translocated on one of the two 14 chromosomes so she has a normal phenotype.



Anaphase I in the woman at the origin of the abortion of a child with trisomy 13

7. Produced gametes



- 8.1 The child of this woman must take from the father a gamete containing a normal chromosome 13 and a normal chromosome 14. To be normal, this child must take from its mother a chromosome 13 and a chromosome 14, which can be linked or free, the chance for each of these two cases is $1/4$, so the chance for the child to be normal is $1/4 + 1/4 = 1/2$.
- 8.2 One of the gametes leading to the state of the child contains two chromosomes 13 and 14 attached to each other, the other contains two free chromosomes 13 and 14. Each of these two gametes has a chance of $1/2$ compared to the other, if the child takes two linked chromosomes 13 and 14, he will have problems similar to those of his mother, then the risk for this child has similar problems is $1/2$.

Exercice 8 A chromosomal translocation

1. The translocation indicated in the text leads to the fusion of two genes, one of chromosome 22 called BCR and the other of chromosome 9 called c-ABL, this fusion results in the formation of a hybrid oncogene of a longer nucleotide sequence leading as a result to the synthesis of a protein that has a larger number of amino acids, this protein is a novel protein formed by the fusion of two peptide sequences together.
- 2.1. In both karyotypes, the chromosomes are of the same number of the same chromosomes of the pair 23 X and Y; similarly, the chromosomes are of the same size pair-to-pair with the exception of chromosomes 9 and 22. One of chromosomes 9 in document 1 has the same size as the two chromosomes 9 in document 2 while the other is shorter; on the other hand, one of the chromosomes 22 in document 1 has the same size as the two chromosomes 22 in document 2 while the other is longer.
- 2.2. Then a translocation was carried out from one part of chromosome 9 to one of the two chromosomes 22 which indicates a risk for him to be affected by leukemia.
3. The FISH technique shows that the c-ABL gene was found on one of chromosomes 9 only, the other copy of this gene is found on one of the two chromosomes 22, indicating that this gene has been translocated with the exchanged fragment validating the threat of M. A.
4. Chromosomal analysis

Phenotypes	Father	x	Normal mother
Chromosomes	9//9 ⁻ 22//22 ⁺	x	9//9 22//22
Gametes	9 22 : ¼	9 22 ⁺ : ¼	9 22 : 1
and proportions	9 ⁻ 22 : ¼	9 ⁻ 22 ⁺ : ¼	

Table of cross:

♀ ♂	9 22 ¼	9 22 ⁺ ¼	9 ⁻ 22 ¼	9 ⁻ 22 ⁺ ¼
9 22 1	9//9 22//22 ¼	9//9 22 ⁺ //22 ¼	9 ⁻ //9 22//22 ¼	9 ⁻ //9 22 ⁺ //22 ¼

Phenotypes and proportions :

Normal child : ¼

Child with a risk of leukemia: ¼

Abnormal children (Loss or gain of chromosomal material) : ½

The risk for the child to be affected by the same abnormality is ¼.

5. As in this case a new protein was produced in the person with a hybrid oncogene then a protein screening of the body allows to find this protein to confirm the condition of M.A.

Exercise 9 Fructosemia

- 1 This disease is recessive since it affects 1/10 000 births, and the percentage of heterozygotes in the human population is 1/50; if the disease is dominant the percentage of heterozygotes must be equal to or smaller than the proportion of individuals affected because all heterozygous individuals are affected, but in this case the proportion of heterozygotes is 1/50 greater than the affected individuals 1/10 000.
- 2 The enzyme fructose-1-P aldolase is an enzyme that transforms fructose-1-P into glyceraldehyde and DHAP, both of which are transformed into glyceraldehyde-3-P, which is transformed into energy by cellular metabolism. A mutation in the gene that codes for aldolase will result in the accumulation of fructose-1-P in liver, small intestine and kidney cells because this molecule cannot follow another metabolic pathway. It is toxic to the cells of these organs.
Symbols: let N be the symbol of the normal allele and d the symbol of the affected allele.
- 3 The father III9 is without a family history, he has a risk of 1/50 of being heterozygous, the mother is affected, she is of genotype dd and she will necessarily give the allele d to the child IV2, the risk for That the father being heterozygous gives the allele d to the child is 1/2; Then the risk for the IV2 child to be affected is $1/50 \times 1/2 = 1/100$.
- 4 If both parents III3 and III4 are without family history, everyone will have a risk of 1/50 to be heterozygous, everyone will have if he is heterozygous a risk of 1/2 to give the allele of the disease to his Child, then the child will have a risk of $1/50 \times 1/50 \times 1/2 \times 1/2 = 1 / 10 000$. But since the two individuals III3 and III4 are of the same family where a case of fructosemia appears, then the risk for them to be heterozygous is higher than 1/50 and for this reason the risk for their child to be affected will be greater than 1 / 10 000.
- 5 The affected woman has a band of 230 bp, the other normal individuals have each band 142 bp and 33 bp, the sum of the sizes of which is equal to 230 bp, then the cut of the 142 bp fragment is carried out on the normal allele, Mutation deleted a site of the Ban I enzyme, so it is a mutation - Ban I.
- 6 The fetuses IV1 and IV2 are normal.

Exercise 10 Breast cancer

1. The allele of the disease is dominant over the normal allele because II-5, healthy man, has affected parents I-1 and I-2. So, the parents have normal allele in hidden state. (C: cancer allele, n: the normal allele).

2. If the allele of the disease is carried by the non-homologous part of the Y chromosome, the transmission is from father to son, affected father I-1 has a normal son II-5. While this is not the case.

If the allele of the disease is carried by the non-homologous part of the X chromosome, the father I-1 should transmit this dominant allele to all his daughters which will be all affected, but II-3 is a healthy girl. While, this is not the case.

If the allele of the disease is carried by the homologous part of X and Y, the boy II-5 of genotype X^nY^n should inherit Y^n from his father I-1. The normal girl II-3 of genotype X^nX^n , should inherit X^n from his father I-1 which would like X^nY^n as genotype and would be normal. While, this is not the case. Therefore, the allele of the disease is located on an autosome.

3. Factorial analysis or logical reasoning: the risk is 1/2.

4. We can obtain directly the mRNA from the not transcribed strand by replacing T by U.

mRNA of normal allele: GAG-UGU-CCC-AUC-UGU-CUG-GAG-UUG

Sequence of amino acids: Gly- Cys- Pro- Ile- Cys- Leu- Glu- Leu

mRNA of mutant allele: GUG-UCC-CAU-CUG-UCU-GGA-GUU-G

Sequence of amino acids: Val- Ser - His- Leu- Ser- Gly- Val

5. The mutation by deletion of 2 successive nucleotides A and G at position 68 and 69 is transcribed in mRNA which translated into an amino acids sequence different from the normal sequence which affects the three-dimensional shape of the protein which becomes non-functional and unable to control the regulation of the cell cycle leading to the onset of breast cancer.

6.1. The percentage of women with breast cancer is zero at the age of 30 years for both groups of women. In Women carrying the BRCA1 and BDCA2 genes this percentage increases with age, reaching 75% by age 80 years. Similarly, in women of the world population, this percentage increases with age but slightly to 10% at the age of 80 years.

6.2. The factors favoring the appearance of breast cancer are:

The age and the presence of mutated genes BRCA1 and BDCA2.

Exercise 11 An enzymatic deficiency

- 1.1.** It is linked to deficiency of aldolase B. This enzyme, located in the liver, small intestine and kidneys, cleaves the fructose-1-phosphate to DHAP and glyceraldehyde in order to allow further glycolysis for the
- 1.2.** This results in an accumulation of fructose-1-phosphate in the liver and kidneys. Since this molecule cannot follow another metabolic pathway, it becomes toxic to these organs.
- 2.1.** Since the couple III (3, 4) is normal and gave birth to affected children (boys 1 and 7 and a girl 3: Generation IV) then the allele that determines the disease was masked or hidden in at least one of the parents. Thus, it is recessive.
 N : normal allele a:Abnormal allele
- 2.2.** The allele responsible for this disease is linked on an autosome because:
 If the gene that determines the disease is bound to the proper part of Y every affected boy must have an affected father but the boy 7 (IV) is affected but his father 3 (III) is normal then this is not the case.
 If the gene that determines the disease is bound to the proper part of X then the affected daughter 3 (IV) should be of genotype X^aX^a , must receive X^a from her mother and X^a from her father 3(III), who will be affected (X^aY), but he is normal.
 If the gene that determines the disease is bound to the common part of X and Y, the affected daughter 3 (IV) of genotype X^aX^a must receive X^a from her mother and X^a from her father and the affected boy 1 (IV) X^aY^a , should receive Y^a from his father who will then of genotype X^aY^a (affected) but he is normal.
- 3.1.** mRNA (normal sequence): AAU GGA CUG GUA CCU AUU GUU GAA.....
 Peptide Sequence: asn- gly- leu- val- pro -Ile -val -glu.....
 mRNA (abnormal sequence): AAU GGA CCU AUU GUU GAA
 Peptide Sequence: asn- gly- pro -Ile- val -glu.....
- 3.2.** A mutation by deletion modified non-functional protein
- 4** The probability of the individual 9 (III) being heterozygous (no family history) is equal to that of the population: 1/5000. The mother is sick and the probability of this couple having a sick child is $\frac{1}{2}$ (fusion of parental gametes that would give birth to an affected child) then the risk = $1/5000 \times 1/2 = 1/10000$. The genetic risk for his cousins to be affected is $1/4$ (parents III 3-4 are heterozygous). This big difference is explained by the fact that consanguineous marriage (parents III 3-4 are close relatives) increases the risk of genetic diseases.

Human genetics

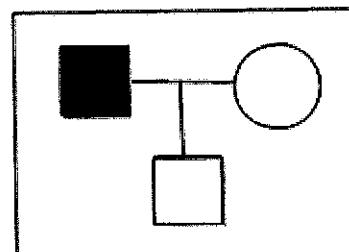
Non-solved exercises

EXERCISE 1 The hemophilia A, sex linked disease

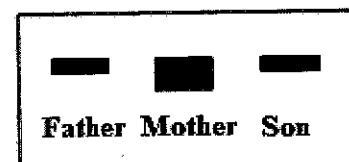
Hemophilia A is a recessive lethal hereditary disease which is characterized by abnormality in the mechanism of the blood coagulation. It results in the absence or the abnormality of a protein implicated in blood coagulation, the factor VIII. Document 1 shows the transmission in a family of this disease.

A test permits visualizing, by an electrophoresis, the gene of this disease without distinguishing between the normal allele and the mutant one. Document 2 shows the electrophoresis result of this family.

1. Explain why some genes are named by the names of diseases.
2. Pick out from the text the name of the protein coded by the gene of hemophilia A.
3. Show that the gene of this disease is carried by the non-homologous segment of the chromosome X.
4. Calculate the probability for the son to have an affected child by the hemophilia if the frequency of heterozygous females is of 1/25.



Document 1



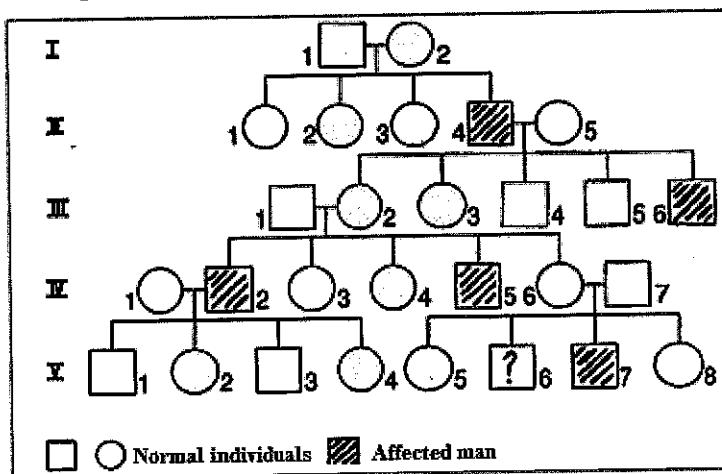
Document 2

In this family, an affected girl by this disease was born. This birth could be explained by an abnormal meiosis in one of the two parents.

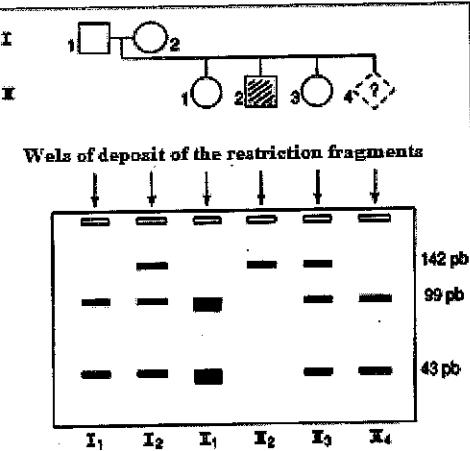
5. Discuss the possibility of the abnormal meiosis in each of the two parents and schematize the different possible meiosis in the two parents leading to the birth of the affected girl.

A portion of 142pb of the gene of this illness is polymorphic at the level of a restriction site for the enzyme Bcl-I, if the enzyme recognizes the site, it cuts the sequence of DNA in two pieces of 99 and 43 bp.

The pedigree of the document 3 shows the transmission of this disease in a family. Individual V1 is married to individual V5. Their pedigree appears in the document 4 with the results of the electrophoresis of the portion of the gene mentioned above.



Document 3



Document 4

6. Calculate the risk for the individual V-6 to be affected by the hemophilia.
7. Determine, by relating the fragments of the DNA obtained by the electrophoresis to the alleles of the gene of the hemophilia, the genotypes of the members of the family in document 4.

EXERCISE 2 Down syndrome

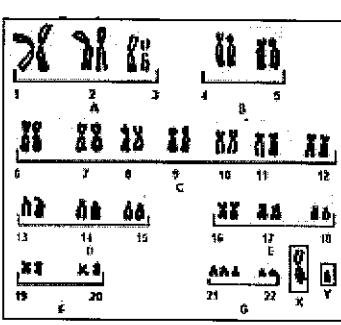
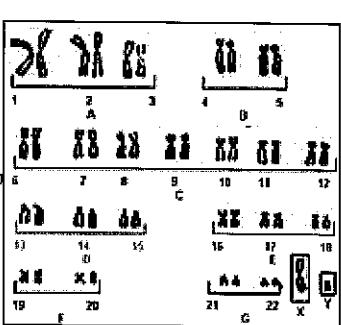
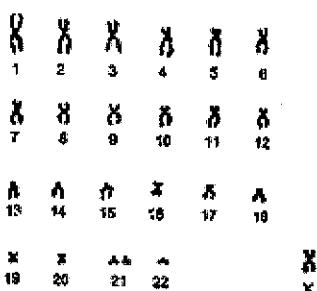
Down syndrome regroups the set of the symptoms which affect the individual having a chromosomal mutation known as trisomy 21. This chromosomal mutation is caused by an extra chromosome 21 in its karyotype.

The opposite documents are presented in order to study the origin of Down syndrome which appears at the individuals having a trisomy 21.

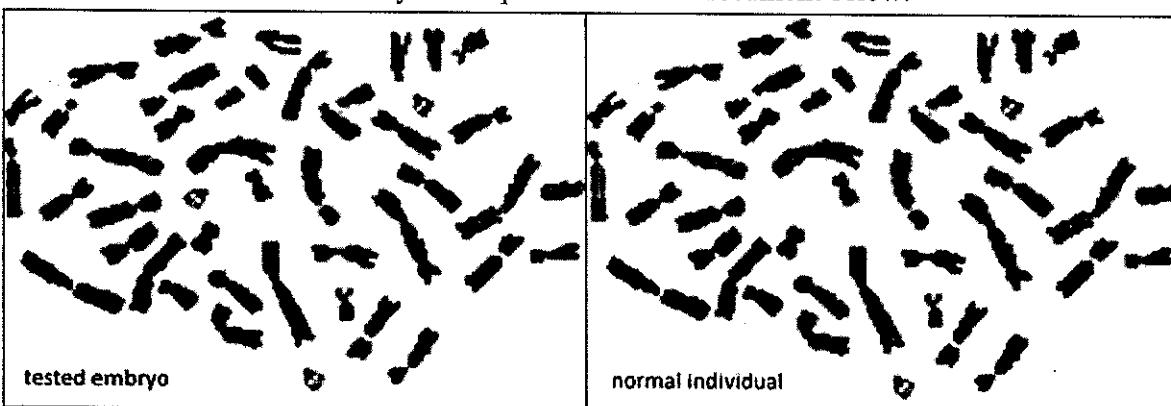
1. Indicate the difference between the two expressions "Down syndrome" and "trisomy 21".
2. Make a comparison of the karyotypes 1a and 1c with the karyotype 1b.

The karyotype of doc 1c corresponds for a cell coming from a man with normal karyotype. This cell is identical to that giving the gamete which lead to the birth of the individual 1a.

3. Determine the type of the cell having the karyotype represented by the document 1c.
4. Explain precisely why the quantity of the DNA of this cell is unequal to Q.
5. Make the labeled diagram of the anaphase of the division at the origin of this cell. (Consider only in the answer the chromosomes concerned with the anomaly and the sexual chromosomes).

<p style="text-align: center;">Document 1a : Human karyotype of an individual having Down syndrome</p> 	<p style="text-align: center;">Document 1b : Normal human karyotype</p> 
Document 1c : Karyotype of a germ cell	
	

In order to test an embryo about a possible Down syndrome; a doctor decides to realize a technique allowing to locate a gene carried by the chromosome 21, the results of the technique of a normal individual and of the tested embryo are represented in the document below.



6. Name this technique.
7. Determine the state of the embryo.

EXERCISE 3 The daltonism and the G6PD

Daltonism is a recessive disease carried by the gonosome X. the gene of the G6PD (enzyme found in the human species) has two codominant alleles A and B that command respectively the synthesis of the form A and the form B of this enzyme.

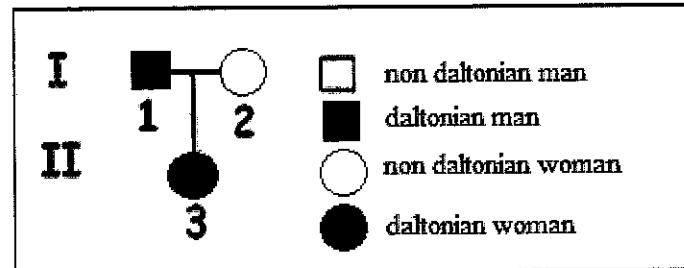
We decide to study the masculine offspring of many heterozygous women for the gene of G6PD with normal vision having daltonian fathers, and married to non daltonian men with G6PD of form B, the results are shown in the table of the document 1.

	Non daltonian with form B	Daltonian with form A	Non daltonian with form A	Daltonian with form B
Number of boys	75	75	4	4

Document 1

1. Show, starting from this table, that the gene of the G6PD is carried by the chromosome X.
2. Make the necessary analysis to verify the obtained results.

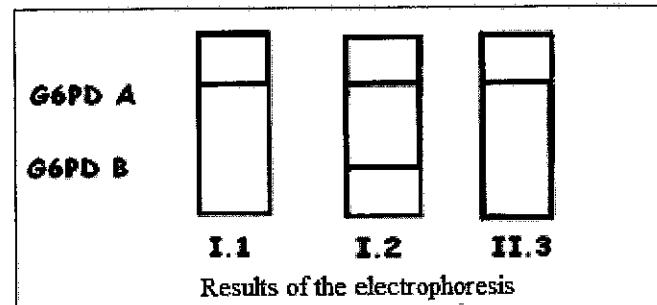
The opposite pedigree shows a family where the mother is heterozygous for the two traits studied above, and has a daltonian father having the enzyme G6PD of form A. the document 3 shows the results of the electrophoresis of the members of this family concerning the G6PD.



Document 2

3. Determine the possible causes of the state of the girl II-3 shown in the documents 2 and 3.

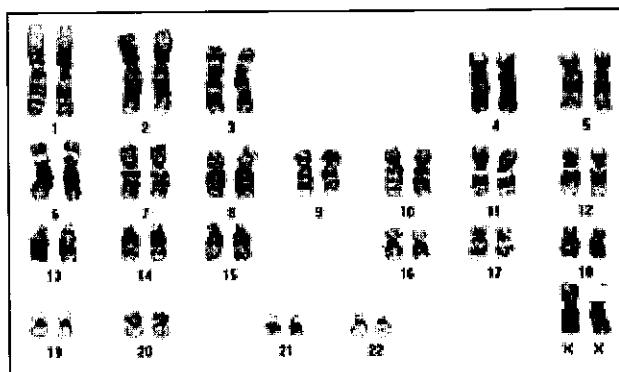
In this family another is born girl having an electrophoresis identical to her mother and has the same phenotype to her sister concerning the daltonism.



Document 3

Since this girl suffers from other troubles in development, a karyotype is realized in order to determine the origin of these troubles. This karyotype is represented in the document 4.

4. How does the analysis in document 4 support the answer to part 4?



Document 4

EXERCISE 4 Two hereditary diseases

A non-daltonian father affected by rickets and his normal wife have three girls affected by rickets and a daltonian boy (Rickets is caused by a dominant allele localized on the chromosome X). This family is expecting another child, but the pregnant woman is afraid of having a fifth child affected by one of the two diseases.

1. Starting from the phenotypes of the members of this family:
 - 1.1. Specify if the chromosomal localization of the allele of rickets is confirmed by the data presented by this family.
 - 1.2. Show that the gene of the daltonism is localized on the chromosome X.

In order to specify the state of the fetus of this family, the doctor decide to make many tests; the first consists of an exam by ultrasound. The other is to test an enzyme, the G6PD that exists in two active forms A and B, and that has a gene carried by the non-homologous segment of the chromosome X. The table below shows the enzymes found in the members of this family.

Father	Mother	Girl 1	Girl 2	Girl 3	Boy	Fetus
A	A and B	A and B	2 A	2 A	B	2B

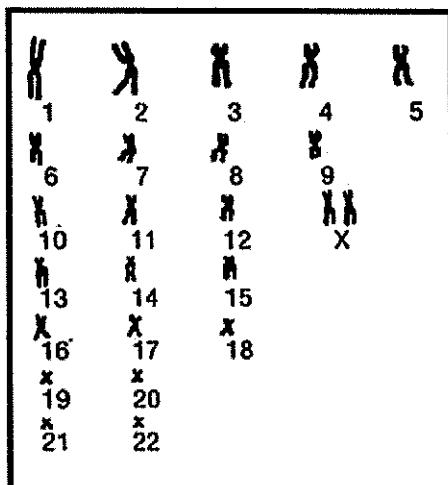
Document 1 the alleles of the enzyme G6PD in the members of the studied family.

2. Draw the pedigree of this family.

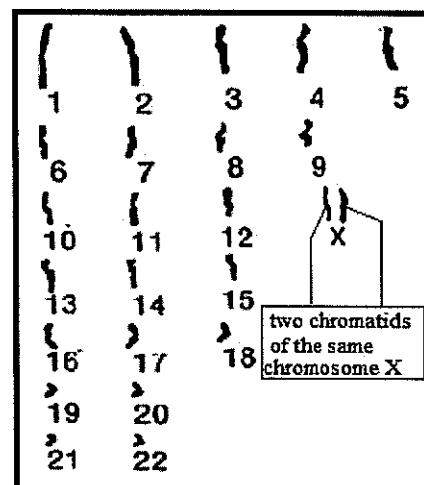
The test by the ultrasound shows that the fetus is a boy.

3. Explain the importance of the ultrasound and the test of the enzymes realized on the members of this family.

The state of the fetus shows an abnormality in an important biological process in relation with the reproduction. The two documents 3 and 4 give the Karyotypes of two female germ cells among which one is similar to the germ cell responsible for the state of this fetus.



Document 2



Document 3

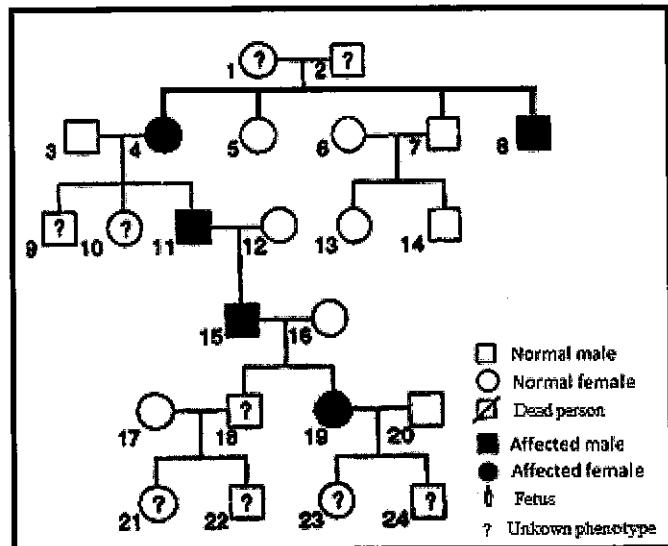
4. Identify these two cells.
5. Determine starting from the cells of the two documents 2 and 3 the one that might be at the origin of the anomaly of the fetus.
6. Schematize the abnormal meiosis leading to each of the two cells of the documents 2 and 3.

EXERCISE 5 A hereditary disease, Huntington chorea

Huntington Chorea is a rare hereditary disease characterized by involuntary movements, progressive intellectual deficits, and various mental health disorders. These symptoms generally appear, at a person, between 40 or 50 years of age.

The pedigree in document 1 shows the transmission of this disease in a family.

1. Specify based on the above text, if there is a risk for a 60 years old individual to get affected by Huntington chorea.
2. Show that the pedigree in document 1 permits to prove that the mode of transmission of this disease is dominant autosomal.
3. Determine the risk to be affected for individual 21 in document 1.

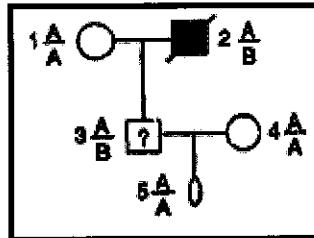


Document 1

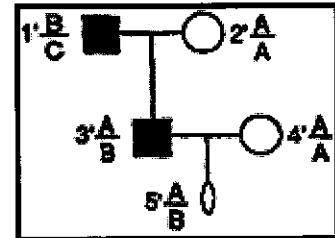
In certain families the determination for a fetus to be carrier of the allele of the disease can be done by the method of the genetic markers. There exists, near the locus gene intervening in the disease, a portion (P) of DNA which can exist in four allelic forms noted A, B, C and D; the portion P can easily be revealed during an examination of the chromosomes.

In a family affected by the disease, the study of the transmission of the alleles of the portion P, in parallel with the transmission of the normal and mutant alleles of the gene of Huntington chorea, allows to associate one of the four allelic forms (A, B, C, or D) with the mutant allele.

The results of this method done in two families are represented on the pedigrees in document 2 and 3.



Document 2



Document 3

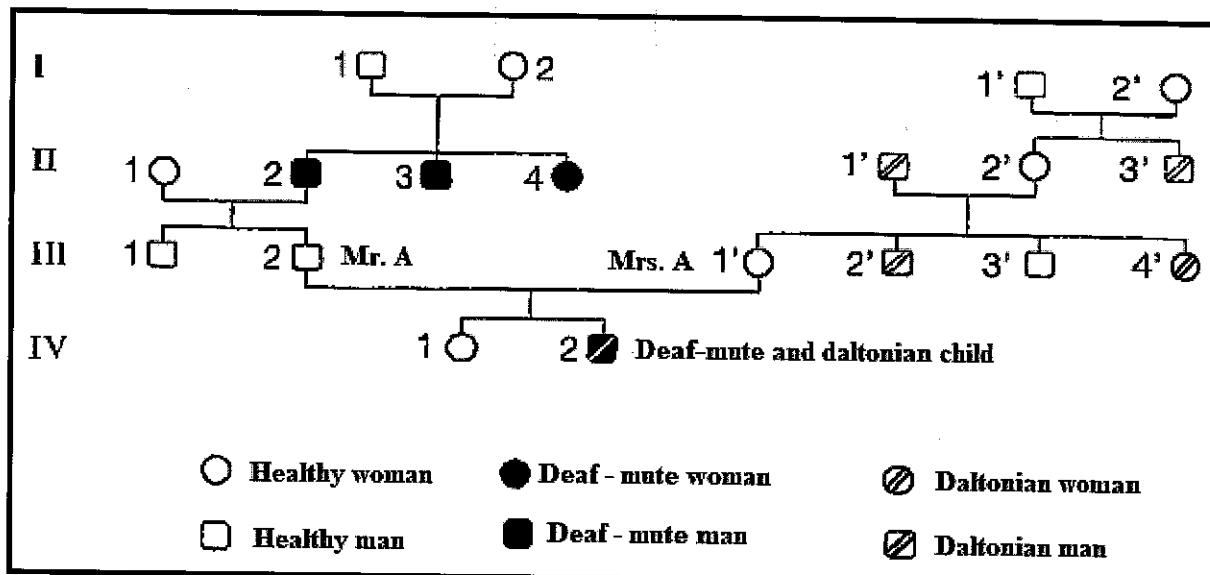
4. Pick out from the text the statement that indicates that the gene of the disease and the portion P of DNA are linked.
5. Write, in chromosomal form, the genotypes of the individuals 1, 2, 1', and 2' in documents 2 and 3 considering at the same time the gene of Huntington chorea and the portion P of DNA.
6. Explain why the fetus of the family in document 3 is likely to be affected by Huntington chorea while the fetus of the family in document 2 is not.

Knowing that the genetic distance between the gene of the disease and the portion P is of 4 cM;

7. Make the necessary factorial analysis to determine the chance of the fetus of family in document 3 to be normal.

EXERCISE 6 Transmission of two hereditary diseases.

Mrs. and Mr. A have two children, a deaf mute and daltonian boy and a healthy girl. The birth of the boy caused them to make a genealogical study of their families. These studies had shown some relation between their families. We also say that the father 1' is not carrier for the allele of daltonism. The gene of deaf mute is carried by an autosome.

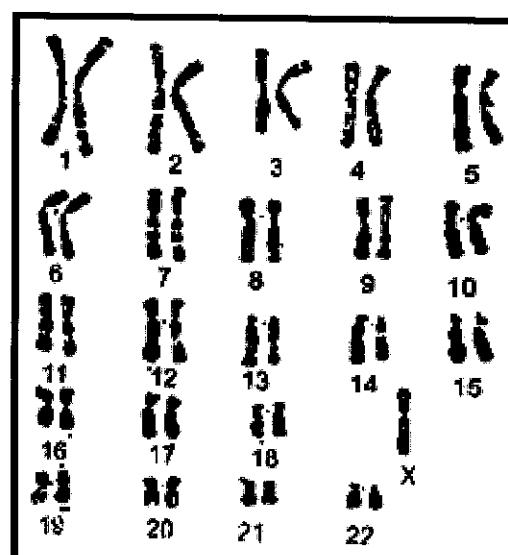


Document 1

1. Specify whether the alleles of these two diseases are dominant or recessive.
2. Show that the gene responsible for daltonism is carried by the non-homologous segment of chromosome X.
3. Indicate if these two genes are linked or independent. Justify.
4. Write the genotypes of III₂, III_{1'}, IV₁ and IV₂ for the two diseases. Justify the answer.
5. Schematize, by limiting to the chromosomes concerned by the two diseases; the meiosis occurring at Mrs. and Mr. A that are the origin of the birth of child IV₂ while indicating the gametes that give birth to this child.
6. Determine, by using a table of cross, the risk for these two parents to have another child affected by the two diseases at the same time.

The couple (1', 2) had given birth to a daltonian girl. The karyotype of this girl is given in document 2.

- 7.1 How does this karyotype explain the daltonian phenotype of this girl?
- 7.2 Schematize the meiosis of the parent at that causes the abnormal karyotype of this girl.



Document 2

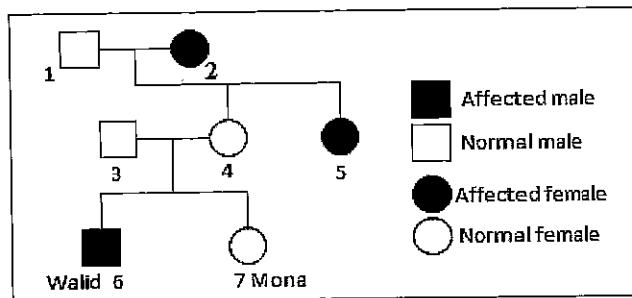
EXERCISE 7 A hereditary disease: the retinoblastoma

Retinoblastoma is a type of cancer characterized by the development of a tumor in the eye. The normal individuals have a gene on chromosome 13 that can suppress cancer while inhibiting the mitosis. This gene, in babies and children, can be submitted to two events provoking this type of cancer:

- A point mutation by substitution that alters the allele of the suppressor gene.
- A deletion of a part of the chromosome 13 containing the suppressor gene.

Concerning the first event, the mutant allele must be presented in two copies to provoke the cancer. Concerning the second event, the deletion of the allele that takes place in the somatic cells provokes the development of cancer.

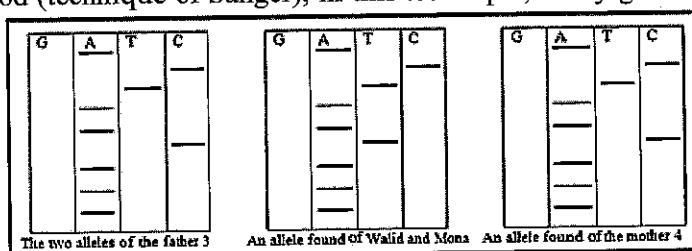
Document 1 shows the pedigree of the family of Mona in which some members are affected by retinoblastoma. The probability of heterozygous in the population is about 1/40.



Document 1: Pedigree of the family of Mona

1. Determine the risk for Mona to have an affected child if her husband is normal.

In order to know if Mona has a risk to develop cancer, some biotechnological methods are applied. The first method aims to detect the presence of the substitution in the allele of Mona, her brother and her parents, we achieve a specialized method (technique of Sanger), in this technique, every gotten band corresponds to the existence, in the sequence of the DNA, of nitrogenous base represented in the column and the order of the bands corresponds to the order of the nitrogenous bases in the DNA.

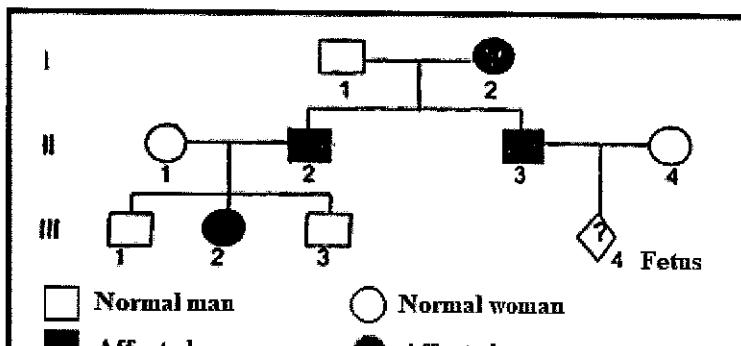


EXERCISE 8 Hereditary disease and prenatal diagnosis

Document 1 shows the pedigree of a family where some members are affected by a hereditary disease.

- Show, knowing that the female II-1 is homozygote, that this disease cannot be recessive.

In order to determine the chromosomal location of the gene corresponding to this disease, we realize an electrophoresis in order to separate the two alleles A₁ and A₂ of the gene. The results are represented in the document 2.



- Explain how do these results permit to say that the mutation leading to the formation of the mutant allele had deleted a restriction site compared to the normal allele of the gene.
- Show starting from the pedigree and the DNA analysis that the corresponding gene is located on the non-homologous segment of the chromosome X.

	Individual II-2	Individual III-3
Allele 1		██████████
Allele 2	██████████	

↑ direction
of migration
of the DNA

Document 2

The couple (II-3, II-4) are expecting a newborn and decides to know if this fetus has a risk to be affected by this disease. A prenatal diagnosis is able to reveal the state do the fetus by a DNA analysis. Yet the doctor decides to make a karyotype.

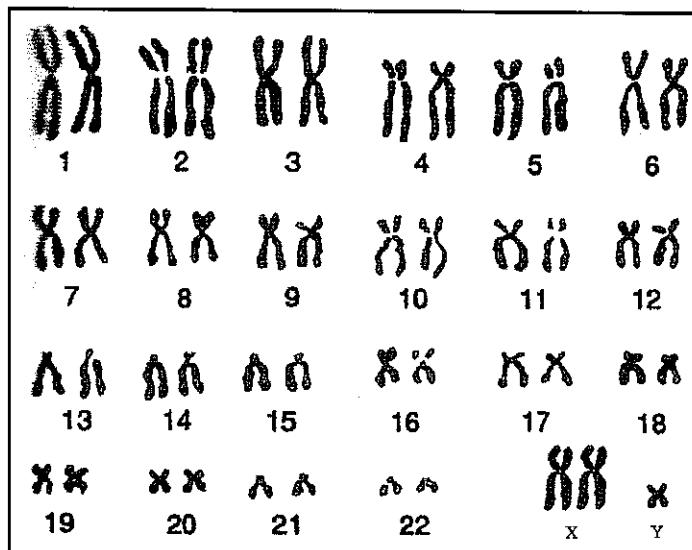
- Explain how a karyotype could clarify its state.

Document 3 shows the karyotype of the fetus.

- Basing on the karyotype; is it necessary to determine the state of the fetus concerning the disease by a DNA analysis? Justify.

A DNA analysis shows that the fetus possesses the two alleles A₁ and A₂.

- Schematize the abnormal behavior of the sexual chromosomes during the gametogenesis at the origin of the state of the fetus.



Document 3

EXERCISE 9 Restriction Enzymes

We have three restriction enzymes that cut the DNA in specific regions called restriction sites indicated in the document 1 with their cleavage sites. We have to study two alleles of the same gene with the sequences of their non-transcribed strands indicated in the document 2. This gene is located on an autosome and its mutant allele is recessive and expressed in an abnormality. The frequency of the heterozygote individuals in the population of this family is 1/25.

G TAAAC (1)	A T AGCTT (2)	G ATTC (3)
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Document 1

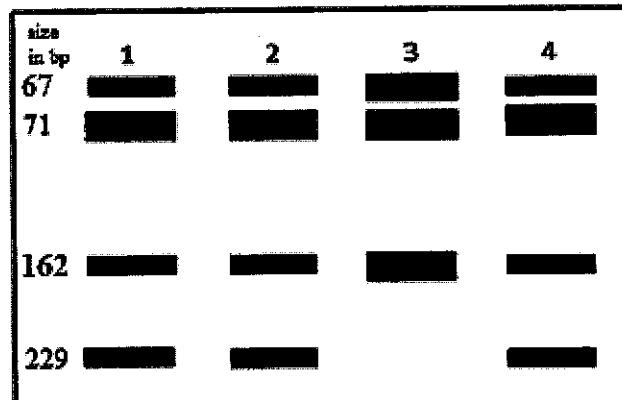
	1	70	121	230	300
Allele a A.....	CAAGCTTA.....	GGAATTCAA.....		TTCAAGCTTACGG.....	C
Allele b A.....	CAAGCTTA.....	GGAATTCAA.....		TTCAACCTTACGG.....	C

Document 2

- Q.** Determine, starting from the restriction enzymes given by the document 1, the one that is able to detect the genetic polymorphism of the given gene (discuss the three given enzymes).

Five individuals of the same family are tested by a DNA analysis using one of the three given enzymes in document 1. This test aims to determine their phenotypes concerning the disease caused by the abnormal allele of this gene. The results of the DNA analysis are given by document 3.

- Q.** Specify, starting from the analysis of these results and by referring to the documents 1 and 2, the phenotype of each member of this family.

**Document 3**

The following pedigree is that of the previously tested family behind, individual 5 is a fetus, for which the parents require a diagnosis.

Knowing that the gene is not considered located on the homologous segment of the chromosomes X and Y.

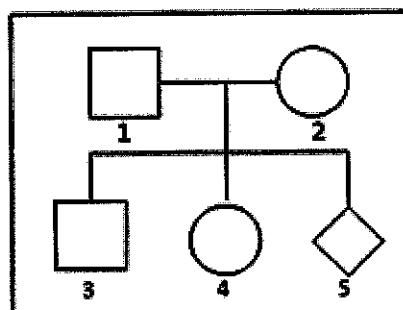
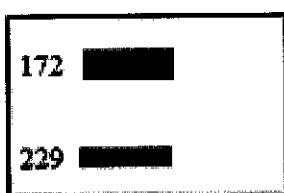
- Q.** Show that the set of the pedigree and the electrophoresis permit to determine the chromosomal localization of the studied gene,

Knowing that the parents do not have a family history concerning this disease.

- Q.** Calculate before and after making the DNA analysis the risk for this family to have an affected child by this disease

Document 5 shows partial extract of the DNA analysis of the fetus.

- Q.** Propose and explanation for the obtained result.

**Document 4****Document 5**

Reproduction and human genetics

Official exercises

Session 2001-1

Exercise 1 (4.5 pts) Inheritance of cystic fibrosis

Cystic fibrosis is a hereditary disease manifested by abnormality in cell exchange, which leads to progressive blockage of respiratory and digestive functions. Document 1 represents the pedigree of a family suffering from this disease.

1. Is the allele responsible for the disease dominant or recessive? Justify the answer.
2. Discuss logically the chromosomal localization of the gene responsible for cystic fibrosis.
3. Write the genotypes of parents 3 and 4, and their children. Justify the answer for each genotype.

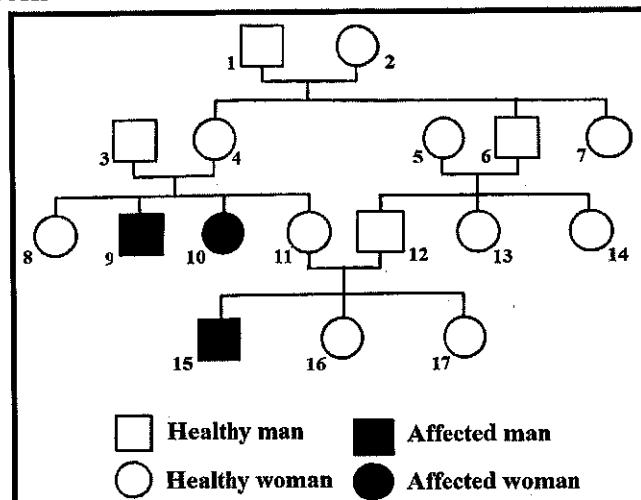
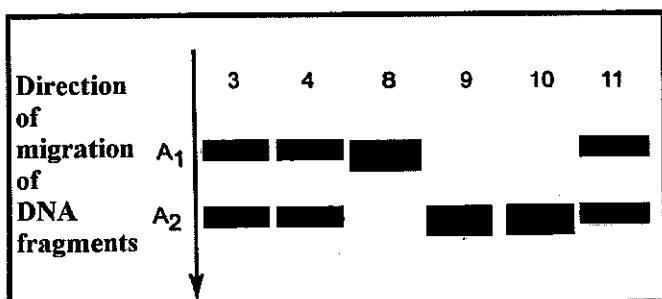
Researchers associate cystic fibrosis to a modification in the structure of a single gene located on a pair of human chromosomes. A technique of chromosome fractioning permits to obtain fragments of DNA, which can be separated by migration on a gel and identified by marking.

Document 2 shows the results of the migration of DNA fragments, termed A1 and A2, issued from the chromosomes of different members in the family presented in document 1.

4. Match each of the fragments A1 and A2 to its corresponding allele or each individual in document 2.

With reference to the phenotypes revealed in the pedigree.

5. Discuss and write the genotypes of the tested individuals, which are presented in document 2.
6. Compare these genotypes of individuals 3 and 4 and their children to those which were obtained previously.
7. What advantage does this genetic marking technique provide to the determination of the genotype of a certain individual?

**Document 1****Document 2**

Exercise 2 (4 pts) A cross of drosophilae

Session 2001-2

A. Crossing wild type drosophilae having striped bodies and brown eyes with mutant drosophilae having black bodies and red eyes, gives an F1 generation of drosophilae all of which are of the wild type.

An F1 female crossed with a male having black body and red eyes gave eggs that hatched into:

- 4% drosophilae having black bodies and brown eyes,
- 46% drosophilae of the mutant type,
- 46% drosophilae of the wild type,
- 4% drosophilae having striped bodies and red eyes.

B. Another homozygote drosophila having black body and vestigial wings was crossed with a drosophila having striped body and long wings. The F1 generation gave 100% drosophilae having striped bodies and long wings.

An F1 Females that were crossed with males having black bodies and vestigial wings gave descendants having four phenotypes of equal proportions.

1. Indicate the dominant alleles and the recessive ones. Justify the answer.
2. Explain the results obtained by each of the two test-crosses mentioned above.

These results provide evidence on the existence of two types of genetic assortments during meiosis in drosophilae.

3. Name these two types of genetic assortments.
4. Illustrate each of the two genetic assortments mentioned above by an explanatory schematic drawing.

Exercise 3 (4 pts) Transmission of albinism

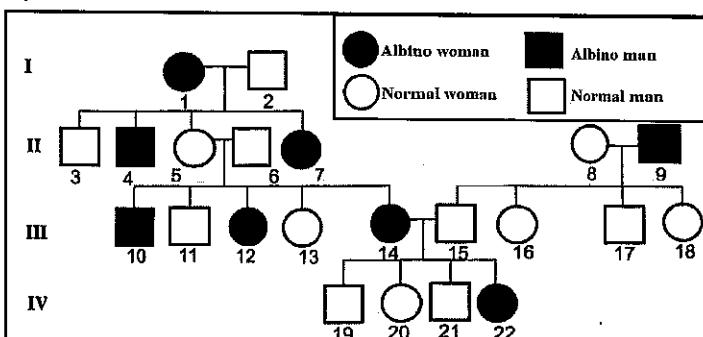
Session 2002-1

Albinism is a hereditary abnormality that results from the absence of melanin in the skin cells, especially in the cells of the hair root. The adjacent document presents the pedigree of a family in which some of its members, shown in black, are affected.

1. Is the allele of the abnormality dominant or recessive? Justify the answer.
2. Discuss logically the chromosomal localization of the gene of albinism.

Woman 22 marries an albino man. Her family predicts that all her children will be albino.

3. Indicate if this prediction is compatible with the answer to part (1)? Justify.

**Document 1**

Woman 22 gave birth to a non-albino girl, Ghada. To eliminate any doubt that the albino man is not the real father of Ghada, we determine the blood groups, ABO, and Rhesus of Ghada and her parents. The father is of group [O, Rh+], the mother is of group [B, Rh-] and their daughter, Ghada, is of group [O, Rh+].

4. After a logical analysis of the obtained results concerning the blood groups and taking into consideration the non-albino phenotype of Ghada, can we confirm that Ghada is the daughter of this couple? Justify the answer.
5. Formulate a hypothesis to explain the non-albino phenotype of Ghada.

N.B. The allele of Rh+ dominates the allele of Rh-.

Exercise 4 (3 ½ pt) chromosomal abnormality**Session 2002-1**

A family in which the father and the mother are phenotypically normal, but their two children present physical abnormalities and mental retardation, which are more or less significant.

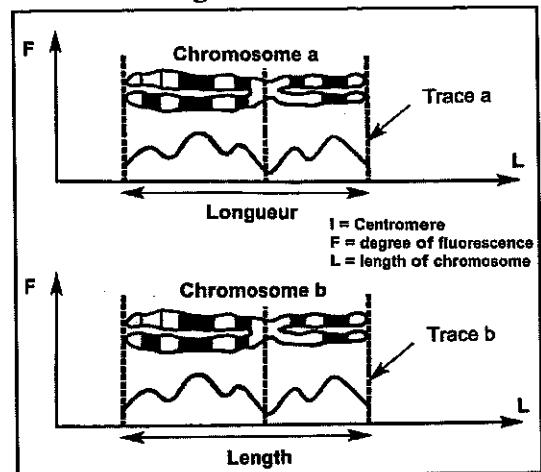
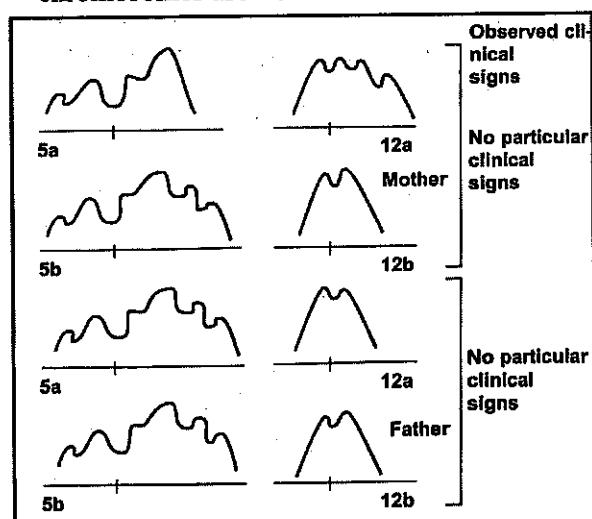
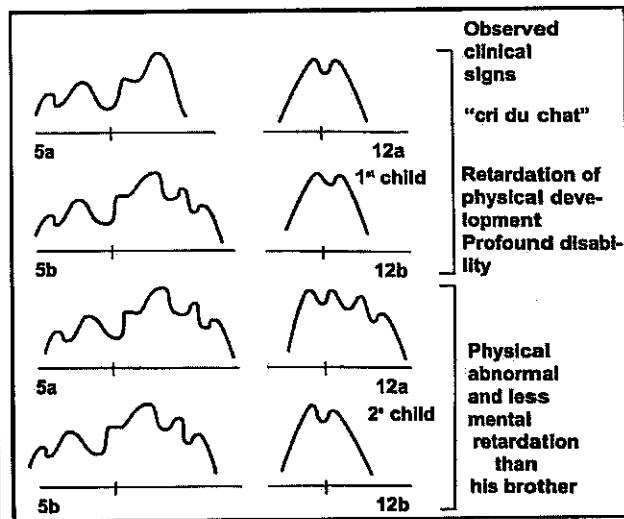
The doctors think that the genetic abnormalities have a chromosomal origin. They proposed to establish the karyotypes of all the members of the family.

To perform this study the chromosomes are chemically treated and fluoresced, then the degree of fluorescence along the length of the chromosome was measured.

Document 1 represents the results obtained for each of the chromosomes a and b of the pair NO 4.

- What information, concerning the length and the degree of fluorescence, does the comparison of chromosome pair N° 4 in document 1 reveal?

Following the same procedure, documents 2 and 3 represent the variations of the degree of fluorescence and length of the pairs of chromosomes 5 and 12 in the members of this family. The remaining pairs of chromosomes are normal.

**Document 1****Document 2****Document 3**

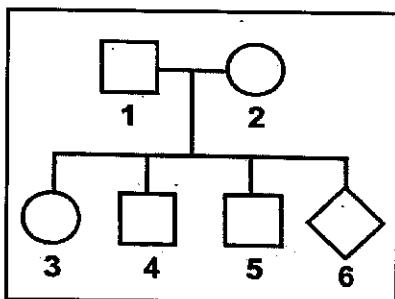
- Compare the pairs of chromosomes 5 and 12 of the mother and the two children to that of the father.
- Conclude the origin of the abnormalities observed in the two children.
- Schematize the pairs of chromosomes 5 and 12 of each member of the family.
- How can you explain the fact that the mother is phenotypically normal?

Exercise 5 (4 pts) A chromosomal abnormality

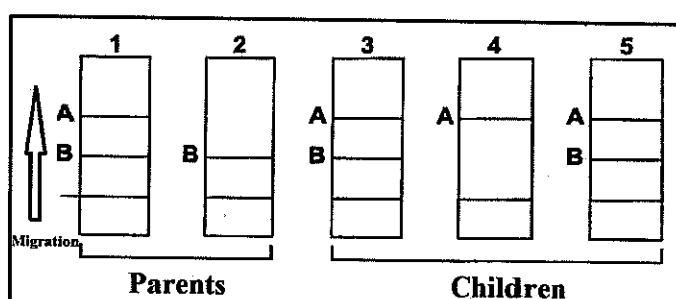
Session 2002-2

We study the relationship between certain sexuality troubles and genetic abnormalities. In a family whose pedigree is shown in document 1, Marwan (5) presents an abnormal development of the breasts and sterility.

A. The members of the family were tested for an enzyme, glucose 6-phosphate dehydrogenase (G6PD), which intervenes in carbohydrate metabolism. The synthesis of this enzyme depends on a gene carried by chromosome X. There are two alleles A and B that code for the two forms of G6PD respectively. Electrophoresis showed that form A of G6PD migrates faster than form B. Document 2 shows the obtained results, called zymograms.



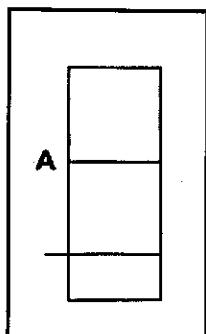
Document 1



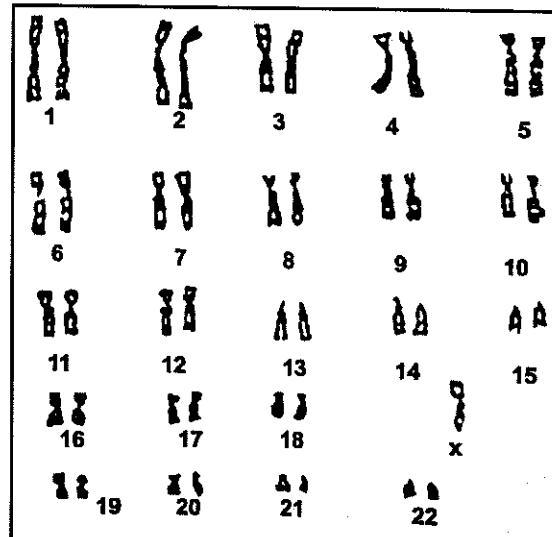
Document 2

1. Formulate the hypothesis to be verified by the performed test.
2. Explain, using all the given information and the acquired knowledge, the cause of the troubles presented by Marwan.
3. What complementary test would confirm the cause of this abnormality?

B. Marwan's parents are expecting a baby. Since the family exhibits the abnormality, the parents were worried and demanded genetic consulting and parental diagnosis. The results of the tests done are shown in documents 3 and 4. Moreover, echography test (detects the genital organs) showed that the fetus was a girl.



Document 3



Document 4

4. Show that the information provided by the three performed technique are complementary to answer the worries of the parents.

Exercise 6 (6 pts) Reproduction of mice

We propose to study certain particularities of sexual reproduction in mice.

We measure the quantity of DNA present in different cells. The results are indicated in document 1.

Cells of the mouse	Liver	Pancreas	Spermatogonium	Sperm cell
DNA Quantity (in pg)	6.199	6.2	6.2	3.1

Document 1

1.1. Compare the obtained results.

1.2. Name the biological phenomenon that is at the origin of the observed difference.

Geneticists crossed a mouse having curly hair and malformed eyes with a mouse having smooth hair and normal eyes. All the individuals of the first generation (F1) have curly hair and normal eyes.

The results of the (F2), issued from the self-cross of the individuals of F1, are the following:

- 42 mice having smooth hair and normal eyes.
- 127 mice having curly hair and normal eyes.
- 41 mice having curly hair and malformed eyes.
- 14 mice having smooth hair and malformed eyes.

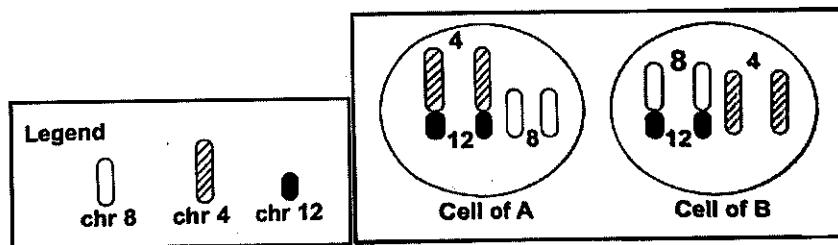
2. Calculate the phenotypic proportions of the F2 generation.

3. Explain the results obtained in F1 and F2.

4. Indicate the type of genetic recombination (assortment) responsible for the phenotypic proportions of F2.

Make a chromosomal representation of the genotype of an individual of F1.

Two mice A and B have two pairs of attached chromosomes (document 2). Upon crossing mouse A with mouse B, we obtain a hybrid individual AB of a normal phenotype yet their descendants may include individuals with trisomy.

**Document 2**

5. Schematize, by restricting only to chromosomes 4, 8 and 12:

- 6.1. The chromosomes of the gametes produced by each of the cells A and B in document 2.
- 6.2. The chromosomes of one cell of the hybrid mouse AB.

Knowing that during meiosis in the mouse AB, there is pairing of homologous chromosomes 4 and 8, the chromosomes 12 are not submitted to pairing:

6. Schematize the chromosomes of the gametes produced by a hybrid mouse AB.

An abnormal gamete of the hybrid mouse AB leads to trisomy 12 after its fertilization with a normal gamete of a mouse with non-attached chromosomes.

7. Indicate this abnormal gamete among the gametes schematized in part 7.

Exercise 7 (7 pts) Lesch-Nyhan syndrome

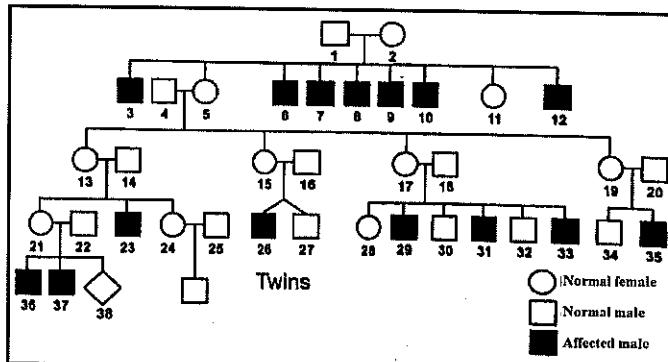
Session 2003-2

Lesch-Nyhan syndrome is a rare form of hereditary paralysis that leads generally to death before puberty.

This disease is characterized, beside other symptoms, by the increased production of uric acid.

The adjacent document presents the pedigree of a family that has some members, figured in black, who are affected.

- Is the allele responsible for the disease dominant or recessive? Justify the answer.
- Discuss, logically, the chromosomal localization of the gene responsible for this sickness.
- Are the twins 26 and 27 identical or fraternal twins? Justify the answer.
- Write their genotypes.

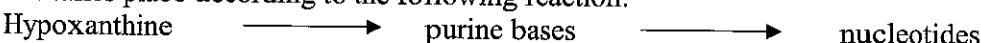


Document 1

We study the mode of action of the mutant allele responsible for Lesch-Nyhan syndrome. In a healthy individual, the nucleotides are continuously hydrolyzed liberating purines and pyrimidines (nitrogenous bases). Part of these bases (purines), are degraded progressively into hypoxanthine, then into uric acid as follows:

Nucleotides → purine bases → hypoxanthine → uric acid.

Most of the hypoxanthine is continuously recovered to synthesize new nucleotides. This synthesis requires certain enzymes such as HGPRT (hypoxanthine - guanine - phosphoribosyl-transferase), and takes place according to the following reaction:



At birth, we notice that the sick individual has a high concentration of uric acid in the blood.

In an experiment, we culture, in the same medium containing hypoxanthine, two types of cells, one is taken from a healthy individual, and the other type is taken from a sick one. The results are presented in the table in document 2.

Based on the given information, and knowing that nucleotides are used in the replication of DNA:

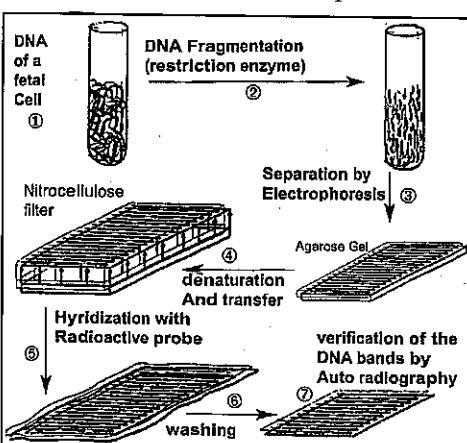
- Determine the cause of the sickness.

Observation	Culture of cells taken from a healthy individual	Culture of cells taken from a sick individual
Multiplication of cells	No multiplication of cells	

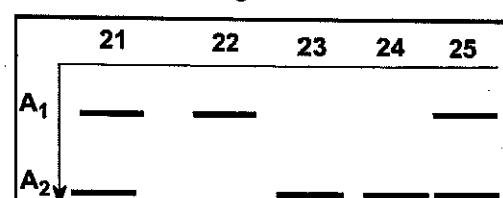
Document 2

In order to prepare a prenatal diagnosis of fetus 38, we cut the DNA of the family of the couple (21, 22) according to the technique schematized in document 3. The results are shown in document 4.

- Write a text describing the technique used in document 3, respecting the chronological order.



Document 3



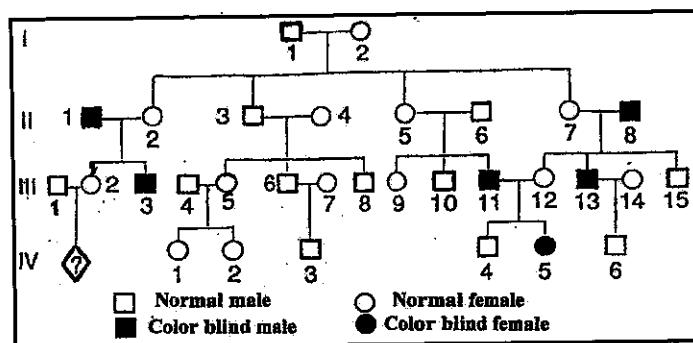
Document 4

- Identify, by referring to document 4, the mutant allele.
- Specify the phenotype of the fetus.

Exercise 8 (6 pts) Inheritance of color blindness

A- Color blindness (daltonism) is a defect in vision of colors. This defect is due to a gene localized on the non-homologous segment of chromosome X. Document 1 represents the pedigree of a family, whose certain members are affected.

1. Indicate if the allele responsible for color blindness is dominant or recessive. Justify the answer.
2. Write the genotypes of the individual (III-2), her parents, and her husband. Justify the answers.



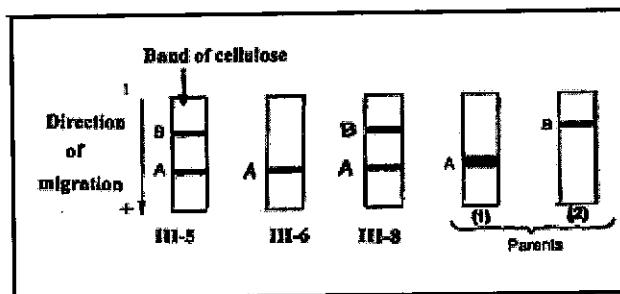
Document 1

Individual (III-2) is expecting a child and is worried if her child will be color blinded.

3. Make a factorial analysis to find the probability of the child to be color blinded.

B- In this family, medical analyses are performed to individual (III-8), who presents physical and sexual troubles. Equally, medical analyses were done to his parents, his sister (III-5) and his brother (III-6).

Among the medical analyses done, was testing for glucose-6-phosphate-dehydrogenase (G6PD), an enzyme whose synthesis depends only on a gene localized on the non-homologous segment of the X chromosome. On this gene locus two alleles may be found; allele A or allele B, that code for the synthesis of G6PD of form A and G6PD of form B respectively. We can distinguish the two forms by electrophoresis. Document 2 shows the electrophoregrams obtained for individuals III-5, III-6, and III-8, and for their parents.



Document 2

4. Compare the electrophoreograms of the two individuals III-5 and III-6.
5. Indicate, to which of the two parents of the individual III-8, each the electrophoreograms 1 and 2 corresponds. Justify the answer.

6.1. Determine the possible disorder that caused the troubles in individual III-8.

6.2. Which one of his parents is responsible for that? Justify the answer.

6.3. Name the phase of meiosis during which the disorder took place.

A study was done on the **male descendants** originated from women, whose fathers are color blinded and have a G6PD of form B. These women are of normal color vision like individual III-2 but their electrophoregram is like that of individual III-5. The husbands of these women are of normal vision and have a G6PD of form A.

7. Indicate the genotype of these women and the genotype of their husbands.

The descendants produced are the following.

- 75 Males with normal color vision and G6PD of form A.
- 71 Males with color blindness and G6PD of form B.
- 4 Males with normal color vision and G6PD of form B.
- 4 Males with color blindness and G6PD of form A.
- 8. How can you explain the obtained results? (a table of cross is not required).
- 9. Calculate the distance between the gene of color blindness and that of G6PD.

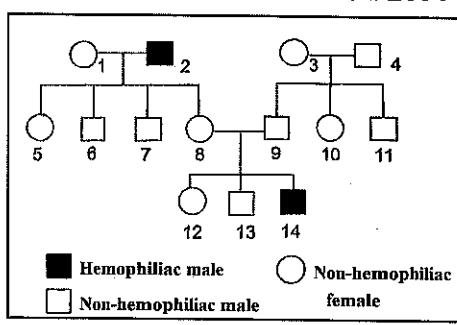
Exercise 9 (6% pts) Transmission of hemophilia

A- Document 1 represents the pedigree of a family whose certain members, colored in black, are affected by hemophilia B. The presence of the mutant allele of this gene in two copies in a genotype provokes the death of the embryo.

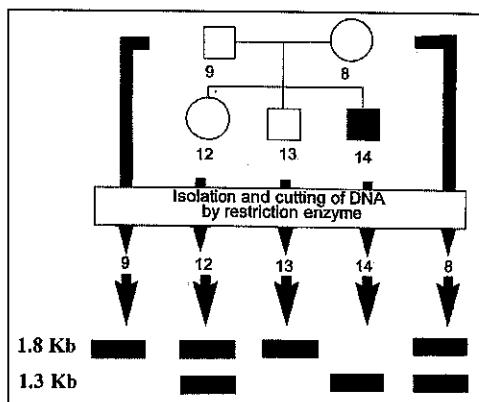
1. Is the allele responsible for this disease dominant or recessive? Justify the answer.
2. Is this gene sex-linked? Justify the answer.
3. Indicate the genotype of each of the individuals 8, 13 and 14. Justify, for each genotype, the answer.
4. Make the necessary analyses to determine the possible phenotypic proportions of the descendants of female 5 in each of the following cases:
 - 4.1. If her husband is not affected by hemophilia.
 - 4.2. If her husband is affected.

We performed a special technique for the analysis of DNA of the couple 8-9 and their children 12, 13, and 14. We obtained different DNA fragments of variable lengths measured in kilo bases (document 2).

5. Specify, by referring to document 2, the allele of the hemophilia B.

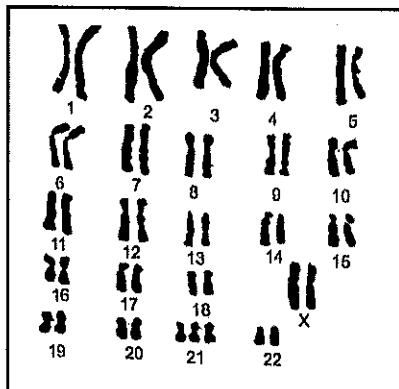


Document 1

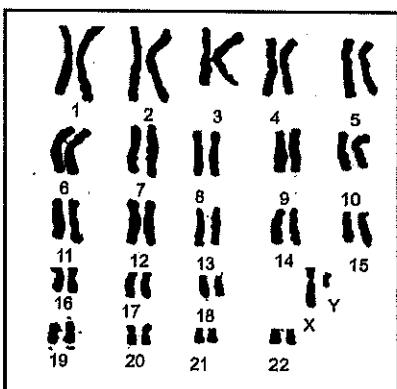


Document 2

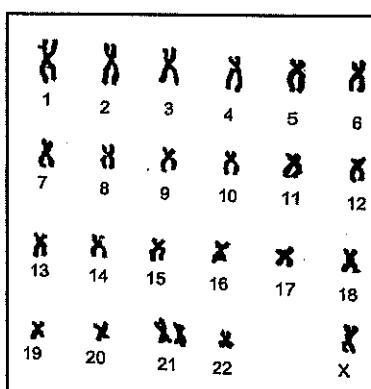
B- Document 3 presents the karyotype of an individual with an abnormality while document 4 presents the karyotype of a normal individual.



Document 3



Document 4



Document 5

- 6.1. Compare the two karyotypes in documents 3 and 4.
- 6.2. What is the abnormality revealed?

Document 5 shows the Karyotype of spermatocyte II that is obtained from a man of a normal karyotype. This spermatocyte II is identical to that, which has allowed the birth of the individual affected by the abnormality (document 3).

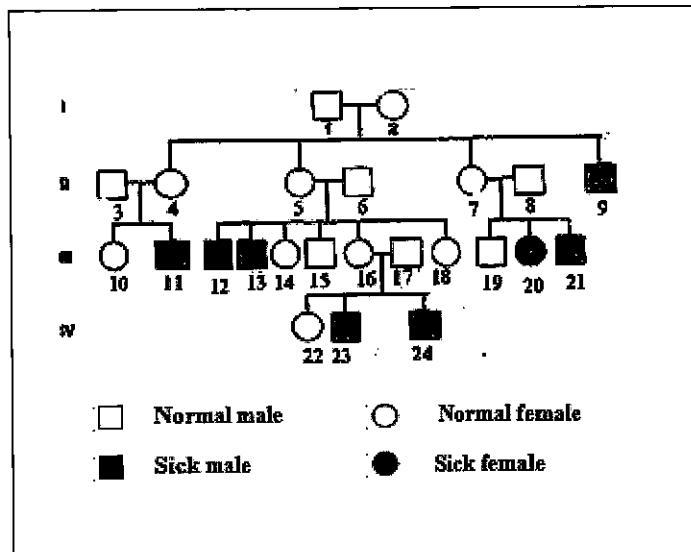
7. What information does document 5 provide?
8. Make a labeled diagram of the anaphase stage of the division, which produces this type of spermatocyte II. (Present, only, the chromosomes X and Y and the concerned chromosomes by the abnormality).

Exercise 10 (5 ½ pts) A hereditary disease

Session 2005-1

Document 1 represents the pedigree of a family with some of its members, shown in black, having a rare hereditary disease that occurs mainly in males and very rarely in females.

- Is the allele responsible for the disease dominant or recessive? Justify the answer.
- Discuss logically the chromosomal localization of the gene responsible for this disease (without considering female 20).
- Illustrate, chromosomally, the genotype of each of the individuals 13 and 16. Justify the answer.



Document 1

Female 20 presents, besides her disease, an abnormality, which is manifested by the absence of menstruation, absence of the development of mammary glands...

To identify this abnormality, we perform the karyotype of female 20, document 2.

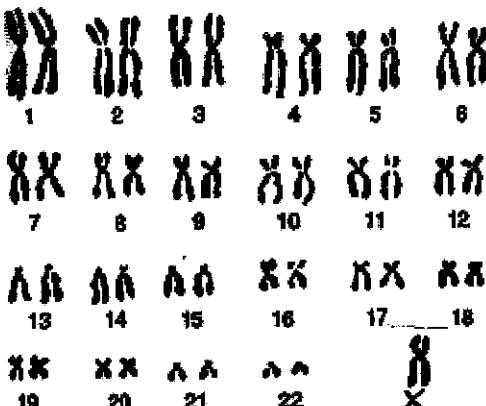
- Write the chromosomal formula of this female.
- Give the name of the abnormality revealed by the karyotype.

Based on the karyotype:

- How can you explain the appearance of the disease in female 20?

Knowing that this chromosomal abnormality results from an error in meiosis during spermatogenesis:

- Schematize the chromosomal behavior of the concerned chromosomes only (consider one case only).



Document 2

Exercise 11 (5 pts) Hemoglobin

Hemoglobin A is constituted of four chains of globin: 2 α and 2 β . The synthesis of β globin is controlled by a gene located on chromosome n°11. This gene exists in several forms of alleles such as allele HbA that leads to the formation of normal hemoglobin A and the allele HbS that leads to the formation of abnormal hemoglobin S.

Only persons possessing two HbS alleles have a disease called sickle cell anemia.

Document 1 presents the percentage of the two types of hemoglobin HbA and HbS in three persons P, M and R.

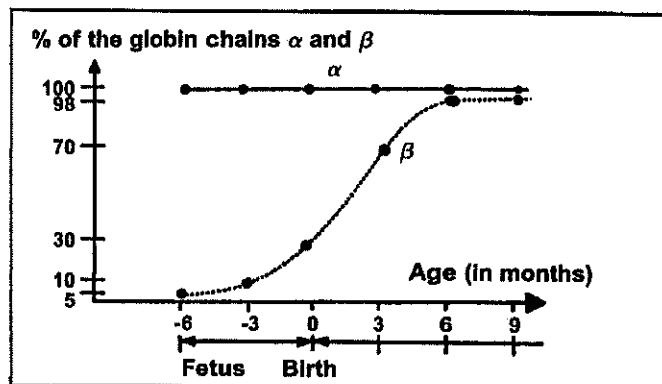
1. Write the genotype of each of these persons. Justify the answer in reference to document 1.
2. Indicate the sick person/s. Justify the answer.

We study the percentage of the α and the β globin chains present in the blood of the fetus from the 6th month before birth until the 9th month after birth. The results are shown in document 2.

3. Construct a table showing the variations of the percentage of blood globin chains as a function of age, revealed by document 2.
4. Justify, in reference to document 2, that sickle cell anemia is not manifested until six months after birth.

	Person P	Person M	Person R
Hemoglobin A	100 %	0 %	50 %
Hemoglobin B	0 %	100 %	50 %

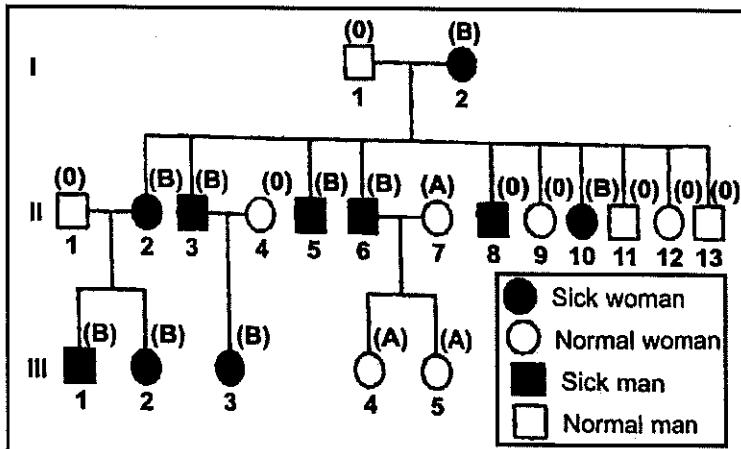
Document 1



Document 2

Exercise 12 (2 ½ pts) Two linked genes in humans

Blood groups of the ABO system are determined by a gene located on chromosome n°9. This gene exists in three alleles A, B, and O. Alleles A and B are codominant to each other but they dominate allele O. The pedigree of the adjacent document shows the transmission of two hereditary characteristics: the blood group and a dominant autosomal genetic disease manifested by reduced fingers and undeveloped patella. Designate the normal allele by "n" and the mutant allele responsible for the disease by "N". (A), (B) and (O) designate the blood groups.



Document 1

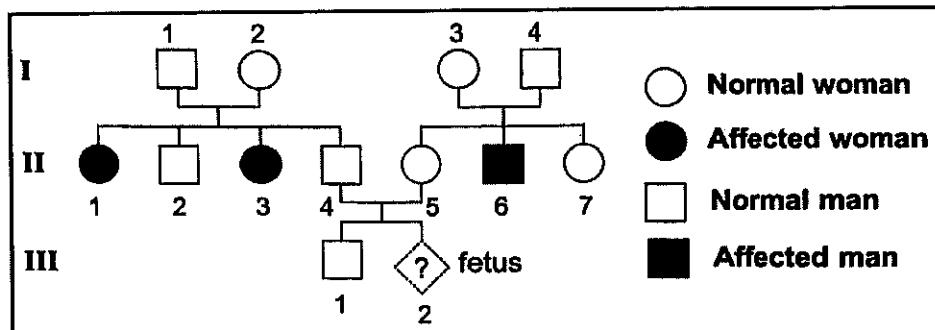
1. What information in the adjacent document reveals that the two studied genes are linked?
2. Schematize chromosomally the genotypes of parents I-1 and I-2, Justify the answer.
3. Indicate the genotype of the person II-8.
4. Explain the appearance of this genotype in the offspring of parents I-1 and I-2.

Exercise 13 (5 pts) Transmission of cystic fibrosis

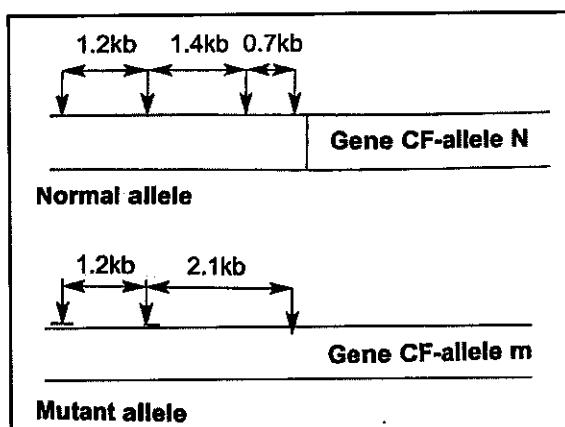
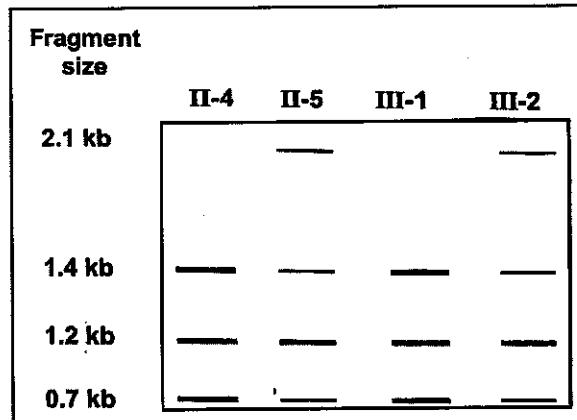
Session 2006-1

Document 1 represents the pedigree of a family of whom some members, figured in black, are affected by a disease called cystic fibrosis, a hereditary disease manifested by respiratory and digestive troubles. This disease is determined by a mutant allele of a gene called CF. This gene is located on chromosome 7, and very close to a non-coding region that has restriction sites recognized by the restriction enzyme Taq 1.

The non-coding region close to the functional dominant allele N has four restriction sites for enzyme Taq 1, while the non-coding region close to the mutated recessive allele d has three restriction sites. The length of the restriction fragments is expressed in kilobase (kb), document 2.

**Document 1**

1. Indicate the possible genotypes of individuals 11-4 and 11-5. Justify the answer.
2. Determine the genetic risk of couple 11-4 and 11-5 to have a sick child.
3. Specify the site at which mutation took place, document 2. Justify the answer.

**Document 2****Document 3**

Document 3 shows the results of electrophoresis of the restriction fragments obtained by Southern blot technique for individuals 11-4, 11-5 and their children.

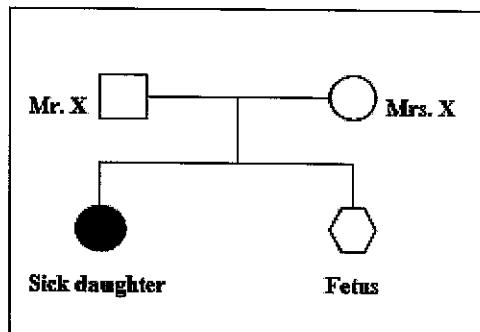
4. Determine the real genotype of each of individuals 11-4, 11-5 and the fetus.
5. Indicate if this couple is in risk of having affected children. Justify the answer.

Exercise 14 (5 pts) Inheritance of sickle cell anemia

Session 2006-2

Mr. and Mrs. X have a daughter suffering from sickle cell anemia, document 1. This hereditary sickness, whose mode of transmission is autosomal recessive, is characterized by an abnormality in the β -globin molecule which leads to the deformation of the red blood cells. Mrs. X is pregnant and the couple demand prenatal diagnosis to know if their second child will be affected by sickle cell anemia.

1. Indicate the genotype of Mr. and Mrs. X and that of their daughter. Justify the answer.
2. Based on logical reasoning, find the probability for this couple to have an affected child.

**Document 1**

Document 2 reveals the sequences of parts of the non-transcribed strands of the β -globin alleles: HbA is the normal allele while HbS is the mutant allele of the β -globin gene responsible for sickle cell anemia.

A direct diagnostic method by radioactive probe is done for this family. Many copies of the parts of the β -globin gene can be obtained from the DNA of each person by this technique. These copies are separated in two lots, and each lot is placed in the presence of a different radioactive probe, document 3; each probe is capable to bind with either allele HbA or HbS. The results of autoradiography are shown in document 4.

Position of the nucleotide	1	10	20
HbA	CTCCTGAGGAGAAAGTCTGCC		
HbS	CTCCTGTGGAGAAGTCTGCC		

Probe n°1	GAGGACACCTCTTCAGACGG
Probe n°2	GAGGACTCCTCTTCAGACGG

Document 2**Document 3**

	Mr. X	Mrs. X	Daughter	Fetus
Probe n°1	[redacted]	[redacted]	[redacted]	
Probe n°2	[redacted]	[redacted]		[redacted]

Document 4

3. Specify, based on document 2, the location of the mutation and its type.
4. Determine, by referring to documents 2 and 3, which allele corresponds to each used probe.
5. Do the results in document 4 confirm the genotypes you have indicated in question a? Justify the answer.
6. Deduce the genotype and the phenotype of the fetus.
7. Justify why prenatal diagnosis is more accurate than a pedigree in detecting a genetic disease.

Exercise 15 (5 ½ pts) Inheritance of Hemophilia A**Session 2007-1**

Hemophilia A, is a genetic recessive disease due to an abnormality of a blood coagulation factor: factor VIII. This factor is the expression of a gene located on the non-homologous segment of chromosome X. We designate, by h, the allele responsible for the disease and by N the normal allele.

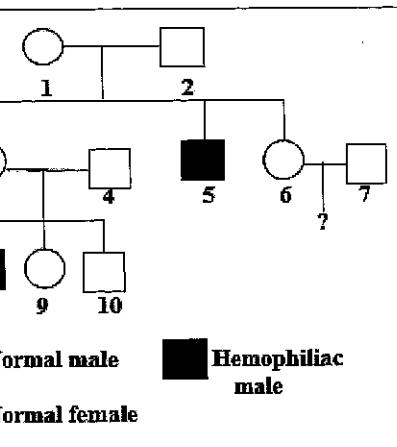
Document 1 reveals the pedigree of a family that expresses this disease. Woman 6 is pregnant and asks for prenatal diagnosis for her fetus.

1. Indicate the genotypes of persons 6 and 7. Justify the choice.
2. Show by logical reasoning, that this pedigree does not permit a sure diagnosis concerning the fetus.
3. Determine the genetic risk of this child to be hemophiliac.

To clarify the diagnostic problem of hemophilia in the fetus, two tests were done. The first test is a karyotype of the fetus, document 2.

4. Does this karyotype solve this problem? Justify the answer.

The second test is the analysis of the DNA of chromosome X. The DNA of the mother, the fetus, and the sick person 8, are subjected to restriction enzymes. The obtained DNA fragments are separated by gel electrophoresis, then hybridized by a probe.

**Document 1**

Because we cannot use an intragenic probe to distinguish the hemophilia allele from the normal allele that codes for factor VIII, we use probe ST14 that can mark a polymorphic zone, very close to this gene. This zone has 10 alleles, but only alleles 3 and 5 are present in this family.

An autoradiography is done and the results are shown in document 3.

5. Specify, starting from the analysis of the obtained autoradiogram, the real genotype of the mother and the fetus.

We estimate a 4% recombination between the polymorphic zone and the gene coding for factor VIII.

6. Indicate, in this case, if the second test is reliable for diagnosing hemophilia in the fetus. Justify the answer.

	Mother	Fetus	Person 8
Allele 3	■■■	■■■	
Allele 5	■■■		■■■

Document 3

Exercise 16 (5 pts) Hemophilia B

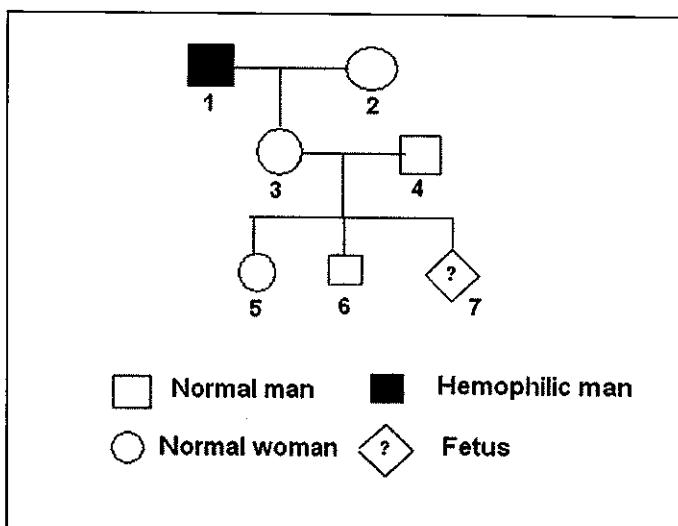
Session 2007-2

Hemophilia B is a recessive lethal disease characterized by the absence of blood clotting, which may lead to significant hemorrhage. It is linked to the absence of a clotting factor, factor IX, whose synthesis is controlled by a gene located on the non-homologous segment of the X chromosome. This abnormality affects boys and not girls.

- Explain the absence of this abnormality in girls?

Document 1 shows the pedigree of a family, one member of whom has the abnormality.

- Confirm that this disease is recessive.
- Determine the genetic risk of the fetus to be hemophilic.

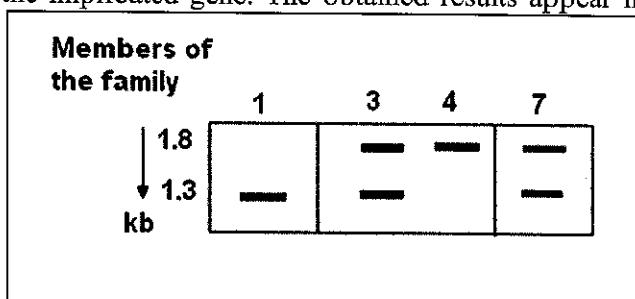


Document 1

Ultrasound scan was done to determine the sex of the fetus. It revealed that it is a boy. The doctor then prescribed analysis of DNA by the method of Southern blotting. The used probe permits to distinguish the mutated and normal forms of the implicated gene. The obtained results appear in document 2.

- Indicate the band that corresponds to the defective allele. Justify the answer.
- Specify, from the DNA analysis, the problem of the child that will be born.

The doctor completed the diagnosis by establishing the karyotype of the fetus, document 3.



Document 2

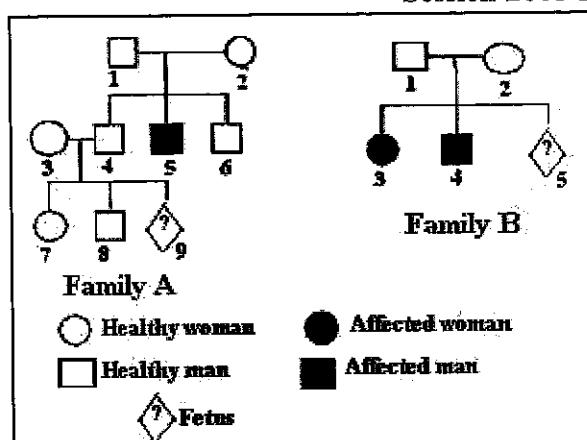


Document 3

Exercise 17 (5pts) A deficit in a hepatic enzyme
 Phenylketonuria is a disease caused by a deficit in a hepatic enzyme – PAH – responsible for the transformation of an amino acid, phenylalanine, into another one called tyrosine. In Europe the risk of being heterozygous is 1/50.

Document 1 shows the pedigrees of two families A and B which some members are affected with this disease. Couples (3, 4) of family A and (1, 2) of family B ask for a prenatal diagnosis.

- Through a rigorous analysis of the pedigree of family B, determine:
 - whether the allele responsible for the disease is dominant or recessive.
 - the location of the gene responsible for the disease.
- Determine the genetic risk for each fetus to be affected with this disease.



Document 1

Three mutations were determined to be at the origin of phenylketonuria. Document 2 shows a part of the codon sequences that correspond to three regions X, Y, and Z of the normal allele, and of the three mutant alleles that are responsible for this disease.

Codon	278.....282.... (Region X)	310.....314.... (Region Y)	406.....410 (Region Z)
RNA	278.....282.... (Region X)	310.....314.... (Region Y)	406.....410 (Region Z)
Normal allele	ACC CCC GAA CCU GAC...	UCU CUG GGU GCA CCU ...	AUA CCU CGG CCC UUC
Mutant 1	ACC CCC AAA CCU GAC...	UCU CUG GGU GCA CCU...	AUA CCU CGG CCC UUC
Mutant 2	ACC CCC GAA CCU GAC...	UCU CCG GGU GCA CCU...	AUA CCU CGG CCC UUC
Mutant 3	ACC CCC GAA CCU GAC...	UCU CUG GGU GCA CCU ...	AUA CCU UGG CCC UUC

Document 2

- For each allele responsible for the disease, locate the mutation and indicate its type.

In order to diagnose the fetuses, the following DNA tests were carried out in both families.

1st test: DNA is extracted from parental and fetal cells and is subjected to restriction enzymes. Hybridization technique is then carried out using two radioactive DNA probes that are complementary to a specific "region X". One of the probes is specific for the normal allele; the other is specific for a mutant allele.

The results are shown in document 3.

Determine out the genotypes of the individuals of family A in document 3.

- Justify that the test performed is not sufficient to establish the diagnosis of family B.

	P	M	E	P	M	E
Normal probe	●	●		●	●	●
Mutant probe	●	●	●			
Family A				Family B		

P= Father M= Mother E= Fetus

Document 3

2nd test: Family B is subjected to a second DNA test yet using other restriction enzymes. This method reveals a restriction site (cleavage site) at the level of region Z, while regions X and Y remain intact. The results of this test are shown in document 4.

- Show the importance of the second test in order to obtain an exact diagnosis concerning the fetus of family B.

	Father	Mother	Fetus
Normal allele	—	—	
Mutant allele	—	—	—

Document 4

Exercise 18 (5 pts) Transmission of phenylketonuria

Session 2008-2

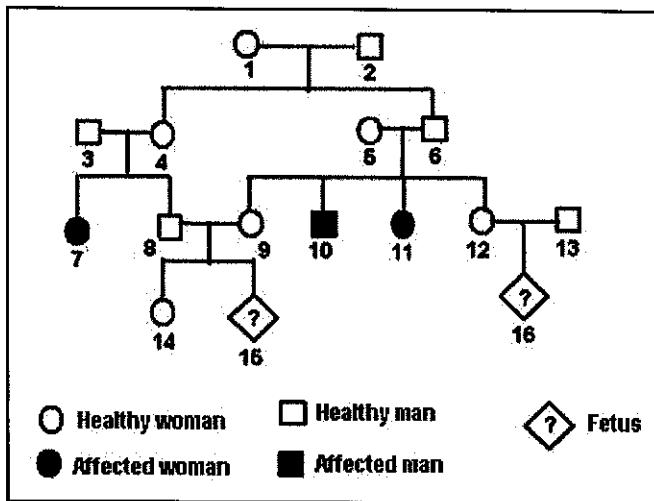
Phenylketonuria is a recessive autosomal disease that affects 1/10,000 of newborns worldwide. This disease is related to a deficiency in an enzyme called PAH. In normal conditions, this enzyme metabolizes phenylalanine into tyrosine, in the presence of a co-factor DHBP. This deficiency leads to an increase in the amount of phenylalanine in the blood accompanied with serious troubles.

A study performed on 1,200 children selected from an isolated community, showed that 30 children were heterozygous for PAH.

1. Calculate the proportion of heterozygous children in this community; and then determine the genetic risk for a child to be affected with phenylketonuria.
2. Compare the genetic risk obtained to the world-wide risk.
3. Formulate a hypothesis that explains the difference between these two risks.

In order to verify the formulated hypothesis, a study was carried out on a family of this community, which pedigree is shown in the adjacent document.

4. Justify, by referring to the pedigree, that the disease is recessive and autosomal.
5. Determine, for each of the fetuses 15 and 16, the risk to be affected.
6. Do the obtained results confirm the formulated hypothesis? Justify the answer.



Daughter 7 marries an affected man. Their first child was normal. All the tests performed confirm that the child is legal, and that the husband, unlike his wife, has a normal amount of PAH.

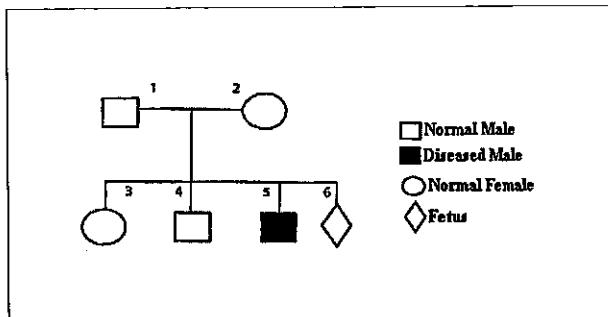
7. Determine the probable cause of the disease of the husband of daughter 7.
8. Justify, genetically, the birth of a normal child by this couple.

Exercise 19 (5 pts) A sex linked disease

Duchene Myopathy is a degenerative disease of muscle fibers which is due to a gene carried on the non-homologous segment of chromosome X.

Boys affected with myopathy do not synthesize the muscle protein, dystrophin, or synthesize an inactive form of dystrophin.

Document 1 represents the pedigree of a family having one member of its family affected with the disease.

**Document 1**

1-Determine, by referring to the pedigree, whether the allele responsible for the disease is dominant or recessive.

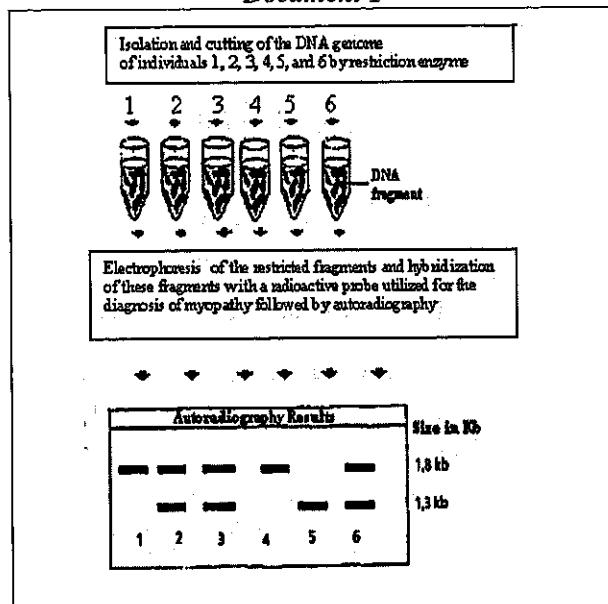
2-Indicate the genotypes of the parents. Justify the answer.

3-Determine the probability of the fetus to be affected.

Parents (1&2) who are expecting a baby want to know whether their fetus is at risk of developing the disease. They consult a doctor who proposes a prenatal diagnostic test by applying Southern Blot technique. The results are shown in document 2.

4-Indicate, by referring to document 1 and the autoradiography of document 2, the allele causing the disease. Justify the answer.

5-Specify the sex and the phenotype of the fetus.

**Document 2**

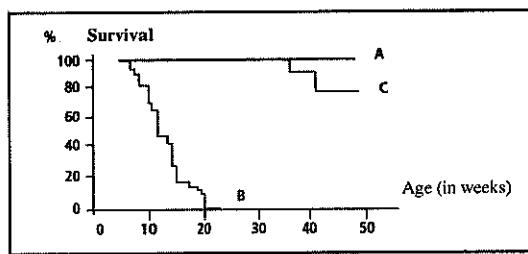
A gene therapy is applied for the first time on mice attaining myopathy similar to Duchene myopathy in humans. This technique consists of injecting the dystrophin gene into a diseased organism by means of a virus vector which is harmless to mice and human species. After this treatment, transversal sections are taken from the diaphragm muscle (respiratory muscle) of 3 groups of mice (A, B and C); then incubated with anti-dystrophin fluorescent antibodies and observed under a fluorescent microscope. The results obtained within 16-18 weeks are shown in document 3.

Mice	Results
A. Normal	Presence of fluorescence
B. Myopathic, non-treated	Absence of fluorescence
C. Myopathic, treated by injecting the dystrophin gene through a virus vector	Presence of fluorescence

Document 3

Document 4 reveals the percentage of survival of the three groups of mice in function of time.

- 6- What can be deduced about the efficiency of the used gene therapy?

**Document 4**

Exercise 20 (5 pts) Spermatogenesis**Session 2009-2**

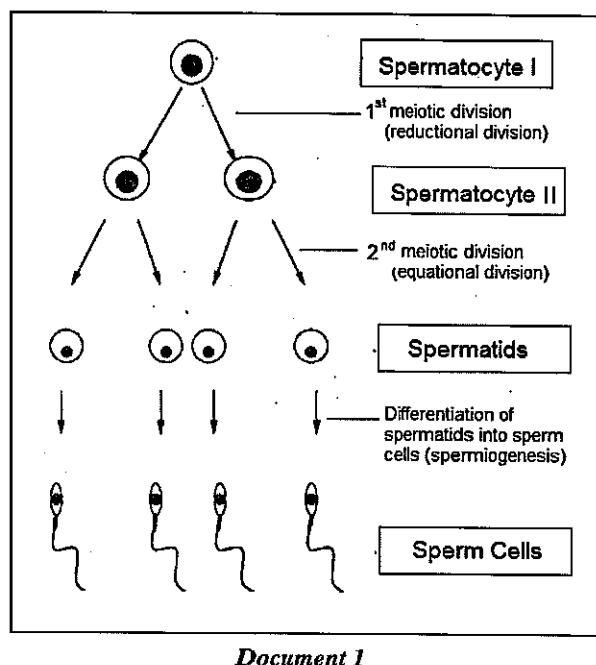
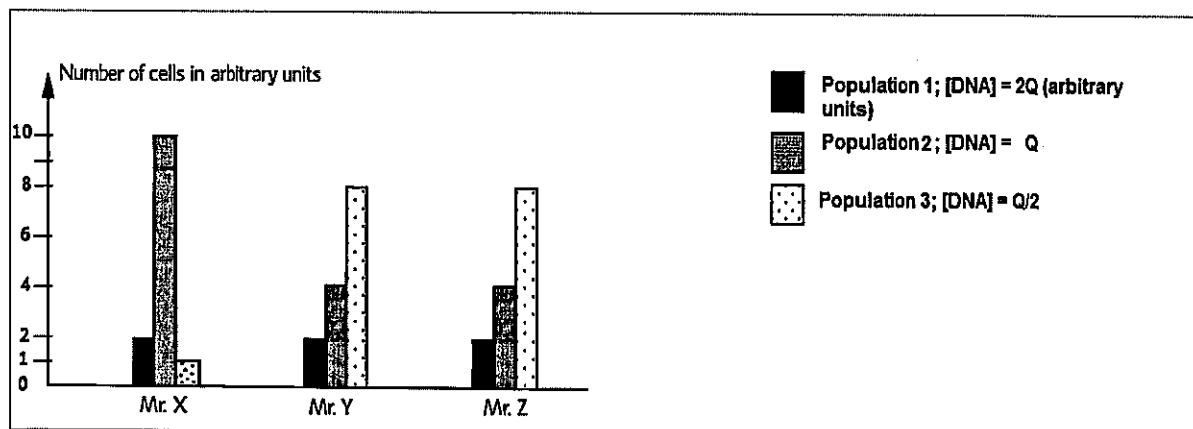
Mr. X and Mr. Y are two adult sterile men. We perform different tests to specify the origin of this defect.

Document 1 shows certain stages of spermatogenesis. The germ cells, whose names are framed in boxes, are found in the wall of the seminiferous tubules.

- 1- Describe the different stages of spermatogenesis represented in document 1.

We perform a quantitative study for the amount of DNA of the germ cells extracted directly, by biopsy, from a fragment of the testicles of these two sterile men and that of a fertile man Mr. Z. Three different populations of germ cells are obtained. The number of each cell population, as well as the amount of DNA in each of them are shown in document 2.

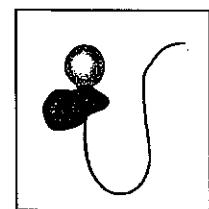
- 2- Indicate the germ cells corresponding to each of the three populations shown in document 2. Justify the answer.

**Document 1****Document 2**

- 3- Explain the variation of the number of germ cells of the three populations in the fertile man Mr. Z.
- 4- Determine, by referring to document 2, the cause of sterility of Mr. X.

Microscopic observations of the semen of Mr. Y showed sperm cells, where the majority of these cells showed an aspect identical to that schematized in document 3.

- 5- Explain the origin of the sterility of Mr. Y.

**Document 3**

Exercise 21 (5 pts) Transmission of hereditary characteristics in drosophilae Session 2010-1

In an attempt to study autosomal heredity in drosophilae, we cross a drosophila of pure race having gray body, red eyes and well-formed wings with another drosophila of pure race having black body, purple eyes and deformed wings. We obtain in F1 100% drosophilae having gray body, red eyes and well-formed wings.

- 1- Indicate the dominant allele and recessive allele for each of the studied genes.

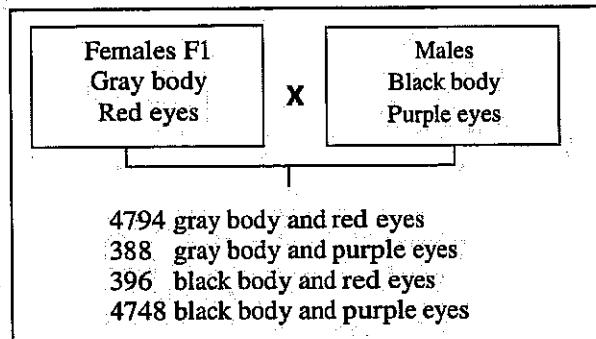
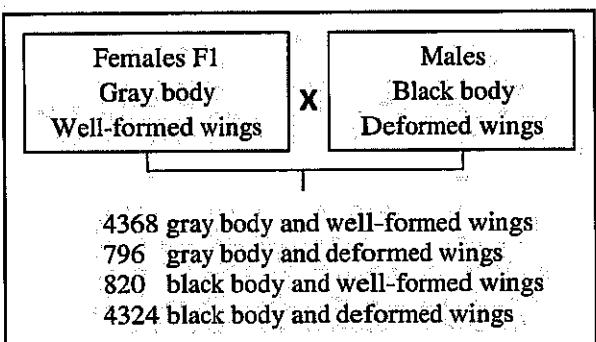
We perform, in drosophilae, two other experimental crosses 1 and 2, represented in the adjacent figures.

- 2- Name the type of the performed crosses.
- 3- Explain the results obtained in the first cross.

The results of the two crosses put in evidence the existence of a certain type of genetic recombination during meiosis in female drosophilae F1.

- 4- Name this type of genetic recombination and illustrate by explanatory schematic drawings the behavior of the corresponding chromosomes of the second cross.

- 5- Determine, by referring to the first and second crosses, whether the genes responsible for eye color and form of wings are linked or independent.
- 6- Calculate the percentage of recombination between the studied genes in each of the two crosses.
- 7- Knowing that the percentage of recombination between the genes of eye color and form of wings is 8%, establish a factorial map which reveals the location of the three studied genes on a chromosome.

First Cross**Second Cross**

Exercise 22 (5 pts) Sexual reproduction in mammals

Session 2010-2

We are interested in studying the events that accompany the sexual reproduction in mammals. These events are studied at cellular and molecular levels.

Female rabbits were mated with sterile males in order to induce ovulation, and then they were inseminated with sperm cells taken from different levels of the genital tract of adult fertile male rabbits. One day following the insemination, the aspect of the cells that were taken from the oviducts was observed under the microscope.

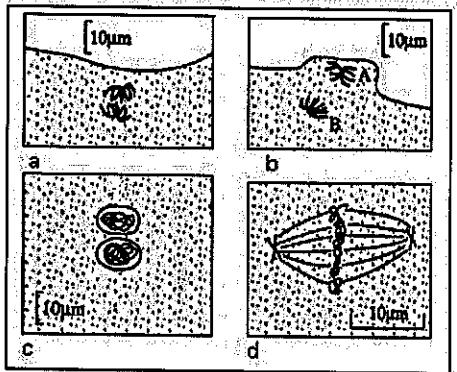
Document 1 presents the percentages of the two main aspects (schema X and Y) observed according to the site where the sperm cells were removed.

Site from where sperm cells were removed.	Aspect of the cells taken from the oviducts one day after the insemination	
	X	Y
Head of the epididymis	100%	0%
Proximal part of the body of the epididymis	85%	15%
Distal part of the body of the epididymis	35%	65%
Vas deferens	8%	92%

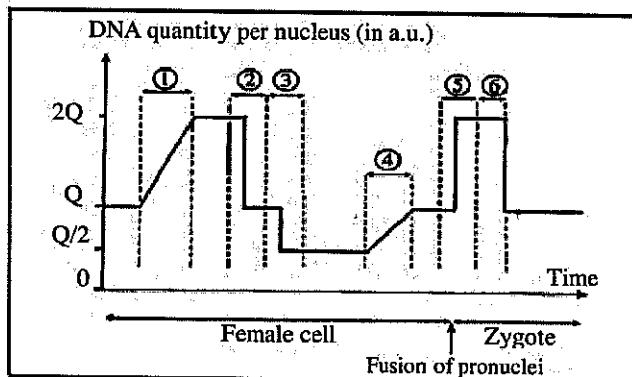
Document 1

- Explain briefly the structural modifications that take place during the passage of the cell from aspect X to aspect Y.
- Determine, by referring to document 1, the role of the epididymis.

Document 2 reveals, in chronological order, some steps of the evolution of the fertilized oocyte II and that of the zygote. Document 3 represents the evolution of the DNA quantity per nucleus of the female cell and that of the zygote.



Document 2



Document 3

- Name the two principal mechanisms of the sexual reproduction in mammals.
- Specify the importance of each of these mechanisms. Justify the answer by referring to document 2.
- Match each of the schema b, c and d of document 2 with a numbered step of the curve of document 3. Justify the answer.

Exercise 23 (5 pts) Retinitis pigmentosa

Retinitis pigmentosa, a hereditary disease, is the main cause of visual impairment (30% of visual deficiencies). The disease starts by affecting night vision and reducing the visual field. It is caused by progressive degeneration of rod cells, which are photoreceptor cells of the retina containing the protein rhodopsin.

To understand the origin of this disease, we study the structure of proteins encoded by different alleles of the rhodopsin gene.

The rhodopsin gene consisting of 1044 pairs of nucleotides encodes a protein of 348 amino acids.

Document 1 represents a portion of the nucleotide sequences of the alleles of the rhodopsin gene and that of the amino acids sequences of the corresponding proteins in individuals with normal phenotype and individuals with retinitis pigmentosa.

Individual's phenotype	Portion of the nucleotides sequence of the allele		portion of the amino acids sequence of the protein	
normal	391 ↓ ...CTG GCC ATC GAG CGG TAC...	408 ↓ ...CTG GCC ATC GAG CTT TAC...	131 ↓ ...Leu-Ala-Ile-Glu-Arg-Tyr...	136 ↓ ...Leu-Ala-Ile-Glu-Leu-Tyr...
Affected with retinitis pigmentosa	391 ↓ ...CTG GCC ATC GAG CTT TAC...	408 ↓ ...CTG GCC ATC GAG CTT TAC...	131 ↓ ...Leu-Ala-Ile-Glu-Leu-Tyr...	136 ↓ ...Leu-Ala-Ile-Glu-Leu-Tyr...

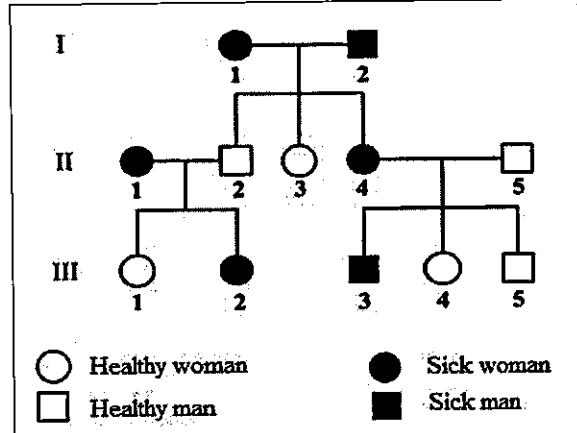
Leu = leucine, Ala = alanine, Ile = isoleucine, Glu = glutamic acid, Arg = arginine, Tyr = tyrosine.

Document 1

- Pick out from the text the cause of retinitis pigmentosa.
- Compare the two nucleotides sequences and the two amino acids sequences presented in document 1. Draw out the origin of this disease.
- Explain how the modifications in the nucleotides sequence of the allele (doc.1) lead to the appearance of the previously mentioned symptoms of retinitis pigmentosa.

Document 2 presents the pedigree of a family having some of its members affected with retinitis pigmentosa.

- Specify if the allele responsible for the disease is dominant or recessive and indicate its chromosomal location. Justify both answers.
- Determine the genotypes of individuals II3 and II4. Woman III2 married her cousin III3.
- Determine the risk for this couple to have children with retinitis pigmentosa.

**Document 2**

Exercise 24 (5 pts) DNA alteration

Session 2011-2

The Xeroderma pigmentosum is a disease that results in skin lesions which can develop into cancerous tumors and eye lesions. We are interested in the causes of this disease and the relative influence of genes and environment on its appearance. The body cells have, in their nucleus, enzymes that can repair DNA whenever this latter shows alterations. One of these enzymes is the ERCC3 which is coded by the gene G-ERCC3.

We present in document 2 the nucleotides sequence of a fragment of the non-transcribed strand of the gene G-ERCC3 of a healthy individual (allele G1) and the sequence of the equivalent fragment of an individual affected by xeroderma pigmentosum (allele G2).

		NUCLEOTIDE POSITION 2									
		U	C	A	G						
NUCLEOTIDE POSITION 1	U	UUU UUC UCC UUA UUG	phenylalanine alanine serine leucine	UCU UAC UCA UCC	serine	UAU UAC UAA UAG	tyrosine stop	UGU UGC UGA UGG	cysteine stop tryptophane	U C A G	NUCLEOTIDE POSITION 3
	C	CUU CUC CUA CUG	leucine	CCU CCC CCA CCG	proline	CAU CAC CAA CAO	histidine glutamic acid	CGU CGC CGA CGG	arginine	U C A G	NUCLEOTIDE POSITION 3
	A	AUU AUC AUA AUG	isoleucine methionine	ACU ACC ACA ACG	threonine	AAU AAC AAA AAG	asparagine lysine	AGU AGC AGA AGG	serine arginine	U C A G	NUCLEOTIDE POSITION 3
	G	GUU GUC GUA GUO	valine	GCU GCC GCA GCG	alanine	GAU GAC GAA GAG	aspartic acid glutamic acid	GGU GGC GGA GGG	glycine	U C A G	NUCLEOTIDE POSITION 3

A: Adenine

U: Uracile

G: Guanine

C: Cytosine

Document 1

Allele	nucleotides sequence of the fragment
G1	1 12 ...AAG AAG AGC AAC...
G2	1 12 ...AAG AAG AGA AAC...

Document 2

	Reference electrophoresis	Individual A	Individual B	Individual C
ERCC3 (coded by allele G1)	—		—	—
ERCC3 (coded by allele G2)		—		—

Document 3

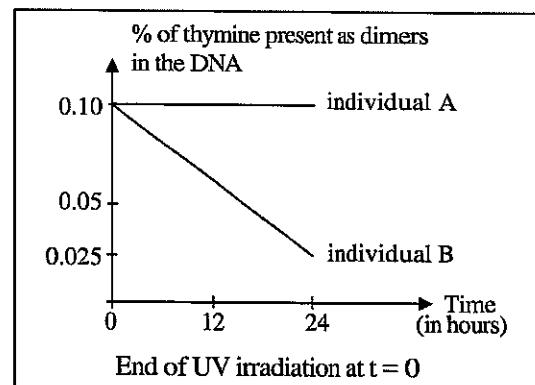
- 1- Determine using the genetic code table (doc.1) the amino acid sequence of the portion of each of the enzymes ERCC3 coded by the allele G1 and by the allele G2.

We can separate, by electrophoresis, the enzyme ERCC3 coded by the allele G1 and enzyme ERCC3 coded by allele G2. Electrophoresis is performed for three different individuals: A, B and C. Individual A is affected with Xeroderma pigmentosum, and individuals B and C are not. The results are presented in document 3.

- 2- Write the genotypes of individuals A, B and C. Justify the answer.
 3- Specify the dominant allele and the recessive one. Justify the answer.

Upon exposure to ultra violet sunlight rays, the DNA of skin cells undergo alterations, particularly the formation of dimers between two successive thymines T-T. We measure the evolution of the percentage of dimers in the two individuals A and B after being subjected to irradiation with ultraviolet rays. The measured results are presented in document 4.

- 4- Analyze the obtained results in document 4.
 5- Based only on the previous given:
 5-1- Explain the results of document 4.
 5-2- Specify the factors that determine the development of the studied disease. Justify the answer.



Document 4

Exercise 25 (5 pts) Analysis of partial karyotypes

Session 2012-1

In the framework of studying human chromosomal abnormalities, we prepare the karyotypes of parents of normal phenotype (document 1) and those of their children (document 2); one of these children has trisomy 21. Only certain pairs of chromosomes, from number 13 to 22, are represented.

- 1-1-** Compare the partial karyotypes of the father and the mother.

- 1-2-** What can you draw out?

- 2-** Explain why the first child is affected with trisomy 21 and the second presents normal phenotype.

- 3-** Schematize, for the mother, the phase of meiosis that is at the origin of trisomy 21 in the first child. (Limit your answer to chromosomes 14 and 21)

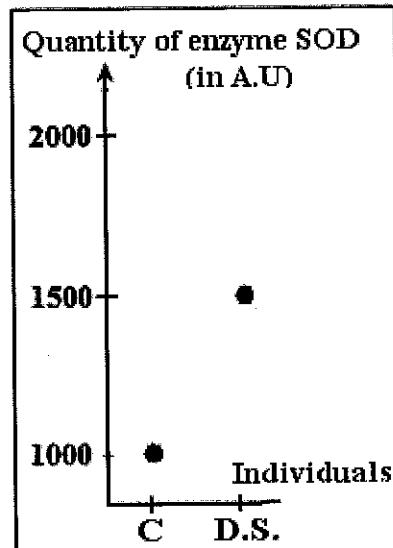
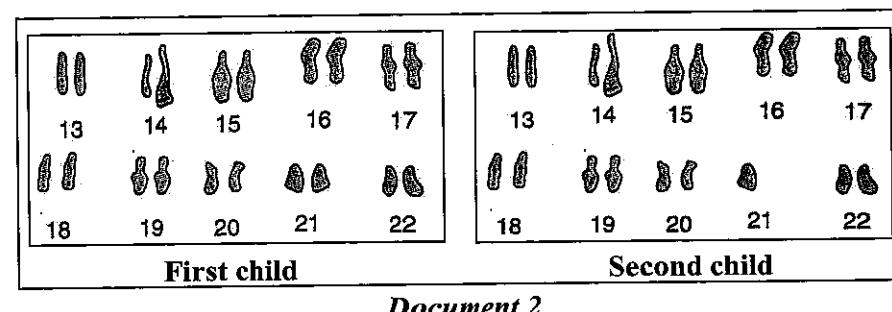
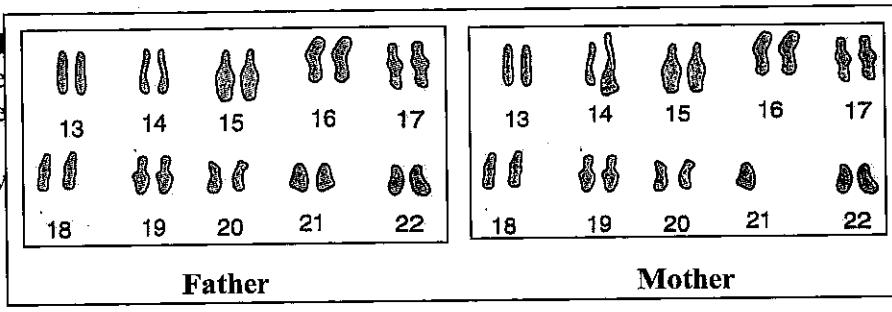
- 4-** Make a chromosomal analysis considering only chromosomes 14 and 21 in order to determine the proportions of normal and abnormal children of this couple.

One of the manifestations of Down syndrome (trisomy 21) is mental retardation. Biochemical analyses relate this manifestation to an abnormally high level of a protein "P" in the brain of individuals with this syndrome. This protein is coded by a gene located on chromosome 21.

- 5-** Propose an explanation concerning the presence of protein "P" in quantities greater than normal in the brain of individuals with Down syndrome.

On the other hand, we measured the level of an enzyme, superoxide dismutase (SOD), in the red blood cells of unaffected control individuals (C) and in those of others with Down syndrome (D.S.). This enzyme is coded by a single gene and is involved in the synthesis of protein "P". Document 3 shows the results of SOD measurement.

- 6-** Determine, with reference to document 3, the probable chromosomal location of the gene coding for the enzyme SOD.

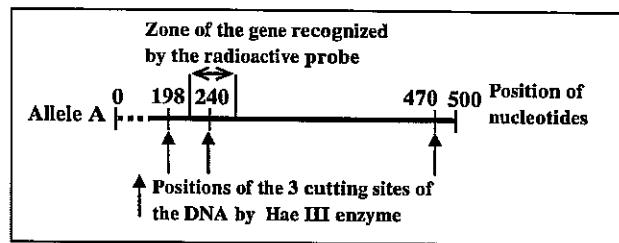
**Document 3**

Exercise 26 (5 pts) Transmission of albinism

Session 2012-2

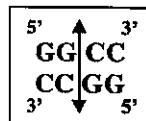
Albinism is a hereditary deficiency characterized by the absence of skin, eyes and hair pigmentation due to the absence of a black pigment: melanin. Tyrosinase is an enzyme involved in the biosynthesis of this pigment. The gene coding for tyrosinase exists in many forms of alleles and is carried by an autosome. Only two alleles are taken into consideration: allele A which codes for an active tyrosinase that is responsible for the synthesis of melanin and allele B that codes for an inactive tyrosinase that does not permit the synthesis of melanin.

Document 1 represents the map of the restriction sites recognized by Hae III enzyme in a portion of 500 base pairs (bp) of the allele A of tyrosinase gene.



Document 1

- Determine the number and the length of the restriction fragments obtained as a result of cutting allele A by Hae III enzyme.



Document 2

Document 2 shows the restriction site of Hae III enzyme.

Document 3 reveals a partial single-stranded sequence of the two alleles A and B of tyrosinase gene.

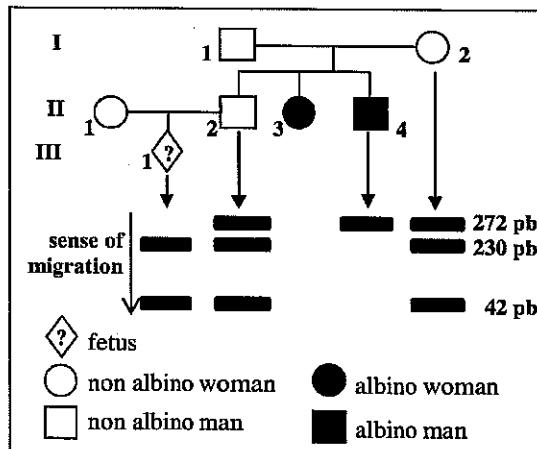
Position of the nucleotides	190	200	210	220	230	240	250
Allele A	5'	...CCACTTGGGCCTCAATTTCCTTACAGGGGTGGATGACCGGGAGTCGTGGCCTTCCGTCT....	3'				
Allele B	5'	...CCACTTGGGCCTCAATTTCCTTACAGGGGTGGATGACCGGGAGTCGTGGCCTTCCGTCT....	3'				

Document 3

- Compare these two sequences. Draw out the position and the type of mutation that took place.
- Determine the consequence of this mutation on the produced restriction fragments upon using Hae III enzyme on allele B.

Document 4 represents the pedigree of a family whose some members show albinism. It also shows the results of the electrophoresis of the restriction fragments obtained following the action of Hae III enzyme on a portion of the tyrosinase gene. These fragments are obtained by the Southern blot technique for four members of the family.

- Specify the respective alleles of individuals I₂ and II₄. Justify the answer by referring to the results of electrophoresis.
- Indicate, referring to document 4, whether the allele of albinism is dominant or recessive. Justify the answer.
- Establish a prenatal diagnosis of albinism for the fetus III₁.

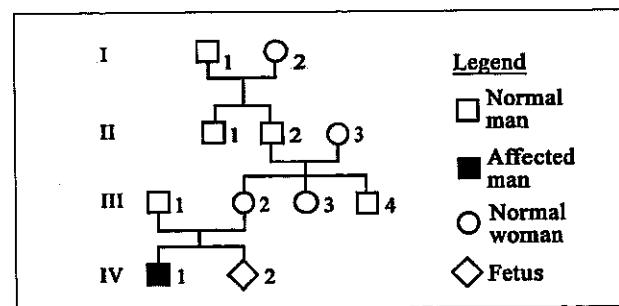


Document 4

Exercise 27 (5 pts) Fragile X syndrome

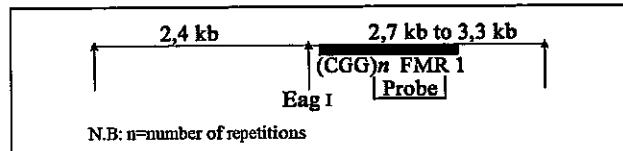
Session 2013-1

Fragile X syndrome is the most common cause of hereditary mental retardation. The gene FMR1 which is responsible for this disease is located on the non homologous segment of the X sex chromosome. The alleles at the origin of the abnormal phenotype are characterized by the repetition of CGG triplets for more than 200 times. Couple III₁- III₂ (document 1), who already had an affected child, expects another one and would like to know if it will be affected or not.

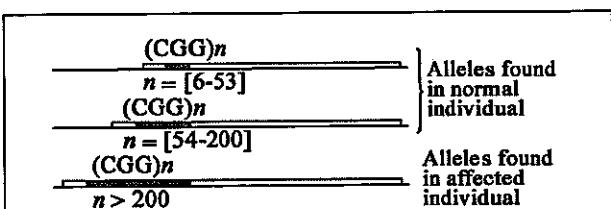
**Document 1**

- Justify that the gene is not localized on Y chromosome.
- Propose an explanation for the appearance of the disease in individual IV1 (document 1).

The fragment of DNA which carries the FMR1 gene is isolated. A very close site to this gene is recognized by the restriction enzyme EagI. For a complicated reason, this site is no more recognized by the enzyme in the mutant allele where the number of repetitions of CGG triplets exceeds 200. Document 2 shows the position of this cleavage site in normal alleles.

**Document 2**

Individuals	III3	III1	III2	IV1	IV2
5,8 kb				—	
3,2 kb			—		
2,8 kb	—	—	—	—	—

Document 3**Document 4**

- Specify, by referring only to document 2, the band(s) corresponding to the allele(s) of the disease and those corresponding to the normal alleles.
- Determine whether the fetus IV2 will be affected or not by the fragile X syndrome.
- Pose the problem that arises from the study of document 3 concerning the origin of the disease in IV1.

Document 4 shows the position and the number of repetitions of CGG triplet for the allele of FMR1 gene. The alleles having a number of repetitions between 54 and 200 are expressed normally but might be subjected to instability during gametogenesis. This instability can be manifested by an increase in the number of triplets.

- Explain, based on what precedes, the real origin of the disease in IV1.

Exercise 28 (5 pts) Origin of Phenylketonuria

Session 2013-2

In hepatic cells, the enzyme phenylalanine hydroxylase, PAH, is responsible for the transformation of phenylalanine into tyrosine. Its absence or its inactivity results in the accumulation (increase in the amount) of phenylalanine in the blood which becomes toxic at a dose exceeding 20mg/dL which leads to the destruction of the nerve cells in individuals affected with phenylketonuria. This disease has different origins and is manifested by irreversible mental retardation.

- Pick out the consequence of the high amount of phenylalanine in the blood.

Document 2 represents a part of the gene coding for the enzyme PAH of a healthy individual and that of the equivalent fragment of an individual suffering from phenylketonuria.

- Determine, using the genetic code table (document 1), the sequence of amino acids of the part of the enzyme PAH coded by each of these two alleles.
- Explain how the modification in the nucleotide sequence of the allele leads to the appearance of phenylketonuria.

Two normal couples had two newborns with high plasma concentration of phenylalanine that exceeds 20mg/dL.

- Indicate if the allele of the disease is dominant or recessive. Justify the answer.

In order to determine the origin of the disease in these two newborns, N_1 and N_2 , these couples consulted a doctor who recommended DNA analysis for all the family members. The obtained results are presented in document 3.

Moreover, the doctor proposed another test, where he injected the newborns with phenylalanine followed by injection of BH_4 , an organic substance normally present in the organism and that is indispensable for the normal activity of PAH. The obtained results are presented in document 4.

- Indicate the possible origin of the disease in the case of the newborn (N_1). Justify the answer by referring to documents 3 and 4.
- Determine, by referring to documents 3 and 4, the possible origin of the disease in the case of the newborn (N_2).

		Nucleotides position 2								
		U	C	A	G					
Nucleotides position 1	U	UUU UUC UUA UUG	phenylalanine leucine	UCU UCC UCA UCG	serine	UAU UAC UAA UAG	tyrosine non-sens	UGU UGC UOA UGG	cysteine non-sens tryptophane	Nucleotides position 3
	C	CUU CUC CUA CUG	leucine	CCU CCC CCA CCG	proline	CAU CAC CAA CAG	histidine glutamine	CGU CGC CGA CGG	arginine	
	A	AUU AUC AUA AUG	isoleucine methionine	ACU ACC ACA ACG	threonine	AAU AAC AAA AAG	asparagine lysine	AGU AGC AGA AGG	serine arginine	
	G	GUU GUC GUA GUG	valine	GCU GCC GCA GCG	alanine	GAU GAC GAA GAG	aspartic acid glutamic acid	GGU GGC GGA GGG	glycine	

A : Adenine

U : Uracil

G : Guanine

C : Cytosine

Document 1

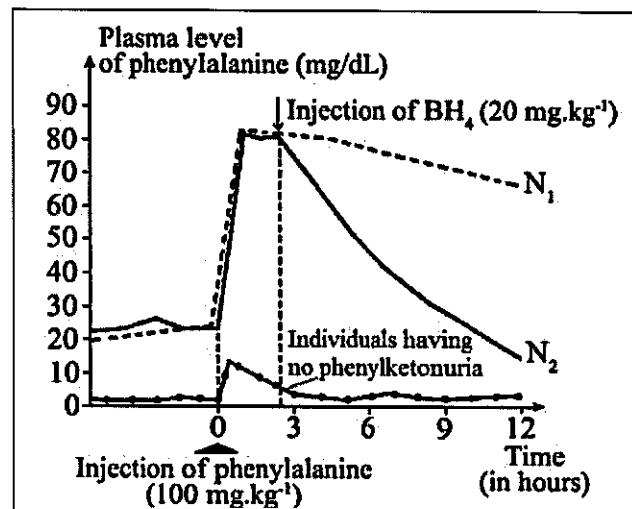
Alleles	Nucleotide sequence of the non-transcribed strand of DNA from codon 277 to codon 283
Normal	TAT ACC CCC GAA CCT GAC ATC
Diseased	TAT ACC CCC AAA CCT GAC ATC

Document 2

Alleles	F ₁	M ₁	N ₁	F ₂	M ₂	N ₂
Normal	—	—	—	—	—	—
Diseased	—	—	—	—	—	—

F: Father M: Mother N: Newborn

Document 3



Document 4

Exercise 29 (5 pts) Transmission of two hereditary traits

Session 2014

Hemophilia A is one of the oldest well-known genetic diseases, it is relatively rare in a population. It is characterized by the deficiency in the antihemophilic factor A, or the clotting factor VIII which is coded by a gene located on the long arm of the X chromosome.

Woman II7 with red hair, having brothers with red hair and suffering from hemophilia A, suspects the presence of a relation between the "red hair color" and the appearance of the disease. She consults a doctor for a genetic advice. Genetic investigation allowed her to construct the pedigree of her family which is presented in document 1.

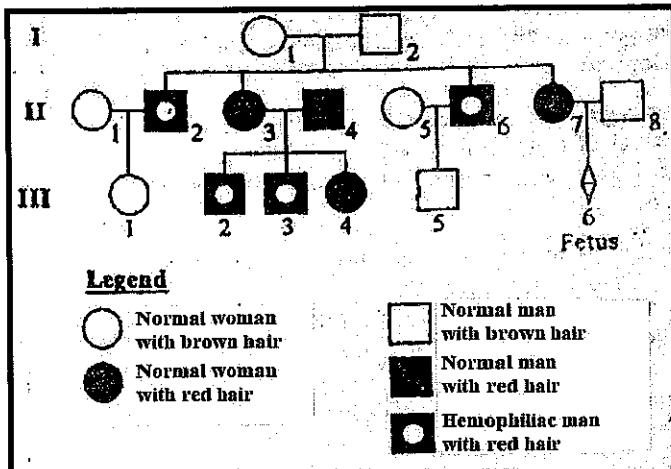
1. Determine the recessive allele and the dominant allele of the gene coding for:
 - 1.1.hemophilia
 - 1.2.hair color
2. Specify the location of the gene coding for the hair color.
3. Show that the suspicions of II-7 concerning the existence of a relation between the hair color and the appearance of the disease are not justified.
4. Determine, for the couple II7-II8, the risk of having a child suffering from hemophilia.
5. Indicate the possible genotypes of a couple that theoretically gives half of the children with red hair and the other half with brown hair and half of boys suffering from hemophilia while all the girls have normal blood coagulation.

In order to complete his diagnosis of hemophilia, the doctor prescribed a karyotype of the fetus and an analysis of the DNA of the gene responsible for hemophilia.

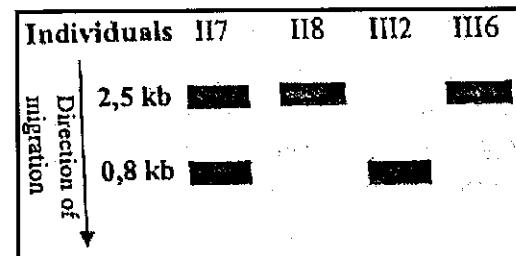
The karyotype of the fetus shows a chromosomal formula of 46, XY.

The DNA of the gene responsible for hemophilia, in the parents II7, II8, the fetus III6 and his sick cousin III2 are subjected to the action of a restriction enzyme, the BCI I. The obtained DNA restriction fragments are then separated by electrophoresis and hybridized with a radioactive probe then visualized by autoradiography.

6. Identify, by referring to document 2, the band that corresponds to the allele of hemophilia A.
7. Determine if the fetus will be affected by hemophilia.



Document 1



Document 2

Exercise 30 (5 pts) Huntington Chorea

Session 2015-1

Huntington Chorea is a serious neurodegenerative hereditary disease. Its first symptoms appear in adults starting from the age of 25 years.

We seek to determine the mode of transmission of this disease as well as its origin.

Document 1 shows the pedigree of a family whose certain members are affected by this disease.

- Indicate whether the allele determining this disease is dominant or recessive. Justify the answer.
- Determine the localization of the gene responsible for this disease.

All the members of this family are over 25 years old except individuals III3 and III5. The latter are willing to get married but are afraid of being affected by this disease.

- Determine the risk for each of individuals III3 and III5 to be affected by this disease.

Studies have shown that the gene coding for the functional protein, huntingtin, exists in many allelic forms that differ by the number of CAG triplets. The number of repetitions of CAG triplet in each allele is studied in healthy individuals as well as in affected ones. The obtained results are presented in document 2.

- Deduce, based on the statistical results of document 2, the origin of this disease.

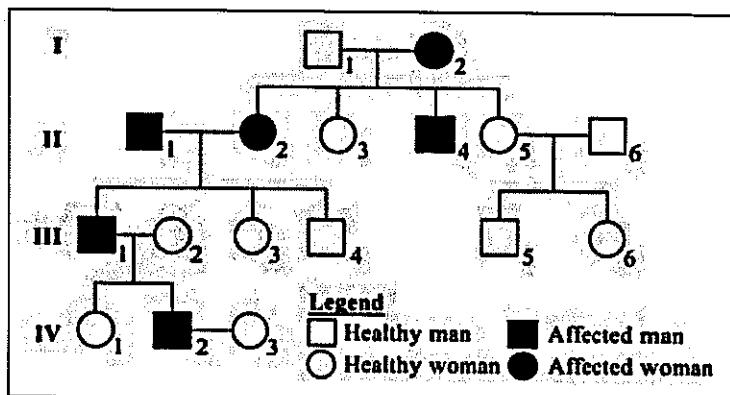
The analysis of the gene in woman III3 has revealed that she possesses two alleles. The number of repetitions of CAG in one of them is 10 and in the other it is 15.

- Specify the real genotype of this woman.

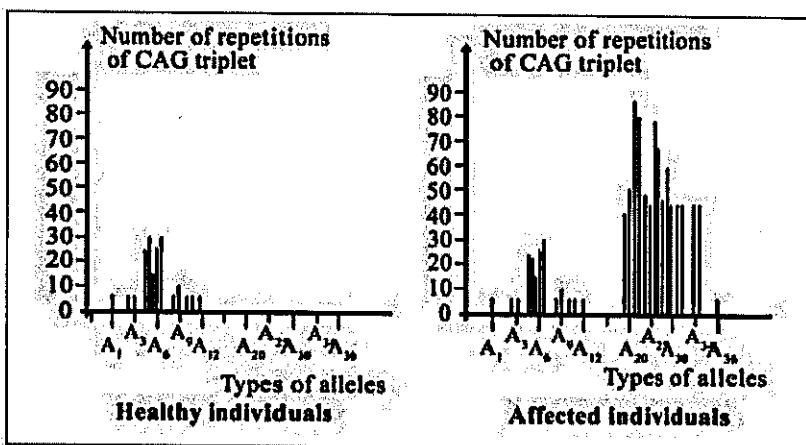
A statistical study has been performed concerning the age of appearance of this disease in function of the number of CAG triplets. The obtained results are shown in document 3.

- Analyze the obtained results.

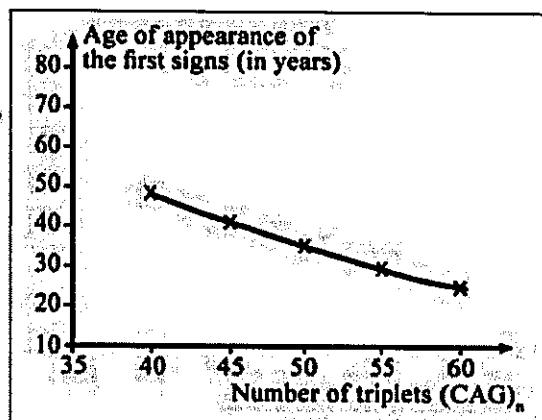
- Conclude the factor that determines the age of appearance of this disease.



Document 1



Document 2



Document 3

Exercise 31 (5 pts) Origin of mental retardation

Alain, son of Riad and Samar, is affected by a mental retardation. This couple who has no family history concerning mental retardation is expecting a second child and wishes to know whether he will be affected like his brother.

- Formulate a hypothesis explaining the appearance of this retardation in Alain.

In order to understand the possible origin of this mental retardation, the following studies are performed.

Blood analysis of Alain concerning substances involved in mental retardation shows a high amount of purines of 118 mmol/L with respect to the normal level of 79 mmol/L.

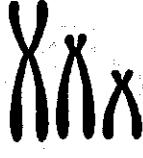
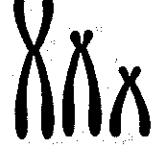
The synthesis of purines is controlled by 5 enzymes. The pathway of this biosynthesis in the body is presented in document 1.

Three cell cultures are performed.

- Culture 1:** nerve cells are cultured in a medium rich in purines. These cells degenerate.
- Culture 2:** cells of CHO mice are cultured in a medium without purines. In these mice, the gene coding for enzyme E2 which is homologous to that of humans, is inactive. These cells degenerate.
- Culture 3:** human cells are fused with cells of CHO mice and Hybridoma are obtained. These hybridoma are cultured in a medium without purines. Spontaneously, some hybridoma lose with time their human chromosomes. Those that lose their chromosome n° 21 degenerate and those that conserve the chromosome n° 21 remain in the medium.

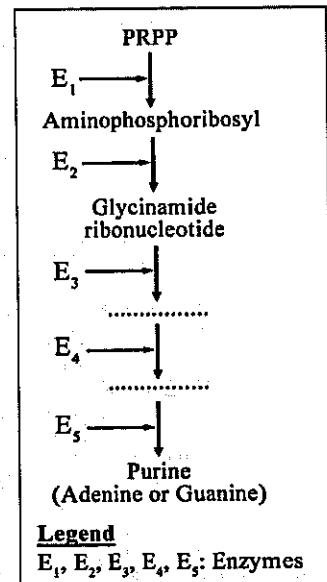
- Interpret the results obtained in cultures 1 and 2.
- Determine the location of the gene studied in this mental retardation.

The karyotype of Alain consists of 46 chromosomes. Document 2 shows the blood level of purines as well as the karyotype of Alain, those of his parents, and that of the fetus. In these karyotypes only the pairs of chromosomes 14 and 21 are schematized; the other pairs of chromosomes are normal.

Alain's Family	Mother : Samar	Father : Riad	Alain	Fetus
Karyotype				
Blood level of purines (in mmol/L)	79	79	118.5	?

Document 2

- Determine, from all what precedes, the origin of the mental retardation revealed in Alain.
- Specify the diagnosis for the fetus.
- Make the factorial analysis to determine the phenotypic proportion of this couple's children who will suffer from a mental retardation identical to that of Alain.

**Document 1**

Exercise 32 (5 pts) Dysuria

Dysuria is a disease that consists of a difficulty in urinating. It's related to excessive formation of urinary calculi ("stones" in urinary tracts). A family, which has twins suffering from dysuria, consults a doctor. He prescribed many tests whose results are represented in document 1.

Document 2 shows the reactions of metabolism of adenine related to the formation of calculi.

Measurements	Control	Twins
Quantity of adenine in urine excreted within 24h	1.5 mg	40 mg
Dihydroxyadenine (constituent of calculi)	Not detected	High quantity
Amount of active enzyme APRT	100 %	0 %

Document 1

- 1- Justify, by referring to documents 1 and 2, the dysuria detected in the twins.

In order to clarify the problem observed in the twins, a more detailed analysis concerning members of their family was performed. The pedigree of their family is shown in document 3.

- 2- Formulate, by referring to document 3, two hypotheses explaining the appearance of the disease in the twins.

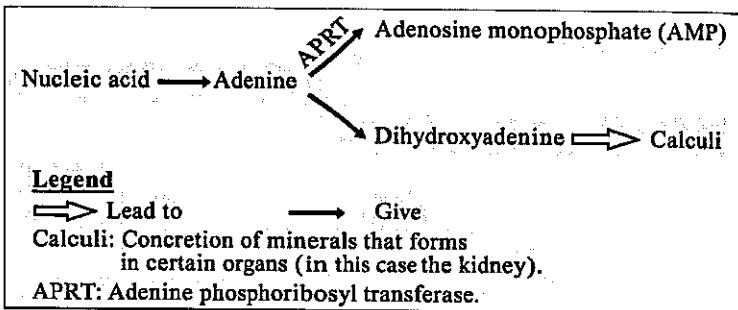
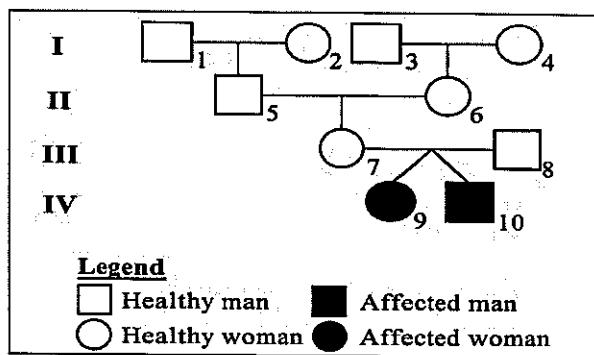
- 3- Knowing that the gene exists only in two allelic versions, specify if the allele responsible for the disease is dominant or recessive.

- 4- Show that this gene is not carried by a sex chromosome.

- 5- Indicate the possible genotype(s) of each of the individuals II5 and III8. Justify the answer.

Blood tests concerning the amount of active enzyme APRT were performed in members of this family. The results are represented in document 4.

- 6- Show, by referring to document 4, that at the molecular level, the two alleles are codominant.

**Document 2****Document 3**

Member of the family	Amount of active APRT
III7	50 %
III8	50 %
II5	50 %
II6	100 %
IV9	0 %
IV10	0 %

Document 4

Exercise 33 (5 pts) Hemochromatosis

Session 2016-2

Hemochromatosis appears after the age of 40 years and is characterized by the accumulation of iron in the body. It is a recessive disease linked to the HFE gene which is located on chromosome 6. This gene has two alleles: the normal allele which codes for a membrane protein that regulates the entry of iron into the cells, and the mutated allele which codes for an abnormal protein that favors the accumulation of iron inside the cells.

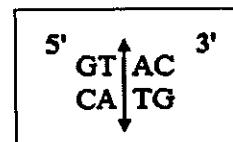
Document 1 presents the partial sequence of nucleotides of the two alleles, the normal and the mutated ones.

Document 2 presents the restriction site of a restriction enzyme Rsa1.

Number of the nucleotide	1	240	250	270	278	387
Normal HFE Allele						
Mutated HFE Allele						

Document 1

- Specify, by referring to document 1, the origin of hemochromatosis.
- Determine for each of the two alleles, the number and the length of the restriction fragments obtained after cutting by Rsa1 enzyme.



Document 2

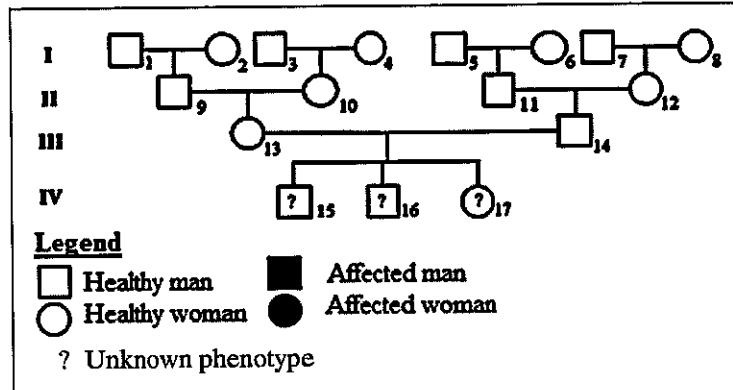
The frequency of heterozygotes in a certain population is 1/10.

A healthy couple, older than 40 years, belongs to this population. This couple would like to know if their three children, who appear healthy, have a risk to develop the disease. That's why they consult a doctor who, as a first step, establishes for this family a pedigree which is shown in document 3.

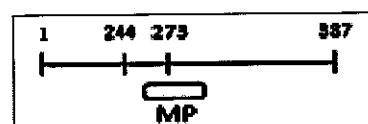
- Calculate the risk for this couple, III13 and III14, to have an affected child.

As a second step, the doctor performs DNA analysis by applying the southern blot technique using the restriction enzyme Rsa1 and a radioactive molecular probe (MP) which is complementary to a specific sequence of HFE gene. This probe can fix to the whole or to a part of the recognized sequence as shown in document 4.

Document 5 shows the results obtained by this technique for certain members of this family.



Document 3



Document 4

Size of DNA fragments (bp)	III13	III14	IV15	IV16	IV17
29	■■■	■■■		■■■	■■■
114	■■■	■■■		■■■	■■■
143	■■■	■■■	■■■	■■■	■■■

Document 5

- Explain the absence of the 244 bp fragment in the electrophoregram presented in document 5.
- Establish the diagnosis for each of the children in document 5.

Exercise 34 (5 pts) Cystic Fibrosis

Session 2017-1

Certain mutations which are at the origin of genetic diseases may protect against other diseases. In order to clarify this observation, the following studies are performed.

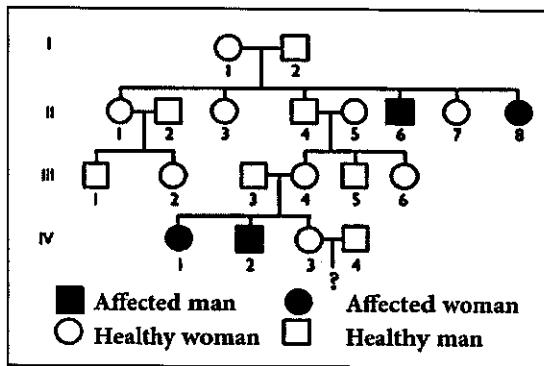
Study 1:

Cystic fibrosis is a severe disease manifested by respiratory and digestive troubles.

The origin of the disease is a mutation of the gene coding for the protein CFTR leading to the modification of amino acid 508.

The protein CFTR is present in the plasma membrane of the cells. It allows the exchange of Cl^- ions and therefore, the exchange of water. The alteration of this protein blocks the passage of the Cl^- ions and water leading to an increase in the viscosity of the mucus, particularly at the level of the lungs and the digestive tract. In a well-defined population, 1 out of 20 persons are heterozygous.

Document 1 shows the pedigree of a family whose some members are affected by cystic fibrosis.

**Document 1****1- Pick out:**

- 1-1 The origin of cystic fibrosis.
- 1-2 The consequences of the mutation at the cellular level.

2- Indicate if the allele responsible for the disease is dominant or recessive. Justify the answer.

3- Determine the chromosomal localization of the gene responsible for cystic fibrosis.

4- Specify the genotype of each of the individuals II8, III3, IV2 and IV3.

5- Determine the risk for couple IV3 and IV4 to have a child affected by cystic fibrosis.

Study 2:

Three lots of mice are genetically modified by integrating the human gene coding for CFTR protein in their genome. The mice of lot 1 are homozygous for the normal allele, the mice of lot 2 are homozygous for the mutated allele, and the mice of lot 3 are heterozygous.

Salmonella typhi bacteria have been ingested by the mice of the three lots. The number of intestinal cells infected by *Salmonella typhi* is estimated. The results are shown in document 2.

The infection by this bacterium leads to Typhoid fever which is manifested by a very serious inflammation of the digestive tract leading to death in the absence of any antibiotic treatment.

6- Justify, referring to what precedes, that some mutations which are at the origin of genetic diseases may protect against other diseases.

	Lot 1	Lot 2	Lot 3
Mice	Homozygous for the normal allele	Homozygous for the mutated allele	Heterozygous for this gene
Results	Numerous infected intestinal cells	No infected intestinal cells	Few infected intestinal cells

Document 2

Exercise 35 (5 pts) Genetics and cancer

Session 2017-2

Billions of cells of the organism, having a limited lifespan, are continuously renewed due to cellular divisions controlled by a system of regulation. The dysfunction of this system of regulation can produce a clone of cells, thus forming a tumor. This latter, is benign as long as it is controlled but it can evolve into malignant tumor: cancer.

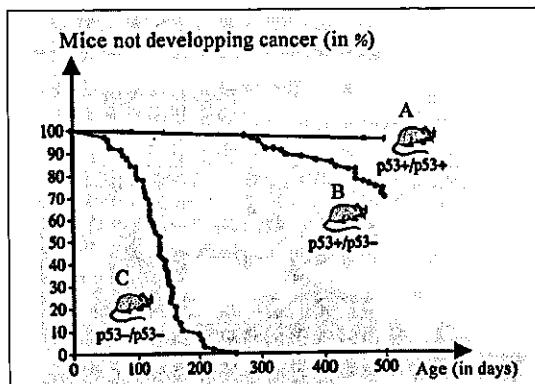
The cancerous cells lose their contact with their neighboring cells; they tend to migrate and colonize in other tissues; this is metastasis.

- 1-** Pick out from the text :
1-1- The cause of the appearance of tumor.
1-2- The definition of metastasis.

In order to better understand the origin of this type of cancer, several studies have been carried out on the gene p53 coding for the protein p53. This protein intervenes in the regulation of the cell divisions.

Study 1:

The development of this type of cancer is studied in three lots of mice as a function of their genotypes concerning the gene p53. Two alleles of this gene, p53+ (normal) and p53- (mutated) are only considered. The results of this study are shown in document 1.



Document 1

- 2- Interpret the obtained results shown in document 1.

Document 2 shows the nucleotides sequence of the non-transcribed strand of each of the two alleles involved in this study.

- Document 2**

3- Specify the type of mutation at the origin of this cancer.
4- Explain how the modification in the nucleotide sequence leads to the appearance of this type of cancer.

Study 2:

Study 2: Researchers have studied the mutations detected in three groups of individuals: individuals of group 1 are non-smokers and non-alcohol consumers, those of group 2 are smokers but non-alcohol consumers and those in group 3 are smokers and alcohol consumers. The results are shown in document 3.

	Group 1	Group 2	Group 3	
Mutations	Number	3	5	7

- 5- Show that the consumption of tobacco is a risk factor for cancer.
6- Justify the high number of individuals affected by cancer in the case of the simultaneous consumption of alcohol and tobacco.

		Group 1	Group 2	Group 3
Mutations detected at the level of gene p53	Number	3	5	7
	Type	Substitution	Substitution	Substitution, deletion and insertion
Result : Number of individuals affected by cancer		Low	Moderate	High

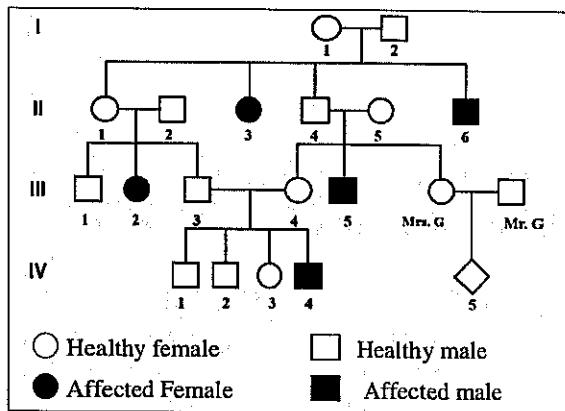
Document 3

Galactosemia is a genetic disease which results from a deficiency in the enzyme transforming galactose to glucose. Several days following the consumption of milk or milk products, the following clinical signs appear: vomiting, diarrhea, On the long term, infants would show retarded growth and later they may have mental retardation.

Mr. and Mrs. G are expecting a child. Mrs. G is worried because several members in her family are affected by this disease as shown in the pedigree presented in document 1.

- Indicate if the allele responsible for the disease is dominant or recessive. Justify the answer.
- Determine the chromosomal location of the gene responsible for this disease.
- Specify the possible genotype(s) of Mrs. G and individual IV-4.

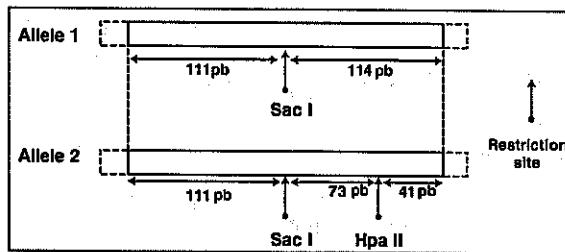
Worldwide, the probability of individuals to be heterozygous for the gene responsible for this disease is 1/100.



Document 1

- Determine the risk for the expected child, IV5, to be diseased.

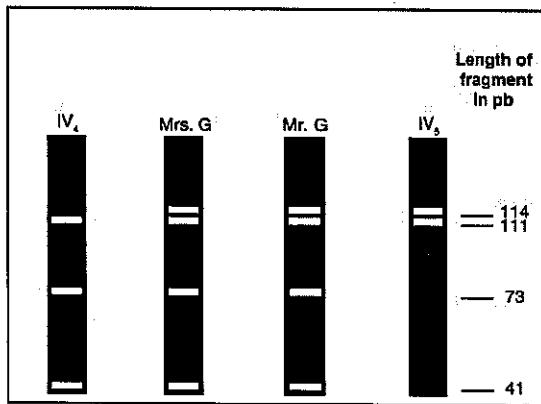
The GALT gene is responsible for galactosemia. Document 2 shows the cleavage sites of two restriction enzymes, Sac I and Hpa II, at the level of a part (from nucleotide 1367 to nucleotide 1605) of two alleles of this gene: Allele 1 and allele 2.



Document 2

Document 3 represents the results of electrophoresis obtained after the combined action of enzymes, Sac 1 and Hpa II on allele 1 and allele 2 of GALT gene of certain family members.

- Indicate, by referring to document 2, the number and size of restriction fragments obtained by the enzymatic digestion of allele 1 and allele 2.
- Determine the allele which corresponds to the mutant one.
- Verify if the fetus IV5 will be affected by galactosemia.



Document 3

Exercise 37 (4.5 pts) Patau syndrome

Session 2018-2

Patau syndrome is caused by an excess of genetic material of chromosome 13 in the cells of the body. It affects one newborn in 10000 births. The affected children show certain abnormalities: small head, malformation of the hands and eyes, as well as various perturbations in the functioning of the organs.

1. Formulate a hypothesis explaining the presence of the excess genetic material in individuals affected by Patau syndrome.

Mr. and Mrs. H, a healthy couple who already have a child affected by Patau syndrome, are expecting another child. They are worried that the fetus might be affected by this syndrome.

The doctor requests certain tests to be performed.

Test 1: The fluorescent in situ hybridization technique (FISH) is applied on fetal cells.

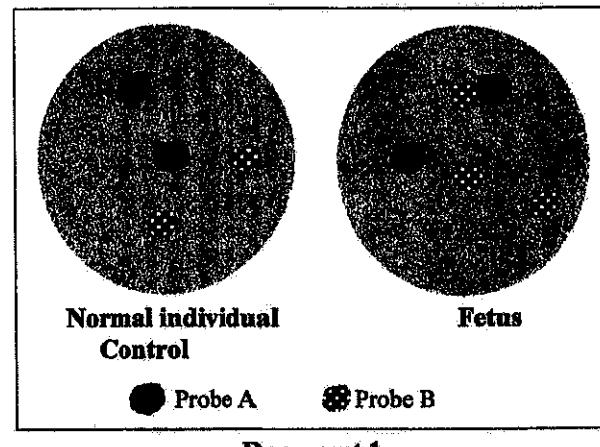
In this prenatal diagnosis technique, two fluorescent single-stranded molecular probes are used:

- Probe A complementary to a specific DNA sequence of chromosome 10.
- Probe B complementary to a specific DNA sequence of chromosome 13 that is involved in Patau syndrome.

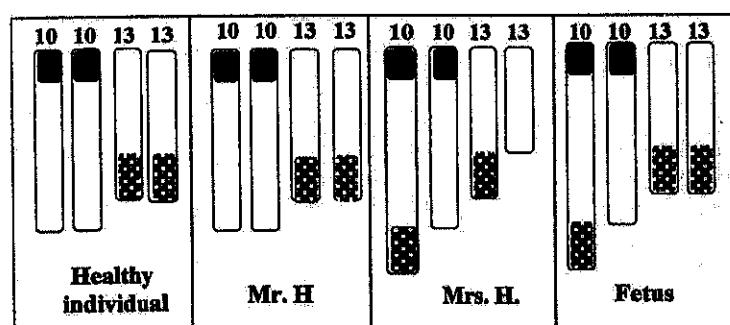
The obtained results are shown in document 1.

Based on the analysis of the results, the doctor assures for the parents that their expected child is affected by Patau syndrome.

2. Justify, by referring to document 1, the doctor's diagnosis.



Document 1



Document 2

Test 2: The doctor orders additional tests for each of the two parents and their fetus. Document 2 shows only the pairs of chromosomes 10 and 13 of the mother, the father, the fetus and those of a healthy individual. The other pairs are all normal.

3. Justify why the mother presents no phenotypic abnormalities.
4. Show that the chromosomal abnormality of the fetus is an abnormality in structure and not in number.
- 5.1. Schematize chromosomes 10 and 13 in the gametes produced by each of the two parents.
- 5.2. Indicate the two parental gametes that are at the origin of the karyotype of the fetus.

Reproduction and human genetics

Official exercises answer key

Exercise 1 (4.5 pts) Inheritance of cystic fibrosis**Session 2001-1**

1. Individuals 9 and 10, with cystic fibrosis, have normal parents; and they receive the alleles responsible for this disease at least from one of their parents in whom these alleles are masked. (0.25 pt)

Therefore, the allele is recessive (0.25 pt).

Symbol of normal allele (N); symbol of cystic fibrosis(c).

2. Location of the gene:

- If the gene is carried by non-homologous segment of chromosome Y, the females can never be sick and all sick males should have obligatory a sick father, this is not the case since the girl 10 is affected, therefore it is not carried by chromosome Y. (0.25 pt)
- If the gene is carried by non-homologous segment of chromosome X, the female 10 should be homozygote Xc/Xc having received chromosome X from her father 3 that would be of genotype Xc/Y and would be sick and this is not the case. (0.25 pt)
- If the gene is carried by the homologous segment of chromosomes X and Y, male 9 would have a genotype Xc/Yc , Yc taken from his father 3; the female 10 would have a genotype Xc/Xc , one of the chromosomes Xc should be taken from her father 3.

The male 3 had given Yc to his son and Xc to his daughter thus he would be of genotype $XcYc$ and would be affected. This is not the case. (0.25 pt)

Then the gene responsible for cystic fibrosis is therefore carried by an autosome (0.25 pt)

3. (3) and (4) have sick children, they are heterozygote: Nc . (0.25 pt)

(9) and (10) are sick of genotype cc , they are homozygote since the recessive allele is expressed only in homozygous state (0.25 pt)

(11) has a sick child, she is heterozygous: Nc . (0.25 pt)

(8) being normal, may be heterozygote Nc or homozygote NN since the dominant normal allele is expressed in homozygote and heterozygote states. (0.5 pt)

4. The individuals 9 and 10 who are sick, are homozygotes cc ; document 2 shows only one thick band of type A2; thus, the fragment A2 corresponds for the allele c and the fragment A1 for the allele N (0.75 pt)
5. The individuals 3, 4 and 11 possess a fragment of DNA of type A1 and a fragment of DNA of type A2. They possess the alleles N and c and hence they are heterozygote. (0.25 pt) There is no more doubt about female 8 being homozygote normal.

Individuals 9 and 10 possess a big patch of A2, they have two copies of allele c and hence they are homozygotes. Individual 8 has a big patch A1, hence it has two copies of N. (0.25 pt)

The result in document 2 confirms the answer of question c. here (0.25 pt)

6. The technique of genetic marking permits us to determine the real genotype of an individual. (0.25 pt)

Exercise 2 (4 pts) A cross of drosophilae

Session 2001-2

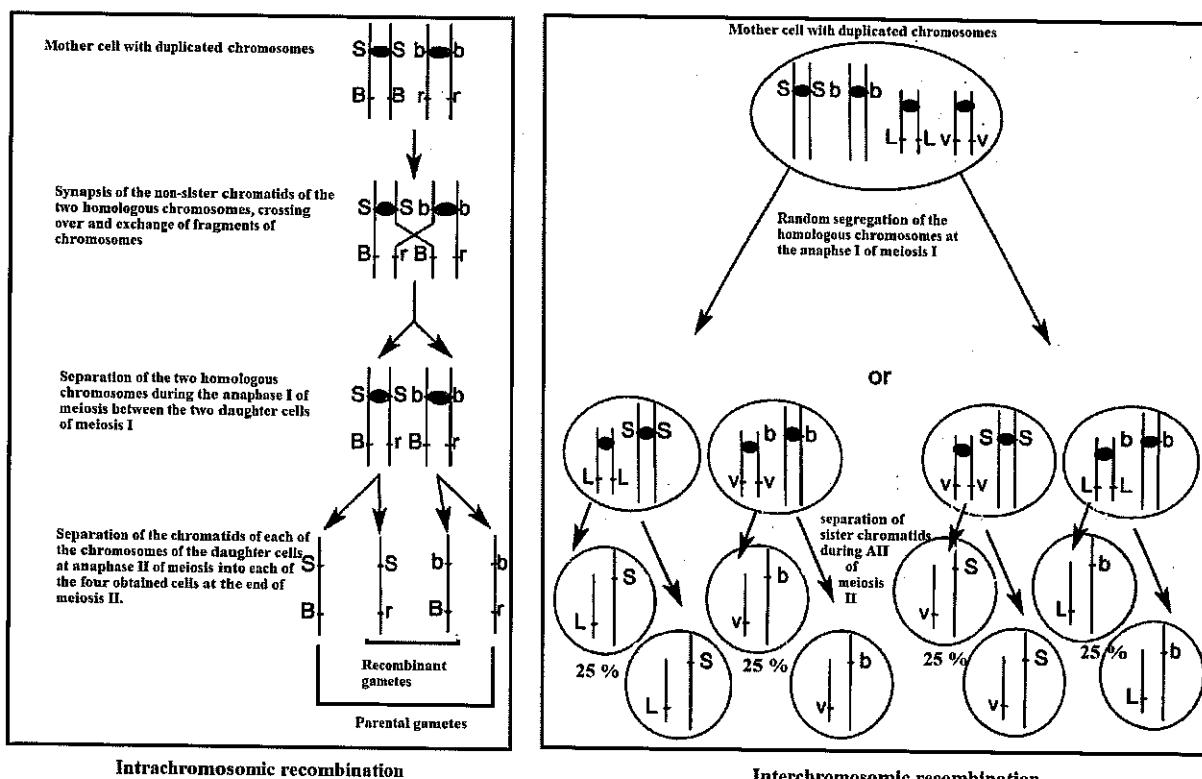
1. The homogeneous generation F1 of wild phenotypes of the first cross permit us to confirm that the alleles of wild drosophilae are dominant over the mutant traits. Therefore, the allele of striped body (S) is dominant over the allele of black body (b), and the allele of brown eye (B) dominant over the allele of red eye (r). (0.5 pt)

Similarly, homogeneous generation of F1 of the second cross and their striped bodies and long wings confirm that the alleles of the striped and long wings dominate the alleles of black bodies and vestigial wings. Therefore, the allele of long wings (L) is dominant over the allele of vestigial wings (v). (0.5 pt)

2. The formation of four phenotypes with unequal proportions by the first test-cross reflects the types of gametes produced by the female F1 and their proportions. Since the male gives only one type of gametes, the female F1 had given four types of gametes of unequal proportions, resulted from intrachromosomal recombination during the first prophase of meiosis I, this is the case of partial linkage where crossing over takes place. (3/4 pt)

The result of the 2nd test-cross between the female F1 and a double recessive male shows that the female produces 4 types of gametes in equal proportions since the male is birecessive gives only one type of gametes, then the diversity of the phenotypes is caused by the gametes given by the female F1. These types of gametes can be obtained by interchromosomal recombination done in the anaphase I of meiosis I by random segregation of homologous chromosomes, thus this is the case of independent genes. (3/4 pt)

3. The genetic assortment of the first cross is intrachromosomal while in the second cross this assortment is interchromosomal. (1 ½ pt)



Exercise 3 (4 pts) Transmission of albinism

1. Individuals 10 and 12 are albino but their parents are non-albino, each of them had received the allele responsible for the abnormality at least from one of the parents, thus the allele of the disease was masked ($\frac{1}{4}$ pt). Thus, the allele responsible for the abnormality is recessive. ($\frac{1}{4}$ pt). (Let N be the symbol of the normal allele and a be the symbol of the allele of albinism).
2. Localization of the gene:
 - If the gene was carried by the non-homologous segment of chromosome Y, the females would never be affected since they do not have chromosomes Y and all affected boys would have the albino fathers since they would have taken the chromosome Y from them, which is not the case since the girl I-1 is affected and the boy II-4 is albino and has normal father. ($\frac{1}{4}$ pt)
 - If the gene was carried by the non-homologous segment of X, girl 12 would be homozygous and her genotype would be Xa/Xa ; she would receive the chromosome X from her father 6, which would be of genotype Xa/Y and would be albino, but this is not the case. ($\frac{1}{4}$ pt)
 - If the gene is carried by the homologous segment of X and Y, man 10 would have the genotype Xa/Ya , Ya would have been taken from his father 6. Daughter 12 would have genotype Xa/Xa , Xa chromosome would have been taken from the father 6, the father's genotype would be Xa/Ya so he would be affected by albinism, but this is not the case. ($\frac{1}{4}$ pt)
 - Thus, the gene of albinism is carried by an autosome.
3. Yes, the prediction is compatible ($\frac{1}{4}$ pt) since the allele of albinism is recessive, woman 22, being albino, is homozygous and her genotype would be a/a . She will transmit the allele of albinism to all her children. The same for her husband, all the children will be of genotype a/a and will be affected. ($\frac{1}{2}$ pt)
4. The daughter whose phenotype [O, Rh-] is homozygous and her genotype is O//O Rh-//Rh- (the alleles O and Rh- are recessive, they are expressed only in homozygous state).
 The father is of group O, his genotype O//O, would transmit an allele O to his daughter.
 The mother whose group is B may have the genotype B//B or B//O. She would have transmitted an allele O to her daughter, that is why she has a daughter who has the genotype O//O, and the phenotype [O].
 The allele Rh- is recessive, the mother who has the phenotype [Rh-] is homozygous and her genotype is Rh-//Rh-, she would certainly transmit the allele Rh- to her daughter.
 The father whose phenotype is [Rh+], may be heterozygous and his genotype is Rh++//Rh- , he would have given an Rh- to his daughter. ($1 \frac{1}{2}$ pt)
 According to the given blood groups, we can say that Ghada is the daughter of the couple. However, we cannot be certain that she is their daughter so, the problem of her non-albino type is not yet solved. ($\frac{1}{4}$ pt)
5. Hypothesis: the non-albino phenotype of Ghada is due to a mutation in the gene of albinism. ($\frac{1}{4}$ pt)
 Or
 The origin of albinism in the mother and the father is not the same, the daughter will receive the normal factor for this phenotype from each one of the parents.

Exercise 4 (3 ½ pt) chromosomal abnormality

Session 2002-1

- The homologous chromosomes of pair 4 have the same length and the same degree of fluorescence. (¼ pt)
- The comparison of the length of the chromosomes and the degree of fluorescence shows that: For the father, the pair of chromosomes 5a and 5b on one hand and the pairs of chromosomes 12a and 12b on the other hand have the same length and the same fluorescence. (1/4 pt)

The mother who appears phenotypically normal (no particular clinical signs) has a karyotype which shows alteration in 2 chromosomes:

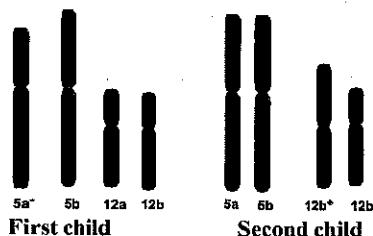
Chromosome 5a is smaller than that of the father and has a low degree of fluorescence. Its homologue 5b is of same size and fluorescence as the chromosomes 5 of the father. Chromosome 12a is longer and has a higher degree of fluorescence, its homologue 12b is of same size and fluorescence as the chromosomes 12 of the father. (½ pt)

The karyotype of the first child shows that chromosomes 12a, and 12b are of same size and fluorescence as those of the father. Chromosome 5b is also with same size of the chromosomes 5 of the father whereas chromosome 5a is smaller, and with less fluorescence. (½ pt)

The karyotype of the second child shows that 5a and 5b are similar to the chromosomes 5 of the father, 12b is of same size as the chromosomes 12 of the father while chromosome 12a is taller with an extra fluorescent fragment. (½ pt)

- We can conclude that the origin of the abnormality is the deletion of a fragment of the chromosome 5a in the first child and the presence of an extra fragment on the chromosome 12a of the second child. (½ pt)

- (½ pt)



- In the mother, a fragment of chromosome 5a is detached and is attached to chromosome 12a (balanced translocation), the genetic material of the mother is normal with no loss or gain so she is phenotypically normal (balanced translocation). (½ pt)

Exercise 5 (4 pts) A chromosomal abnormality

Session 2002-2

A.

- Hypothesis: The abnormality is due to a chromosomal mutation in the gonosomes. (1/2 pt)
- Based on the given and the zymograms we notice that a woman possesses two different G6PD enzymes corresponding for two different alleles of the gene because she has two chromosomes X each carries an allele coding for G6PD while a normal man possesses a single enzyme G6PD corresponding for one allele because he possesses a single chromosome X. Marwan is a man, he should have a single allele. Whereas the zymograms show that he has the two forms A and B of G6PD enzymes which implies that he possesses the two alleles A and B, thus, he has a sex chromosome abnormality which is XXY which explains the troubles that he presents. (1 ½ pt)
- Karyotype. (½ pt)

B.

- Echography alone does not show any abnormality, it only allows to diagnose a feminine sex. On the other hand, the Zymogram provides information about the alleles carried by the sex chromosome of the fetus. The female fetus cannot be of a genotype XA/XA (G6PD of form A) since the mother XA/XA gives her an XA whereas the father cannot give her unless XB. The problem revealed by the Zymogram is clarified by the karyotype that shows only one chromosome instead of two. The fetus thus, is a female with Turner syndrome XO. (1 ½ pt)

Exercise 6 (6 pts) Reproduction of mice

Session 2003-1

- 1.1. The liver cell, the pancreatic cell and the spermatogonium have the same DNA quantity (6.2 Pg). The sperm cell has half the DNA quantity of all the other cells (3.1 pg). (½ pt)

- 1.2. The phenomenon that ensures this reduction is meiosis. (1/2 pt)

2. Calculation of the F₂ proportions: (½ pt)

$$42/14 = 3; 127/14 = 9,07 \approx 9; 41/14 = 2,92 \approx 3; 14/14 = 1$$

Total = 3 + 9 1 = 16. Then the proportions of the F₂ generation are:

3/16 mice with smooth hair and normal eyes

9/16 mice with curly hair and normal eyes

3/16 mice with curly hair and malformed eyes

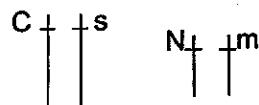
1/16 mice with smooth hair and malformed eyes.

3. The F₁ is homogeneous (uniform) since the parents are of pure race or homozygous. All of the F₁ individuals receive from the curly and malformed eyed parent gametes carrying an allele of curly hair and another of malformed eyes that are dominant over the alleles received from the parent of smooth hair and normal eyes that are recessive. Let (C) be the symbol of the allele of curly hair which is dominant; let (s) be the symbol of the allele of smooth hair that is recessive. Let (N) be the symbol of the allele of normal eye that is dominant and let (m) be the symbol of the allele of mal-formed eye that is recessive (1 pt)

The F₂ shows 4 phenotypes whose proportions are: 9/16, 3/16, 3/16, 1/16. This is obtained because the two studied genes are independent, the hybrid F₁ produces 4 gamete varieties, each, of equal probability (¼) due to the random segregation of homologous chromosomes during anaphase I of meiosis, that are at the origin of 16 possible combinations of genotypes and for the obtained proportions of phenotypes. (1 pt)

4. The assortment is interchromosomal. (½ pt)

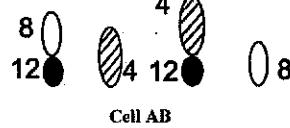
5. (½ pt)



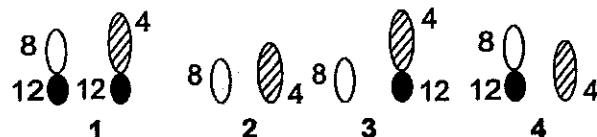
- 6.1. (½ pt)



- 6.2. (¼ pt)



7. (½ pt)



8. Gamete 1. (¼ pt)

Exercise 7 (7 pts) Lesch-Nyhan syndrome**Session 2003-2**

1. The allele responsible for this sickness is recessive, because individuals 3 who is affected has normal parents where the allele of the disease is masked at least in one of them. Let N be the normal allele, and s the allele of Lesch Nyhan syndrome. (3/4 pt)
2. The sickness had affected male individuals only; therefore, the allele of the disease is carried by sex chromosomes (gonosomes) and nor by autosomes neither by the homologous segment of X and Y.

Gene s is not carried by the chromosome Y, otherwise the father of sick boys would be affected, and this is not the case (father 1 is not affected, but he has an affected boy 3). Therefore, allele s is carried by the non-homologous segment of the chromosome X. (3/4 pt)

- 3.1. They are fraternal (non-identical) twins (1/4 pt) because one of them is sick, while the other one is healthy (1/4 pt)
- 3.2. Since the sickness is transmitted genetically, their genotypes must be different. Boy 26 is sick and his genotype is $X^S//Y$ (1/4 pt) whereas boy 27 is healthy and his genotype is $X^N//Y$. (1/4 pt)
4. Cellular multiplication had taken place in the case of healthy cells, but not in the case of sick cells (document 2). Since multiplication is linked to the presence of nucleotides, this would indicate that the absence of multiplication was due to the absence of the nucleotides essential for the replication of DNA.

The text shows that HGPRT is necessary to the transformation of hypoxanthine into purine bases and then into nucleotides, and since the sick person presents a high amount of uric acid, this would imply that this person lacks enzyme HGPRT. So, the real cause of the sickness is the deficiency of functional HGPRT (1 ½ pt)

5. The DNA extracted from a fetal cell is fragmented by means of restriction enzymes. The DNA fragments are separated by electrophoresis in agarose gel, then denatured and transferred to a nitrocellulose filter, where they are hybridized with a radioactive probe. The hybridized DNA fragments are verified by autoradiography after washing. (1 ½ pt)
- 6.1. Woman 21 is healthy and has the two alleles A1 and A2. Man 22 is healthy and has allele A1 only. So, A1 is the normal allele. Children 36 and 37 are sick boys; they have allele A2 only, which is the sickness allele. (1 pt)
- 6.2. The fetus has the two alleles; it would be a healthy carrier since the normal allele is dominant. (1/2 pt)

Exercise 8 (6 pts) Inheritance of color blindness**Session 2004-1****A-**

1. The allele responsible for the abnormality is recessive. The couple II-5 and II-6 are normal and have a boy III-11 who is color blinded. This indicates that the allele (d) is masked in the mother and transmits it to her son III-11. Consider (N) to be the normal allele and (d) the allele for color blindness.
2. The genotype of III-2: $X^N X^d$, heterozygote. She receives X^d from her father and X^N from her mother since she is normal.

The genotype of the mother: $X^N X^d$ since she is normal having the allele N and her son III-3 is color blinded having taken the allele d from her.

Since the phenotype of the male is revealed by his genotype:

The genotype of the father: $X^d Y$ since he is color blinded.

The genotype of her husband: $X^N Y$ since he is normal.

$$\begin{array}{c} \text{P} \quad X^N Y \quad \times \quad X^N X^d \\ \gamma \text{ P: } \frac{1}{2} X^N \quad \frac{1}{2} Y \quad \frac{1}{2} X^N \quad \frac{1}{2} X^d \end{array}$$

Children: $X^N X^N \frac{1}{4}$, $X^N X^d \frac{1}{4}$, $X^N Y \frac{1}{4}$, $X^d Y \frac{1}{4}$.

The probability is $\frac{1}{4}$ of the descendants or is $\frac{1}{2}$ of the boys.

B-

4. The electrophoregram of III-5 presents two distinct bands of same thickness of forms A and B, on the contrary that of III-6 presents only one band A of the same thickness of each of the two bands of forms A and B of III-5.
5. The electrophoregram 1 corresponds to the mother because she possesses one thick band that corresponds to two alleles A carried by two chromosomes X. The electrophoregram 2 corresponds to the father since he has one band B that corresponds to one allele B carried only by the one X chromosome of the father.
- 6.1. The electrophoregram of III-8 reveals the presence of two alleles A and B which indicates that he possesses chromosomes X. and since the pedigree shows that III-8 is a boy, so this indicates that he possesses two X chromosomes and one chromosome Y, thus, he has trisomy XXY.
- 6.2. The father is at the origin of the trouble in III-8 since his mother can only give him X^A , thus his father, who is supposed to give him only chromosome Y, has given him also the chromosome X^B which he carries.
- 6.3. The abnormality is done during anaphase I of meiosis.
7. Genotype of women that are color blinded like III-2: $X^{dB} X^{NA}$

Genotype of husbands: $X^{NA} Y$

8. Since the gene of color blindness and of the G6PD are localized on chromosome X, then the male descendants should be distributed into two phenotypes instead of four each having taken one of the two chromosomes X of the mother. Therefore, the four obtained phenotypes originate from 4 types of female gametes as a result of crossing over during meiosis done in the mothers.
9. Frequency of crossing over = $\frac{(4+4) \times 100}{75 + 71 + 4 + 4} = 5.2\%$

The frequency of crossing over indicates that the distance between the studied genes is 5.2 CM.

Exercise 9 (6½ pts) Transmission of hemophilia**Session 2004-2**

- The allele responsible for hemophilia is recessive because individual 14 is hemophiliac and his parents, 8 and 9 are healthy. The mutant allele is present at least in one of the parents but is not expressed. (½ pt) Symbols: h for hemophilia and N for healthy or non-hemophiliac.
- Yes, because the presence of the mutant allele this gene in two copies provokes the death of the embryo. The hemophiliac boys thus, possess one allele h which is possible only if gene is found on a segment of a sex chromosome, which has no homologous segment on the other, this segment is the non-homologous segment of chromosome X, since in case of the non-homologous segment of the chromosome Y the father will have affected boys to which he inherits chromosome Y carrying the mutant allele. In this case the girls will not be affected since when they possess two mutant alleles on the two chromosomes X they have, they will die. So only boys will be affected, then this disease is sex linked. (¾ pt)
- Individual 8 is X^N/X^h since she is healthy. Being healthy, she must have the allele N, but since her father is sick, he gives her an X^h .

Individual 13 is X^N/Y since he is healthy, his chromosome X carries allele N.Individual 14 is X^h/Y since he is sick, his chromosome X carries allele h. (1 ¼ pt)

- The genotype of female 5 is the same as that of female 8: X^N/X^h since her father is sick.

4.1. 1st case: $X^N/X^h \times X^N/Y$
 $\frac{1}{2} X^N \quad \frac{1}{2} X^h \quad \frac{1}{2} X^N \quad \frac{1}{2} Y$

Table

σ	$\frac{1}{2} X^N$	$\frac{1}{2} Y$
φ		
$\frac{1}{2} X^N$	$\frac{1}{4} X^N X^N$	$\frac{1}{4} X^N Y$
$\frac{1}{2} X^h$	$\frac{1}{4} X^N X^h$	$\frac{1}{4} X^h Y$

2nd case: $X^N/X^h \times X^h/Y$
 $\frac{1}{2} X^N \quad \frac{1}{2} X^h \quad \frac{1}{2} X^h \quad \frac{1}{2} Y$

Analysis of table

- $\frac{1}{2}$ healthy girl
 $\frac{1}{4}$ healthy boy
 $\frac{1}{4}$ hemophiliac boy (¾ pt)

Table

σ	$\frac{1}{2} X^h$	$\frac{1}{2} Y$
φ		
$\frac{1}{2} X^N$	$\frac{1}{4} X^N X^h$	$\frac{1}{4} X^N Y$
$\frac{1}{2} X^h$	$\frac{1}{4} X^h X^h$	$\frac{1}{4} X^h Y$

Analysis of table

- $\frac{1}{3}$ $X^N X^h$ healthy girl
 $\frac{1}{3}$ $X^N Y$ healthy boy
 $\frac{1}{3}$ $X^h Y$ hemophiliac boy (¾ pt)

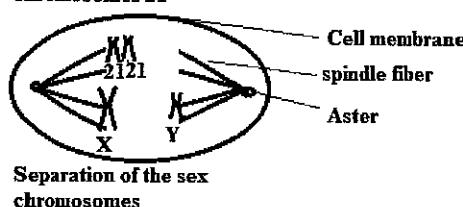
- The comparison between electrophoreses 9 and 13 of healthy men on one hand and the electrophoresis of individual 14, hemophiliac man, on the other hand, permits to say that the radioactive band of 1.3 kb, which characterizes the allele h, is responsible of hemophilia

B-6.1. The karyotype of the normal individual shows the sex chromosomes X and Y. All its autosomes are present in pairs. The karyotype of the affected individual shows the two sex chromosomes X. All its autosomes are present in pairs except chromosome 21 that exists in three copies. All the chromosomes of these karyotypes of same sizes pair by pair. (¼ pt)

- Trisomy 21. (¼ pt)

- Document 5 presents the karyotype of spermatocyte II. Each chromosome exists in one copy except chromosome 21, which exists in two copies. (½ pt)

- (1pt) **Non separation of the two chromosomes 21**



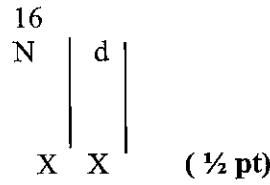
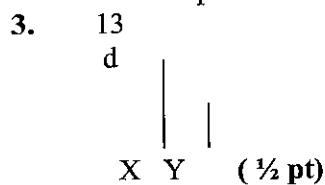
Exercise 10 (5 ½ pts) A hereditary disease

1. The allele responsible for the disease is recessive. Individual 9, sick, his parents are healthy (couple 1-2). This individual receives the allele responsible for the disease from his parents who have the allele which is masked. (½ pt)

Let "N" be the symbol of the normal allele, and "d" the symbol of the allele of the disease.

2. Localization of the gene:

- Since the disease affects mainly the males and very rarely the females, hence the most probable hypothesis is that the gene is sex linked.
- If the gene is carried on the part of Y that has no homologue on X, the transmission should be from fathers to sons. In this case all the boys should have sick fathers. Thus, male 9 who is sick has a normal father 1, which is not the case. Hence the allele responsible for the disease is carried on the X that has no homologue on Y. (1 pt)



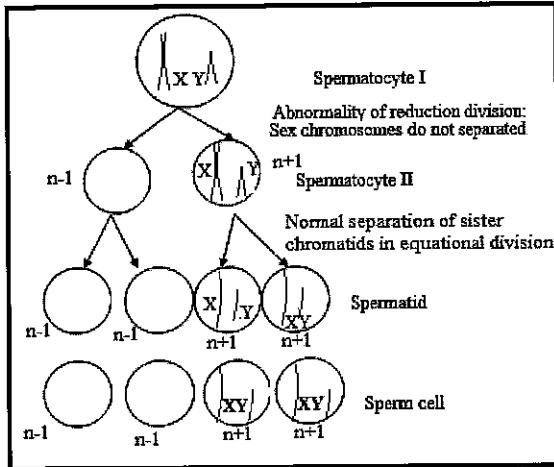
13: Since he is a sick boy, has one chromosome X, carrying the allele d. (½ pt)

16: Since she is a healthy girl and has two sick boys 23 and 24, she must be heterozygote carrying the sick allele. Thus this female must be $X^N X^d$. (½ pt)

4. Chromosomal formula = $44 + X$ (½ pt)
 5. Turner syndrome (½ pt)
 6. This karyotype shows one chromosome X that should have surely the mutant allele, and since she does not have another normal allele on a homologous, the disease is expressed in this female. (½ pt).

7.

Or



abnormality in the equational division.

Exercise 11 (5 pts) Hemoglobin**Session 2005-2**

1. The genotype of individual P is: HbA//HbA. (1/4 pt). Knowing that the gene that codes for hemoglobin is located on an autosome (chromosome no 11), each individual must have two alleles of the gene and since the blood of individual P does not possess except HbA (100%), then this individual possesses the allele HbA twice. (1/4 pt)

The genotype of individual M is: HbS//HbS. (1/4 pt). This individual possesses 100% HbS, then he possesses the allele HbS twice. (1/4 pt)

The genotype of individual R is: HbA//HbS. (1/4 pt). He possesses the two hemoglobin in equal quantities. (1/4 pt)

2. Individual M is sick because he possesses 2 alleles HbS. (1/2 pt)

3. (2 pt)

Age (months)	-6	-3	0	3	6	9
α chain	100	100	100	100	100	100
β chain	5	10	30	70	98	98

Percentage of the blood globin chains in function of age

4. The abnormal synthesis of the β chain is responsible for sickle cell anemia. Since this synthesis is not completed until the age of 6 months, then we cannot confirm before 6 months that the individual is homozygote for the gene responsible for this synthesis and possesses 2 alleles HbS. (1 pt)

Exercise 12 (2 ½ pts) Two linked genes in humans**Session 2005-2**

1. The document reveals that the majority of affected individuals in the second generation are of blood group B like their mother while all the other normal individuals, except individual 8, are of blood group O like their father (1/2 pt)

2. (1/2 pt)



Pair of chromosomes 9
of I-1

Pair of chromosomes 9
of I-2

Justifications:

I-1 is normal of blood group O, since the two traits are recessive, then he is homozygote for both. (1/4 pt)

I-2 has normal children (n/n) of blood group O (O/O), he is then heterozygote for these two characters. (1/4 pt)

3. The genotype of II-8 is ON//On. (1/4 pt). The father cannot give this child unless the alleles O and n, the mother gave him the alleles O and N.
4. We can explain the appearance of this genotype by the phenomenon of crossing over during the formation of the mother's gametes. The two non-sister chromatids exchange the alleles N and n; thus, two new recombinant gametes are produced, one carrying the two alleles B and n and the other carrying the two alleles O and N. (1/4 pt)

Exercise 13 (5 pts) Transmission of cystic fibrosis**Session 2006-1**

1. II-4 and II-5: N//N or N//d, since they are phenotypically normal, each should have a dominant allele N and another allele that can be either N or d since the normal allele is dominant expressed in homozygote and heterozygote. (1 pt)
 2. II-4 and II-5 have the normal phenotypes but each has an affected sister or brother. The risk for each of the two parents to be heterozygous is $\frac{2}{3}$ and the risk for two heterozygous parents to have an affected child is $\frac{1}{4}$ since each one has a risk of $\frac{1}{2}$ to give a gamete carrying the allele of the disease, therefore, the risk of having a sick child is $\frac{2}{3} \times \frac{2}{3} \times \frac{1}{4} = \frac{1}{9}$. (1 pt)
 3. Mutation has occurred at the site between 1.4 kb and 0.7 kb, because, the mutant allele, shows one fragment of 2.1 kb instead of two fragments 1.4 kb and 0.7 kb. (1 pt)
 4. II-4 has two of each fragment 1.4 kb, 1.2 kb, and 0.7 kb. These fragments correspond to the normal allele. Thus, he is homozygous normal of genotype N//N ($\frac{1}{2}$ pt)
- II-5 has one fragment 2.1 kb and 1.2 kb, which corresponds to the mutant allele, and fragments 1.4 kb, 1.2 kb, and 0.7 kb, which correspond to the normal allele. Thus, he is heterozygous normal of genotype Nd. ($\frac{1}{2}$ pt)
- Fetus III-2 has a fragment 2.1kb, which implies that he has received the mutant gene from his mother. He also has fragments 1.4 kb, 1.2 kb, and 0.7 kb, which correspond to the normal allele that he received from his father, Thus, he will be normal heterozygous of genotype N//d. ($\frac{1}{2}$ pt)
5. This couple has no risk of having affected children because the two parents are not heterozygous and the father II-4 who is homozygous gives only one type of gamete N that will lead for all the children to be normal ($\frac{1}{2}$ pt)

Exercise 14 (5 pts) Inheritance of sickle cell anemia**Session 2006-2**

1. N is the symbol of Normal and s is the symbol of the sickled.
- Mr., and Mrs. X: N//s ($\frac{1}{4}$ pt). They have normal phenotype but have a sick child, the parents carry the allele of the sickness which is masked. ($\frac{1}{4}$ pt)
- The daughter: ss ($\frac{1}{4}$ pt) having sickle cell anemia, a recessive sickness cannot appear unless when it is pure. ($\frac{1}{4}$ pt)
2. Both parents are heterozygotes, since half of their gametes have the sick allele s
The probability for this couple to have sick children is $\frac{1}{2} \times \frac{1}{2} = \frac{1}{4}$ ($\frac{1}{2}$ pt)
 3. The mutation is located on position 7. It is a mutation by substitution because the two alleles of the β -globin gene have the same sequence of nucleotides but differ at position 7 where adenine is replaced by thymine. (1 pt)
 4. The radioactive probe binds to the part of the alleles by complementing with the nitrogenous bases.

Probe n°1:**GAGGACACCTCTTCAGACGG****Complementary DNA:** **CTCCTGTGGAGAAGTCTGCC**

This DNA is that of HbS, thus, probe 1 permits visualizing the mutant allele while probe 2 permits visualizing the normal allele (1 pt)

5. Yes, in the two parents the two probes are visualized, which confirms that the parents are heterozygotes of a genotype Ns ($\frac{1}{4}$ pt). With respect to the daughter we visualize only probe 1 that corresponds to the mutant allele, which confirms that she has the genotype ss ($\frac{1}{4}$ pt).
6. The DNA of the fetus does not permit to visualize except probe 2, which corresponds to the allele N. Thus, the fetus has the genotype N//N and he has a normal phenotype ($\frac{1}{2}$ pt).
7. Prenatal diagnosis is more accurate because it depends on the gene itself and gives the real genotype of the concerned person. On the other hand, the pedigree permits to detect the phenotype and the possible genotype ($\frac{1}{2}$ pt)

Exercise 15 (5 ½ pts) Inheritance of Hemophilia A

Session 2007-1

1. Woman 6 : Normal woman but having a hemophiliac brother, her mother is carrier of genotype $X^N X^h$, she can be either homozygous $X^N // X^N$ or heterozygous $X^N // X^h$ since she had taken X^N from her normal father and he can take X^N or X^h from her mother. (¾ pt)
Man 7: $X^N // Y$; normal man and having only one X, thus he carries the normal allele. (½ pt)
2. The child to be born can be either a girl or a boy. If it was a girl, this pedigree permits a sure diagnosis; she will be normal because her father can give her only X^N . But if he was a boy, the diagnosis is sure if the mother was homozygous and he will be normal, but if the mother is heterozygous we cannot determine whether the boy is normal or hemophiliac because his mother can give him either X^N or X^h . (1pt)
3. If this child was a girl, the risk is null.

If this child was a boy, its phenotype depends on the allele provided by his mother. The possibility of the mother of being heterozygote is $\frac{1}{2}$. If she was heterozygous there is a possibility of $\frac{1}{2}$ for giving him X^h and since we do not know the sex of the fetus there is a chance of $\frac{1}{2}$ to take Y from his father and become affected boy. Hence the genetic risk becomes $\frac{1}{2} \times \frac{1}{2} \times \frac{1}{2} = 1/8$. (1pt)

4. No, because the karyotype reveals that it is a boy. If it was a girl the problem would have been solved since the girl will take surely X^N from her father and becomes normal. (½ pt)
5. Person 8, has only allele 5. Being hemophiliac, we can say that allele 5 is linked with allele h that codes for hemophilia.

Mother 6, who is normal, has the two alleles 3 and 5 each one is on an X chromosome. Since allele 5 is linked with allele h, then allele 3 must be linked with the normal allele N. She is thus, healthy but has the allele h, her genotype is $X^N // X^h$. (¾ pt)

The fetus has only allele 3, thus he received X^N from his mother and Y from his father, thus, he will be normal of genotype $X^N // Y$. (½ pt)

6. No, because there is a possibility of crossing over between the polymorphic zone and the gene. Non-sister chromatids of the two homologous X chromosomes will exchange segments leading to the formation of a chromosome X on which allele 5 is linked with the normal allele N and another chromosome X on which allele 3 is linked with the hemophiliac allele h. Thus, the fetus will be hemophiliac even if his autoradiogram shows the presence of allele 3. (½ pt)

Exercise 16 (5 pts) Hemophilia B

Session 2007-2

- 1- The allele of hemophilia is lethal in the homozygous state. The girl has two X chromosomes. If she is $X^h X^h$, she dies before birth. (0.5pt)
- 2- The disease is carried by the X chromosome. The sick individual 1 has only one X, which carries the allele responsible for hemophilia, which he will certainly transmit it to his daughter 3. Girl 3 is normal. She carries an X chromosome having the allele without expressing it. Hence the disease is recessive. (0.5pts)
- 3- Fetus 7 has a heterozygous mother she is able to give two gametes each of a proportion 1/2, one carrying the normal allele and the other carrying the affected one. If it is a boy, there is a risk of $\frac{1}{2}$ to have the X chromosome carrying the allele of hemophilia. If it is a girl, the risk is null because her healthy non-hemophilic father cannot give her except one normal X. (0.5pt)
- 4- The 1.3 kb-band, because document 2 reveals that individual 1, who is a sick man and has only one X, has only one band of 1.3 kb. (0.5pt)
- 5- The fetus is a boy as revealed by ultrasoung, hence he has only one X chromosome, then he must have only one band of DNA, but according to document 2 he presents two bands. Therefore, it is a boy with 2 X. (0.5pt)
- 6- Fetus 7 is a non-hemophilic boy (doc 2), but he has XXY (doc 3). Thus, he will have Klinefelter syndrome. (0.5pt)
- 7- The abnormality of meiosis had taken place during the anaphase of the reductional division by nondisjunction of chromosomes XX or XY, because upon the analysis of DNA there are two different bands that correspond to two X and not to two chromatids of the same X chromosome. In this case the father or the mother could be at the origin of this abnormality. (1pt)

Exercise 17 (5pts) A deficit in a hepatic enzyme**Session 2008-1**

- The pedigree of family B reveals that normal parents 1 and 2 have a daughter (3) and a boy (4) both affected. This means that the allele responsible for the disease was masked at least in one of the parents so it is recessive (**0.25pt**).

Let N be the symbol of the normal allele and d be the symbol of the affected allele.

The allele is not transmitted by sex chromosomes because if it was Y-linked on the non-homologous segment of Y the daughter (3) could not be affected since she does not have a chromosome Y; this is not the case (**0.25pt**).

If the allele was X-linked on the non-homologous segment of X, the daughter (3) would be of genotype X^d/X^d and would have inherited from the father (1) the X chromosome carrying the allele responsible for the disease, this father would have been affected of genotype X^d/Y ; this is not the case. (**0.25pt**).

If it was linked on the homologous segment of X and Y, then the father (1) should be affected of genotype X^d/X^d should have been affected in order to give an X^d and a Y^d respectively to his daughter (3) and his son (4) both carrying the affected allele; this is not the case. (**0.25pt**).

Therefore, the allele responsible for this disease is autosomal. (**0.25pt**)

- The risk for family A: Mother 3 is healthy with no family history of phenylketonuria, then the probability to be heterozygous is 1/50 and in this case, half of the gametes carry the mutant allele. Father 4 is healthy but has an affected brother, then the probability to be healthy and heterozygous is 2/3 and to be healthy homozygous is 1/3. However, if the father is healthy and heterozygous, half of his gametes carry the mutant allele. Then the risk will be:

$$\frac{2}{3} \times \frac{1}{2} \times \frac{1}{2} \times \frac{1}{50} = \frac{1}{300}. (\textbf{0.5pt})$$

The risk of family B: Parents are necessarily heterozygous, then the half of the gametes carry the allele of the disease and the probability of having an affected child is $\frac{1}{4}$. Then the risk is $\frac{1}{4}$.

- Mutant allele 1: Mutation at the level of region X of the gene; 1st nucleotide of codon 280 where G is replaced by A. The nature of this mutation is substitution.

Mutant allele 2: Region Y of the gene; 2nd nucleotide of codon 311 where T is replaced by C. Mutation by substitution.

Mutant allele 3: Region Z of the gene; 1st nucleotide of codon 408 where C is replaced by T. Mutation by substitution. (**1pt**)

- In family A, the parents carry a normal allele and an allele that has a mutation at the level of region X; they are heterozygous Nd. The fetus has a mutation at the level of region X on both alleles. Therefore, the fetus will be homozygous dd. (**0.5pt**)
- Test 1 shows that the individuals of family B are all normal and homozygous. However, the pedigree shows that the parents are normal and heterozygous. Moreover, this test was performed only at the level of region X, while the mutation can affect regions Y or Z. (**0.5pt**)
- The 2nd test allows detecting the presence of a morbid allele in family B at the level of region Z. If it was only referred to the 1st test, the diagnosis of the fetus would have been "healthy" which is not the case. (**0.5pt**)

Exercise 18 (5 pts) Transmission of phenylketonuria

Session 2008-2

- 1- The proportion of heterozygotes: $30/1,200 = 1/40$. (**0.25 pt**)

It is an autosomal transmission, for get a child of normal parents to be affected, parents must be heterozygous. The probability of each parent to be heterozygous is $1/40$, the probability for heterozygous parents to have a sick child is $\frac{1}{4}$. Therefore, the risk of the birth of a child affected with phenylketonuria in this community is $:1/40 \times 1/40 \times 1/4 = 1/6400$. (**0.5pt**)

- 2- The obtained risk $1/6400$ is greater than the world wide risk $1/10,000$ (**0.25pt**)

Hypothesis: A consanguineous marriage (intermarriage) in this community increases the risk of phenylketonuria. (**0.25pt**)

- 3- Couples (3-4) and (5-6) are normal and have affected children (7, 10 and 11). This implies that each of the parents carries the hidden allele of the disease. Thus, the allele is recessive. (m is the symbol of the abnormal allele). (**0.25pt**)

The disease is autosomal.

- If it was sex-linked and the gene carried by the segment of Y that has no homologue with X, then all the affected individuals should be boys who must have the same phenotype as the father. Child 10 is affected while his father 6 is not, this is not the case.

- If the gene is carried by the X segment that has no homologue on Y, daughters 7 and 11 must have a genotype Xm/Xm and each of the parents transmits one Xm , this is not the case since the father of each daughter is normal.

- If the gene is carried by the homologous segment of X and Y, affected children 10 and 11 should have the genotypes Xm/Y and Xm/Xm respectively. The father must give Xm to his daughter and Ym to his son, and in this case his genotype should be Xm/Ym and he should be affected, this is not the case. (**0.75pt**)

- 4- Fetus 15: The two parents of this fetus is normal; however, the grand parents of the fetuses are heterozygous. Thus, the probability for each of the fetuses' parents to be heterozygous is $2/3$. The risk for both parents to be heterozygous is $2/3 \times 2/3$, and the risk to have an affected child is $\frac{1}{4}$. Therefore, the risk for fetus 15 to be affected is $2/3 \times 2/3 \times 1/4 = 1/9$ (**0.5pt**)

Fetus 16: his/her mother has the same risk as his/her sister 9 to be heterozygous: $2/3$. The father is a member of the community and the risk to be heterozygous is $1/40$. If the parents of this fetus are heterozygous, then the probability to have an affected child is $\frac{1}{4}$. Therefore, the risk of fetus 16 to be affected is: $2/3 \times 1/40 \times 1/4 = 1/240$. (**0.5pt**)

- 5- Yes, fetus 15 has a risk of $1/9$ to be affected, this is greater than the risk in the case of fetus 16 ($1/240$). The parents of fetus 15 are cousins of the same family, which presents the disease. On the contrary, only the parents of fetus 16 do not belong to the same family. Therefore, the hypothesis is valid and the

intermarriage favors the appearance of the disease. (**1pt**)

- 6- The husband of daughter 7 is affected and yet with a normal PAH amount. Therefore, it can be said that the disease must have an origin other than PAH. Based on the data, PAH converts phenylalanine into tyrosine in the presence of a co-factor DHBP. This makes us say that the probable cause of the disease in the husband of daughter 7 is due to an absence or a deficiency in DHBP. (**0.75 pt**)

- 7- A normal child inherits the normal allele of the PAH gene from the father and the normal allele of the DHBP gene from his mother. This is why he has a normal phenotype. (**0.5 pt**)

Exercise 19 (5 pts) A sex linked disease**Session 2009-1**

- 1- Couple 1 and 2 who are normal have a child No.5 affected by the disease. This means that the allele causing the disease(m) is recessive masked by the dominant normal allele (N) found at least in one of the parents. (½ pt).
- 2- Father 1 is healthy and possesses the normal allele N on chromosome X. Thus, his genotype is X^N/Y (½ pt). Mother 2 is also normal and possesses the allele m on one of her chromosome X since she had a diseased boy. Thus, she is heterozygous with genotype $X^N X^m$ (½ pt).
- 3- The father produces two gametes of equal probabilities X^N ½ and Y ½. The mother produces two gametes of equal probabilities ½ X^N and ½ X^m . All the girls would inherit an X^N from their father and would be normal. Therefore, the probability to have affected girls is Zero. (¼ pt). The boys inherit Y chromosome from their father and either an X^N ½ or X^m ½ from their mother. This means that the probability to have affected boys is ½ or 50% from all boys or ¼ or 25% of all children. (¼ pt).
- 4- The autoradiography of boy 5, who is affected (from pedigree) shows one band at the level of fragment 1.3Kb. Thus, this fragment corresponds to allele of the disease (½ pt).
- 5- The autoradiography of fetus 6 reveals two fragments, one fragment at the level of 1.3 kb which corresponds to the mutant allele and another fragment at the level of 1.8 kb which corresponds to the normal allele. This means that the fetus possesses 2 X chromosomes and would be a girl with normal phenotype since X^N dominates X^m . (1 pt).
- 6- Document 3 reveals the absence of fluorescence in the non-treated myopathic mice (group B) and its presence in both normal mice (group A) and diseased mice treated with dystrophin gene (group C). This means that dystrophin is absent in the affected non-treated mice and present in the normal and the treated mice (½ pt).
 Document 4 shows that the % of survival in groups A and C is constant at 100% from week zero to week 37. This % of survival remains constant at 100% in group A until week 50, but decreases to about 75% in mice C 0.75 times less. On the other hand, the % of survival in the diseased non-treated mice which was 100%, similar to mice A and C at week 10, decreases sharply to reach Zero at week 20 (½ pt). This means that the gene treatment improves the survival of the myopathic mice (½ pt). Therefore, treatment by the introduction of the dystrophin gene has allowed for the synthesis of dystrophin in the muscle cells of the diaphragm and has improved the survival of the myopathic mice and thus this treatment is efficient. (1/2 pt).

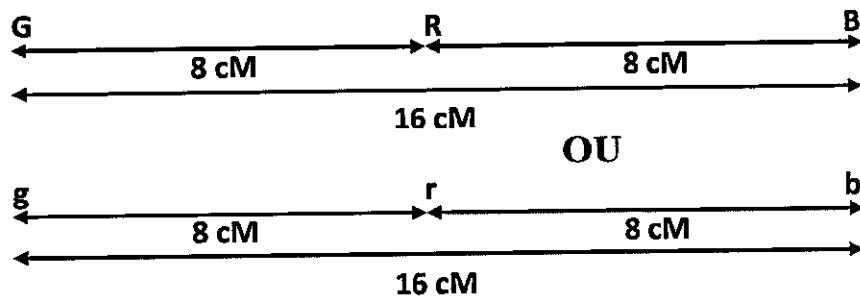
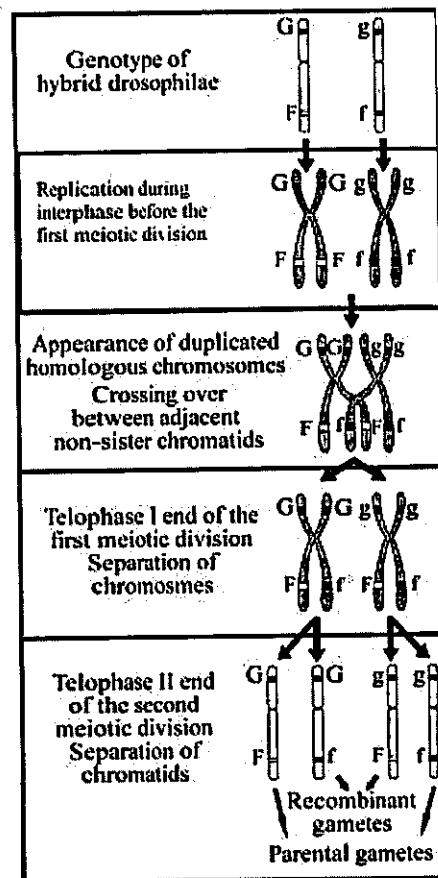
Exercise 20 (5 pts) Spermatogenesis

Session 2009-2

- 1- During the first meiotic division (reductional division), spermatocyte I produces two spermatocytes II that are subjected to the second meiotic division (equational division), each producing two spermatids. Then, the spermatids differentiate into sperm cells (spermiogenesis). (1/2 pt)
- 2- Population 1 corresponds to spermatocytes I because the quantity Q is duplicated during the S phase of interphase and becomes $2Q$ in spermatocyte I that has $2n$ chromosomes of 2 chromatids each. (½ pt)
Population 2 corresponds to spermatocytes II because after the reductional division of meiosis we obtain spermatocytes II that have n chromosomes each of 2 chromatids corresponding to the quantity Q of DNA. (½ pt)
Population 3 corresponds to spermatids or sperm cells because after the equational division of meiosis, we obtain 4 cells (spermatids) each having n chromosomes of one chromatid each corresponding to the quantity $Q/2$ of DNA. This same quantity remains constant after spermiogenesis that gives sperm cells. (½ pt.)
- 3- In the fertile man, the number of germ cells is doubled from 2 to 4 then to 8 passing from population 1 to population 3 because the number of cells is doubled after each meiotic division. Each spermatocyte I produces 2 spermatocytes II and each spermatocyte II produces 2 spermatids (1-2-4) (1/2 pt)
- 4- In the sterile man X, the number of spermatocytes I is the same as in the fertile man (2 a.u.), but the number of spermatocytes II in the sterile man is much higher than that in the fertile man ($10 \text{ a.u.} > 4 \text{ a.u.}$). On the other hand, the number of spermatids or sperm cells in the sterile man is abnormally lower than that in the fertile man ($1 \text{ AU} < 8 \text{ AU}$). Therefore, not all spermatocytes II had divided into spermatids during meiosis. Hence, the cause of sterility in man X is an abnormal meiosis, which is blocked at the stage of spermatocytes II leading to an insufficient number of sperm cells (oligospermia) (1 pt)
- 5- Document 2 reveals that in the sterile man Y, the number of cells of the three populations is the same as in the fertile man Z; this indicates that meiosis took place normally in man Y, that is why he has a normal number of spermatids and sperm cells, therefore, oligospermia did not happen. (1/2 pt) On the other hand, document 3 reveals one type of sperm cell that has a normal flagellum and a normal head, but the middle piece is larger than in the normal sperm cell. This is due to the non elimination of residual cytoplasm. (½ pt) Hence, the origin of sterility of man Y is the abnormal spermiogenesis (1/2 pt)

Exercise 21 (5 pts) Transmission of hereditary characteristics in drosophilae Session 2010-1

- 1- The allele gray color (G) is dominant over the allele black color (g) which is recessive ($\frac{1}{4}$ pt), the allele red eye (R) is dominant over the purple eye color (r) which is recessive ($\frac{1}{4}$ pt) and the allele well-formed wing (F) is dominant over the allele deformed wings (f) which is recessive ($\frac{1}{4}$ pt)
- 2- Back cross or test cross ($\frac{1}{4}$ pt)
- 3- This back cross is neither the case of independent segregation because it didn't give four equal phenotypes nor the case of absolute linkage because this cross didn't give 2 equal phenotypes. This back cross gives 4 unequal phenotypes, so it's the case of partial linkage followed by crossing over (1pt). Moreover, the genes are linked in Cis, since the parental phenotypes, which occur in higher percentages, show the dominant allele Gray linked with the dominant allele red on one chromosome, and recessive alleles linked on homologous chromosome. ($\frac{1}{4}$ pt)
- 4- Intra-chromosomal recombination or crossing over ($\frac{1}{4}$ pt)
Drawing (1 pt)
- 5- These genes are linked because the gene for body color is linked to the gene for eye color (first cross), and the gene for body color is linked to the gene for wing shape (second cross). Therefore, the gene determining eye color and shape of wings are linked. ($\frac{1}{2}$ pt).
- 6- % of recombination between the genes G and R :
 $784 \times 100 / 10326 = 7.59\% \text{ or } \approx 8\%; (\frac{1}{4} \text{ pt})$
 % of recombination between genes G and F:
 $1616 \times 100 / 10308 = 15.67\% \text{ or } \approx 16\% (\frac{1}{4} \text{ pt})$
- 7- Factorial Map ($\frac{1}{2}$ pt)



Exercise 22 (5 pts) Sexual reproduction in mammals

Session 2010-2

1. The scheme X represents an oocyte II blocked at metaphase II after having released the first polar body. Once fertilized by a sperm cell this cell releases the content of its cortical granules forming the fertilization membrane (1/4pt) and resumes meiosis II(1/4pt) releasing the second polar body(1/4pt). The male and female pronuclei are formed(1/4pt). This is how the cell passes from aspect X to aspect Y.
2. The percentage of non fertilized cells of scheme X is 100% while that of fertilized cells of scheme Y is 0% when the sperm cells are collected from the testicles; however as we proceed through the epididymis the percentage of scheme X decreases to reach 8% while the percentage of the scheme B increases to reach 92%. This shows that the testicles produce sperm cells with no fertilization capacity and that this fertilization capacity became more and more important as the sperm cells proceed through the epididymis.(3/4pt)

Therefore the epididymis is the site where the sperm cells acquire their fertilization capacity. (1/4pt)

3. 3-1- Meiosis (1/4pt) and fertilization (1/4pt).

3-2- meiosis allows the reduction of the chromosomal number to obtain haploid cells.(1/4pt) This is revealed in scheme a and/or b that show anaphase II and the separation of chromosomes into two haploid sets. (1/4pt)

Fertilization restores the diploid state of the species (1/4pt) This is revealed in scheme c that shows the male and female pronuclei before their fusion (1/4pt)

4. Scheme b corresponds to step 3 (1/4pt) since there is separation of the two haploid lots of chromosomes each with one chromatid. This corresponds to the second meiotic division where the DNA quantity is reduced from Q to Q/2. (1/4pt)

Scheme c corresponds to step 4 (1/4pt) since it shows the male and female pronuclei just before their fusion. The female pronucleus undergoes replication of its DNA resulting in an increase of its DNA quantity from Q/2 to Q (1/4pt)

Scheme d corresponds to step 6 (1/4pt) since it shows the metaphase of the first mitotic division of the zygote having $2n$ chromosomes with 2 chromatids each and that corresponds to a DNA quantity of $2Q$ (1/4pt)

Exercise 23 (5 pts) Retinitis pigmentosa

Session 2011-1

1	It is caused by progressive degeneration of rod cells which are photoreceptor cells of the retina containing the protein rhodopsin.	0.25
2	The allele of the individual with normal phenotype and that of the affected individual are identical except at their nucleotides 404 and 405: the normal allele has two GG nucleotides, while the allele responsible for retinitis has two nucleotides TT. The two amino acids sequences are identical except at their 135th amino acid: arginine (Arg) in the sequence of the normal individual and leucine (Leu) in the sequence of the affected individual. Thus the modification of the nucleotides sequence of the rhodopsin gene is translated in a modification of the protein which is at the origin of the disease.	1
3	The mutation by substitution of nucleotides 404 and 405 of the DNA was transcribed at the mRNA level by a new codon that results in a new amino acid leucine instead of arginine. This new amino acid sequence affects the three-dimensional structure of the protein rhodopsin, which becomes non-functional. Since this protein exists in the rod cells (photo receptor cells), the change in its function is manifested by impaired night vision in a person with retinitis pigmentosa.	0.75
4	The allele of the disease is dominant with respect to the normal allele since the healthy man II2 has both his parents I1 and I2 affected by retinitis pigmentosa, thus, the parents carry the normal allele which is masked by the allele of the disease. (D = allele of the disease, n = normal allele) The allele of the disease is localized on an autosome. Since: If the allele of the disease is carried by the non -homologous segment of chromosome Y then, it should be transmitted from father to son, however sick father I2 has a healthy son II2. Therefore, the allele is not carried by the non homologous segment of chromosome Y. If the allele of the disease is carried by the non homologous segment of chromosome X, then the sick father I2 should transmit this dominant allele to all his daughters who will be all sick, however daughter II3 is healthy ,thus the allele is not carried by the non homologous segment of X chromosome. If the allele is carried by the homologous segment of chromosome X and Y, then boy II2 who is normal (recessive) should have received Y chromosome carrying the normal allele from his father . Similarly, girl II3 who is normal should have inherited X chromosome carrying the normal allele from her father . Therefore, their father should have the genotype XnYn and would be normal which is not the case. Therefore, the allele is not carried by the homologous segment of chromosomes X and Y.	1.25
5	II3 has a normal phenotype; since the normal recessive allele is only expressed under homozygous state then her genotype is: n/n II4 is diseased , and has a healthy child that should have inherited one normal allele from each of the two parents, thus she carries the normal allele which is masked by the allele causing the disease. Therefore she is heterozygous D/n.	1
6	III2 and III3 have necessarily inherited the normal allele from their healthy father and are thus heterozygous D//n, each of them gives two types of gametes 1/2n and 1/2 D.	0.75

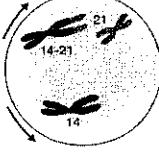
Exercise 24 (5 pts) DNA alteration

Session 2011-2

1	<p>mRNA resulting from the transcription of the allele G1: AAG AAG AGC AAC Amino acid sequence of the polypeptide coded by the allele G1: Lysine – Lysine – Serine – Asparagine</p> <p>mRNA resulting from the transcription of the allele G2: AAG AAG AGA AAC Amino acid sequence of the polypeptide coded by the allele G2: Lysine – Lysine – Arginine – Asparagine Or We can obtain it directly from the non transcribed strand of DNA by replacing T by U. thus, we obtain the same sequence for both the mRNA and the DNA non-transcribed strand.</p> <p>Amino acid sequence of the polypeptide coded by the allele G1: Lysine – Lysine – Serine – Asparagine Amino acid sequence of the polypeptide coded by the allele G2: Lysine – Lysine – Arginine – Asparagine</p>	0.75
2	<p>The genotype of individual A G2//G2 (0.25 pt) because the result of his electrophoresis shows one type of enzyme ERCC3 that is coded by allele G2. (0.25 pt)</p> <p>The genotype of individual B is G1//G1 (0.25 pt) because the result of his electrophoresis shows one type of enzyme ERCC3 that is coded by allele G1. (0.25 pt)</p> <p>The genotype of individual C is G1//G2 (0.25 pt) because the result of his electrophoresis shows the two types of enzymes. (0.25 pt)</p>	1.5
3	<p>The allele G1 is dominant (0.25 pt) and the allele G2 is recessive (0.25 pt) because individual C who is heterozygous of genotype G1//G2 is not affected by Xeroderma Pigmentosum, Allele G2 is masked and not expressed phenotypically in the presence of allele G1 which dominates allele G2(0.25 pt).</p>	0.75
4	<p>The percentage of thymine dimers in the DNA remains constant (0.10%) through the 24 hours in individual A affected by xeroderma, while it decreases from 0.10% to 0.025% through the 24 hours in the healthy individual B after their exposition to ultraviolet irradiation.</p>	0.5
5-1	<p>Individual A (doc. 3) affected with xeroderma has no functional enzyme ERCC3 which is responsible of repairing the DNA alterations . The thymine dimers formed due to the exposition to ultra violet radiation cannot be repaired in this individual and thus the percentage of dimers T-T remains stable (0.25 pt). In the healthy individual B, which possesses functional ERCC3 enzyme, the altered DNA formed by ultraviolet irradiation is gradually repaired by this enzyme thus the percentage of thymine dimers decreases (0.25pt)</p>	0.5
5-2	<p>Two factors determine the development of Xeroderma pigmentosum:</p> <ul style="list-style-type: none"> - The genetic factor(0.25pt): the disease develops only in homozygous individuals with two mutant alleles of a gene coding for the enzyme ERCC3 involved in the repair of DNA damage(0.25pt); - The environmental factor(0.25 pt): exposure to sun ultraviolet rays provokes the alteration of DNA(0.25 pt). 	1

Exercise 25 (5 pts) Analysis of partial karyotypes

Session 2012-1

1	<p>1-1- All chromosome pairs are similar in the father and the mother with the exception of pairs 14 and 21 (1/4pt)</p> <p>Concerning the pair 14: the two chromosomes of this pair are of the same size in the father while in the mother, one of them has the same size as those of the father while the second is longer than the others. (1/4pt)</p> <p>Concerning the pair 21: there are two chromosomes of the same size in the father while in the mother a single chromosome 21 is found and has same size as those of the father. (1/4pt)</p> <p>The number of chromosomes in the given partial karyotype of the father is 20, higher than that of the mother, 19 chromosomes.</p> <p>1-2- We can conclude that the karyotype of the father is normal while that of the mother of normal phenotype shows a chromosomal abnormality for pairs 21 and 14 where there is translocation of a complete chromosome 21 on chromosome 14. (1/2pt)</p>	1 1/4																																								
2	<p>The first child has trisomy 21 because he has three chromosomes 21, two free chromosomes 21 (pair 21) and one chromosome 21 translocated on a chromosome 14 (14^{21}) (1/2pt)</p> <p>The second child has normal phenotype because he has 2 chromosomes 14 and two chromosomes 21, one is free and the other is translocated on chromosome 14, so the genetic material is conserved. (1/2pt)</p>	1																																								
3	<p>Scheme of chromosomal behavior</p>  <p>Anaphase I</p>	1/2																																								
4	<p>Chromosomal analysis</p> <table style="margin-left: auto; margin-right: auto;"> <tr> <td>Phenotypes</td> <td>normal mother</td> <td>x</td> <td>normal father</td> </tr> <tr> <td>Chromosomes</td> <td>$14^{21} // 14 \ 21 /$</td> <td>x</td> <td>$14 // 14 \ 21 // 21$</td> </tr> <tr> <td>Gametes</td> <td>$14 \ 21 : \frac{1}{4}$</td> <td>$14^{21} : \frac{1}{4}$</td> <td>$14. \ 21 : 1$</td> </tr> <tr> <td>and their proportions</td> <td>$14^{21} \ 21 : \frac{1}{4}$</td> <td>$14 : \frac{1}{4}$</td> <td></td> </tr> </table> <p>Table of cross</p> <table border="1" style="width: 100%; text-align: center;"> <thead> <tr> <th>Gametes</th> <th>♀</th> <th>$14 \ 21$</th> <th>14^{21}</th> <th>$14^{21} \ 21$</th> <th>14</th> </tr> <tr> <th>♂</th> <td></td> <td>$\frac{1}{4}$</td> <td>$\frac{1}{4}$</td> <td>$\frac{1}{4}$</td> <td>$\frac{1}{4}$</td> </tr> </thead> <tbody> <tr> <td>14 21</td> <td></td> <td>$14 // 14 \ 21 // 21$</td> <td>$14^{21} // 14 \ 21 /$</td> <td>$14^{21} // 14 \ 21 /$</td> <td>$14 // 14 \ 21 /$</td> </tr> <tr> <td>1</td> <td></td> <td>$\frac{1}{4}$</td> <td>$\frac{1}{4}$</td> <td>$\frac{1}{4}$</td> <td>$\frac{1}{4}$</td> </tr> </tbody> </table> <p>Phenotypes and proportions: Children with trisomy 21: $\frac{1}{4}$ Children with monosomy 21: $\frac{1}{4}$ Children with normal phenotype: $\frac{1}{2}$ The proportion of getting normal children is $\frac{1}{2}$ and that of abnormal children is $\frac{1}{2}$.</p>	Phenotypes	normal mother	x	normal father	Chromosomes	$14^{21} // 14 \ 21 /$	x	$14 // 14 \ 21 // 21$	Gametes	$14 \ 21 : \frac{1}{4}$	$14^{21} : \frac{1}{4}$	$14. \ 21 : 1$	and their proportions	$14^{21} \ 21 : \frac{1}{4}$	$14 : \frac{1}{4}$		Gametes	♀	$14 \ 21$	14^{21}	$14^{21} \ 21$	14	♂		$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	14 21		$14 // 14 \ 21 // 21$	$14^{21} // 14 \ 21 /$	$14^{21} // 14 \ 21 /$	$14 // 14 \ 21 /$	1		$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	1
Phenotypes	normal mother	x	normal father																																							
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1		$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$	$\frac{1}{4}$																																					
5	<p>The individual with Down syndrome has three copies of chromosome 21 instead of 2. Since the gene coding for protein P is located on chromosome 21, the affected individuals have three alleles of this gene that will code for this protein and this explains the high level of protein P.</p>	1/2																																								
6	<p>In the RBCs of control individuals, the amount of enzyme SOD is equal to 1000 au, while it is higher (1500a.u.) in the RBCs of the individuals with Down syndrome. Then individuals with Down syndrome synthesize 1500/1000 or 1.5 times more enzyme SOD than healthy individuals. Since 1000 corresponds to the presence of 2 chromosomes 21 in a normal individual and 1500 corresponds to the presence of 3 chromosomes 21 in the case of trisomy 21, then one can say that the gene coding for the enzyme SOD is located on chromosome 21.</p>	3/4																																								

Exercise 26 (5 pts) Transmission of albinism

Session 2012-2

1	Allele A has 3 restriction sites of enzyme Hae III at the level of the nucleotide numbers 198, 240 and 470. Therefore, the enzyme cuts the allele into 4 fragments (1/4 pt). The length of each fragment is: a fragment of 198 base pairs (bp) (before the site 198), a fragment of 42 bp (between sites of 198 and 240), a fragment of 230 pb (between the sites of 240 and 470) and a fourth fragment which length is 30 bp (beyond the site 470) (1/2 pt).	3/4
2	The nucleotide sequences of the portions of the two alleles are identical except at the level of the nucleotide number 242 where nucleotide C in allele A is replaced by the nucleotide T in allele B(1/2 pt). It is a mutation by substitution (1/4 pt) at the level of nucleotide 242(1/4 pt).	1
3	Hae III enzyme cuts the DNA when encountering the sequence GGCC. The cutting is done between GG and CC (document 2). Document 3 shows that the restriction site at the level of the nucleotide 240 does no longer exist for allele B due to the mutation by substitution. Instead of the GGCC sequence for allele A there is a GGCT sequence for allele B. As a result, the enzymatic treatment of allele B will give: a fragment of 198 base pairs (bp) (before the site 198), a fragment of 272 bp instead of the two fragments (42 and 230 bp) a third fragment which length is 30 bp (beyond the site 470).	3/4
4	Document 4 gives the disposition of the fragments revealed by autoradiography for the four family member. Individual I ₂ has two alleles A and B (1/4 pt) because the electrophoresis results show three fragments: 272 pb that corresponds to allele B and 42 and 230 pb that correspond to allele A(1/4 pt). Individual II ₄ has two alleles B (1/4 pt) because the electrophoresis results show only the fragment of 272 pb that corresponds to allele B (1/4 pt).	1
5	Albinism allele is recessive with respect to the normal allele (1/4 pt) because individual I ₂ having the two alleles A and B is of normal phenotype. Therefore allele A alone is expressed and allele B is masked (1/2 pt). Or Because II ₃ and II ₄ children with albinism arise from normal parents I ₁ and I ₂ , then the allele of albinism is masked in the parents. Therefore allele B determining albinism is recessive with respect to the dominant allele A.	3/4
6	The fetus III ₁ possesses only the fragments of 230 and 42 pb that correspond to the allele A. So, the fetus III ₁ does not have except the allele A and he will not be albino but of normal phenotype.	3/4

Exercise 27 (5 pts) Fragile X syndrome

Session 2013-1

1	If the allele is located on the non homologous segment of Y, then every affected boy should have an affected father. However, child IV1 is affected but his father III1 is not. So, the gene is not located on the non homologous segment of Y.	0,5
2	Given that the gene is carried by the non homologous segment of chromosome X, the sick IV1 inherits obligatory the chromosome Y from his father and a chromosome X from his mother. Thus the mother with normal phenotype should carry an allele of the disease on one of its X gonosomes without expressing it. Therefore the possible origin of the disease of IV1 is a recessive allele masked form by the normal allele in the mother.	0,75
3	<p>Document two shows that the normal allele is cut by the restriction enzyme Eag1 into two fragments, and the probe fixes only on the fragment giving 2.7 to 3.3 kb. This means that the bands 2.8kb or 3.2kb correspond to a normal allele (0.75).</p> <p>The allele for the disease which has a number of repetitions of triplets that exceeds 200 can no more be cut by the restriction enzyme Eag1 and therefore, one fragment which length is more than 5.7kb is obtained. This mean the band of 5.8kb corresponds to the allele of the disease.</p> <p>Or</p> <p>The affected child IV1 possess only one allele of the gene since the gene is carried on non homologous segment of X and the male has one X chromosome. Document 3 presents only one band of length 5.8kb. Therefore, the latter band corresponds to the allele of the disease.</p> <p>The same reasoning for the normal male III1 indicating that the band which length is 2.8kb corresponds to the normal allele.</p> <p>Woman III2 who is normal possesses 2 alleles for the gene since she has two X chromosomes. Document 3 shows 2 bands of lengths 2,8 and 3,2 kb respectively that correspond according to document 2 to the normal alleles. Thus, the band 3,2kb corresponds to the normal allele.</p>	1,5
4	<p>Doc 3 shows that the fetus has only one band of 2,8kb length same as his normal father III1 thus he is normal.</p> <p>Or</p> <p>The fetus has only one band of 2,8kb length that corresponds to one of the fragment produced by the action of Eag1 on a normal allele thus he will be normal.</p>	0,75
5	<p>How come that the disease appeared in child IV1 although both of his parents carry only normal alleles?</p> <p>Or</p> <p>Both parents of IV1 have only the normal alleles, so where does the allele of the disease of child IV1 come from?</p>	0,75
6	The origin of the disease in child IV1 is due to an abnormality that occurred during meiosis in the mother. Actually the mother has two normal alleles, one of them has a large number of repetition that is subjected to an expansion of triplet CGG to more than 200 during oogenesis. This gamete carries the allele of the disease which upon fertilization has given birth to the affected child IV1.	0,75

Exercise 28 (5 pts) Origin of phenylketonuria

Session 2013-2

1	It is toxic, leads to the destruction of the nerve cells and is manifested by irreversible mental retardation.	0,5
2	Portion of the amino acids sequence of the enzyme: We establish the mRNA sequence by replacing T by U Normal mRNA: UAU ACC CCC GAA CCU GAC AUC Amino acids sequence : Tyr-Thr-Pro-Glu-Pro-Asp-Ile Diseased mRNA: UAU ACC CCC AAA CCU GAC AUC Amino acids sequence : Tyr-Thr-Pro-Lys-Pro-Asp-Ile	1
3	The mutation by substitution at the level of the first nucleotide of the 280th codon of the DNA where G is replaced by A is transcribed at the level of mRNA by a new codon which is translated into a new amino acid, lysine instead of the glutamic acid. This new amino acid sequence affects the tridimensional structure of the enzyme PAH which becomes inactive (nonfunctional). Since this enzyme is responsible for the transformation of phenylalanine into tyrosine. This transformation doesn't occur any more leading thus to the accumulation of phenylalanine which in high amount becomes toxic and causes phenylketonuria.	1
4	The allele of the disease is recessive with respect to the normal allele. Since normal parents gave birth to an affected child, thus they carry the allele of the disease that is masked in the parents. Let N be the symbol of the normal allele. Let m be the symbol of the allele coding for the disease.	0,5
5	The origin of the disease in the case of N1 is a mutation that leads to the synthesis of an inactive PAH (non-functional). Document 3 shows that affected N1 is homozygous of genotype m/m. And document 4 shows that a slight decrease in the plasma level of phenylalanine in N1 from 80 to 70 mg/dL after the injection of 20 mg/Kg of BH4. This implies that even in the presence of functional BH4, the PAH remains nonfunctional.	1
6	Document 3 shows that the affected newborn N2 is homozygous of genotype N/N. His allele codes for a normal PAH. Document 4 shows that in N2, the constant plasma level of phenylalanine of 80 mg/dL decreases after the injection of 20 mg/Kg of BH4 to 15 mg/dL value that is inferior to the reference level of 20 mg/dL. Thus BH4 acts in N2 by decreasing the plasma level of phenylalanine toward its normal value. The PAH in the newborn N2 is functional but needs the presence of BH4 to be activated. Hence, his disease in N2 can be due to the absence of BH4 or to the presence of non-functional BH4.	1

Exercise 29 (5 pts) Transmission of two hereditary traits

Session 2014

	<p>1.1 Since both parents I₁ and I₂ are healthy and they give affected individuals II₂ and II₃, meaning that the allele of the disease is masked in the parents. Therefore allele of hemophilia is recessive and the normal allele is dominant.</p> <p>W.r.t: hair color:</p> <p>1.2 Both parents I₁ and I₂ are with brown color and they give boys II₂ and II₄ and girls with red hair color, meaning that the allele of red hair is masked in the parents. Therefore allele of red color is recessive and that of brown color is dominant.</p> <p>N: Healthy / h: hemophilia</p> <p>B: Brown / r: red</p>
1)	<p>Suppose it is Y-linked, in this case every boy with red hair should take the Y' from his parent but II₂ is with red color having a father I₂ with brown color, which is not the case.</p> <p>Suppose it is X-linked, in this case the female II₇ with red color (X^rX^r) inherited one X^r from her father I₂ who should be X^rY with red color hair, which is not the case since he's with brown hair color.</p> <p>Suppose it is carried by the homologous part of X and Y, in this case the boy II₂ with red color of genotype X^rY^r inherited the Y^r chromosome from the father I₂, also the girl II₇ with red color of genotype X^rX^r inherited one X^r chromosome from her father I₂ in this case the father's genotype should be X^rY^r with red color, but it is not the case.</p> <p>Therefore allele of hair color is carried by an autosomal chromosome.</p>
2)	<p>In case the two alleles are linked, then the two boys II₂ and II₆ of genotype X^hY^r, inherited the X^h chromosome from mother I₁ whose genotype should be X^hY^N since she is healthy with brown color, then she will transmit X^h to her girl II₃ that is normal with red hair having a genotype of X^NX^h, so she will take the X^N chromosome from the father I₂ who should be X^NY^r normal with red color, which is not the case.</p>
3)	<p>For the male II₈; he is normal, so his genotype is X^NY.</p> <p>For the female II₇; she is normal, having her father normal and her mother I₁ normal carrier, so the probability for II₇ to be normal carrier is 1/2.</p> <p>In case the child is girl the risk is zero.</p> <p>In case the child is a boy the risk is the probability for II₇ to be heterozygous (x), probability to inherit x^h (x) probability for the fetus to be boy: $\frac{1}{2} \times \frac{1}{2} \times \frac{1}{2} = \frac{1}{8}$</p>
4)	<p>Female: rr X^NX^h and Male: Br X^NY Or Female: Br X^NX^h and Male: rr X^NY</p>
5)	<p>The DNA analysis of the sick individual III₂ shows a band at the level of 0.8 kb, on the other hand the BNA analysis of the normal male II₈ reveals a band at the level of 2.5 kb. Thus the band of size 0.8 kb corresponds to the allele of hemophilia A.</p>
6)	<p>Taking into consideration that the karyotype shows that the fetus is a boy, and since the DNA analysis of III₆ reveals one band at the level of 2.5 kb that corresponds to the normal allele. Therefore the fetus will not be affected by hemophilia.</p>

Exercise 30 (5 pts) Huntington Chorea

Session 2015-1

1	The allele of the disease is dominant with respect to the healthy allele, since normal children III3 and III4 have affected parents II1 and II2. Thus the normal allele is carried at least by one of the parents and masked by the allele of the disease. Let H be the symbol of the dominant allele of the disease and n the symbol of the recessive normal allele.	1/2
2	If the allele is carried on the non-homologous segment of the chromosome Y, the disease would be transmitted from father to son, but the affected son II4 has a healthy father II1. Thus the gene is not carried on the non-homologous segment of the chromosome Y. If the gene is carried by the non-homologous segment of the chromosome X, the healthy girl IV1 must be homozygous of genotype Xn//Xn; she should have inherited the normal allele from her father III1 who should be healthy of genotype Xn//Y. But her father is affected. Thus the gene is not carried by non-homologous segment of X. If the gene is carried by the homologous segments of X and Y, healthy girl III3 should have inherited Xn from her father II1; the healthy boy III4 should have inherited Yn from his father II1. Father II1 should be healthy of genotype XnYn which is not the case (II1 is affected). thus the gene is not carried by the homologous segments of X and Y. Therefore, the gene is carried by an autosome.	1/4 1/4 1/4 1/4
3	The mother II2 is affected by the disease and is heterozygous since she inherited the allele H from her mother and the allele n from her homozygous healthy father who produces only one type of gametes having the allele n. Thus she produces two types of gametes of equal probabilities: $\frac{1}{2}$ H and $\frac{1}{2}$ n. The affected father III1 is heterozygous since he already has a healthy homozygous son III4 to whom he must have transmitted the recessive allele n. Thus he produces two types of gametes equal probabilities: $\frac{1}{2}$ H and $\frac{1}{2}$ n. Since the affected allele of the disease is dominant, it is sufficient for III3 to have at least one allele of the disease in order to be affected. The genotype of III3 can be either H//H $\frac{1}{4}$ or H//n $\frac{1}{2}$. Thus the risk for III3 to be affected is 3/4 of the children. Couple II5- II6 is healthy and recessivity is a criterion of purity. These parents produce only one type of gametes carrying the normal allele n. Thus all their children will be healthy. Therefore the risk for III5 to be affected is null.	1/2
4	In healthy individuals, the number of repetitions of CAG varies between 8 and 30 for the types of alleles A1 till A12. Thus these alleles are associated to the normal phenotype. However, affected individuals present two groups of alleles: the first is identical to that present in healthy individuals with a number of repetitions of CAG between 8 and 30. The second group corresponds to alleles having a number of repetitions of CAG between 39 and 70. Thus these alleles which have a number of repetitions of CAG higher than 39 are associated to the disease. The origin of the disease is the high number of repetitions of CAG greater than 39	1
5	The real genotype of III3 is n/n or A ₆ //A ₉ . Since she has two alleles with a number of repetitions CAG that is respectively 10 and 15 which is less than 30 repetitions and thus correspond to the group of alleles of healthy individuals. These two alleles are among the ones that determine the normal phenotype.	3/4
6-1	The average age of appearance of the disease decreases from 49 years to 25 years, when the number of repetitions of CAG triplet increases from 40 to 60.	1/2
6-2	The factor determining the age of appearance of the disease is the high number of repetitions per allele(>40).	1/4

Exercise 31 (5 pts) Origin of mental retardation

Session 2015-2

1	Hypothesis: The mental retardation of Alain is due to a recessive allele masked in parents. Or The mental retardation of Alain is due to the mutation of a gene implicated in the mental development and that occurred during his conception. Or The mental retardation of Alain is due to a chromosomal aberration (that occurred during meiosis in one or in both parents).	3/4															
2	Nervous cells degenerate in the culture medium rich in purines (culture 1). Similarly cells which are unable to synthesize purines degenerate in the medium lacking purines (culture 2). This shows that the synthesis of purines in amounts far from its normal range is responsible for the degeneration of cells.	1/2															
3	Culture 2 shows that the cells of CHO mice having inactive E2 are unable to synthesize purines and degenerate. Culture 3 shows that the hybridoma having lost their chromosome 21 degenerate. And since the degeneration of nervous cells may lead to a mental retardation, this allows us to say that the gene coding for E2 is localized on the chromosome 21 and that its inactivation is responsible for the mental retardation.	1/2															
4	Culture 1 shows that cells degenerate in a medium rich in purines, and Alain possesses a high purines level of 118 mmol/L. This excessive synthesis is due to an additional allele. However the karyotype of Alain shows two free chromosomes for each of the pairs 14 and 21 with one chromosome 14 that is longer than its homologous. And since the allele coding for E2 is carried by the chromosome 21. This can be explained by the presence of an additional chromosome 21 linked to the chromosome 14. Thus the origin of the mental retardation of Alain is a linked trisomy 21 leading to a high enzymatic activity of E2.	1															
5	The fetus is normal, since as his normal father he possesses a free chromosome 21 and another chromosome 21 linked on the chromosome 14. He has conserved his genetic material, he has two alleles coding for the enzyme E2 and consequently will have a normal amount of purines of 79 mmol/l indicating a normal mental activity.	3/4															
6	Factorial analysis Phenotype : normal mother X normal father Genotype : 14/14 21//21 14//14 ²¹ 21// Gametes and proportions : 14 21 14 21 , 14 , 14 ²¹ , 14 ²¹ 21 1/4 , 1/4 , 1/4 , 1/4 Table of cross: <table border="1"> <thead> <tr> <th></th> <th>14 21 1/4</th> <th>14 1/4</th> <th>14²¹ 1/4</th> <th>14²¹ 21 1/4</th> </tr> </thead> <tbody> <tr> <td>1421 1</td> <td>14//14 21//21 1/4</td> <td>14//14 21// - 1/4</td> <td>14//14²¹ 21// - 1/4</td> <td>14//14²¹ 21// 21 1/4</td> </tr> <tr> <td></td> <td></td> <td></td> <td></td> <td>Mental retardation like that of Alain</td> </tr> </tbody> </table> <p>The Phenotypic proportion of children suffering from mental retardation like that of Alain is 1/4</p>		14 21 1/4	14 1/4	14 ²¹ 1/4	14 ²¹ 21 1/4	1421 1	14//14 21//21 1/4	14//14 21// - 1/4	14//14 ²¹ 21// - 1/4	14//14 ²¹ 21// 21 1/4					Mental retardation like that of Alain	1 1/2
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				Mental retardation like that of Alain													

Exercise 32 (5 pts) Dysuria

Session 2016-1

1	The difficulty of urinating in the twins is due to the presence of urinary calculi. The result shows that the amount of active APRT enzyme necessary for the transformation of adenine into adenosinemonophosphate is null (document 1). This blocks the transformation and leads to the accumulation of adenine and its elimination in high amounts in the urine, $40 \text{ mg} > 1.5 \text{ mg}$ (in control). The absence of APRT provokes the formation of dihydroxyadenine in high amounts (not detected) forming calculi leading to urinary difficulties in the twins.	1
2	Hypothesis : The disease is due to a recessive allele carried by the parents. The disease is due to congenital malformation. The disease is related to the mutation of the gene coding for APRT in the twins. The disease is due to a chromosomal aberration	1
3	Individuals IV9 and IV10 suffer from dysuria and descend from normal parents III7 and III8. So, the allele responsible for the disease is carried by the parents but it is masked. Therefore, the allele responsible for the disease is recessive, whose symbol is d, with respect to the normal allele whose symbol is N.	3/4
4	If the gene is located on the non-homologous part of Y, then the disease is transmitted from the father to son, but the male IV10 is diseased while his father is normal. Therefore, the gene is not located on the non-homologous part of Y. If the gene is located on the non-homologous part of X, then the diseased female IV9 having 2X chromosomes should carry 2 alleles for the disease. She should inherit one allele from each parent. So, her father III8 should be carrying the allele responsible for the disease and would be sick but this is not the case. Therefore, the gene is not located on the non-homologous part of X. If the gene is located on the homologous part of X and Y, then parent III8 must be sick and his genotype must be X^dY^d in order to give his daughter IV9 X^d and his son IV10 Y^d . But he is not sick. Therefore, the gene is not located on the homologous part of X and Y. Therefore, this gene is not carried by a sex chromosomes.	3/4
5	The possible genotypes of I1 is N/N or N/d since the normal allele is dominant and can be expressed in the homozygous or heterozygous state. The genotypes of III8 is N/d since the diseased twins IV9 and IV10 who exhibit the recessive phenotype have genotype dd. The recessive allele is only expressed in the homozygous state. They have surely inherited one allele for the disease d from their father III8 and since he is normal he has the allele N.	1
6	Since the gene is carried by an autosome and it has only two allelic versions then the presence of three different amounts of APRT, 100%, 50% and 0% shows the presence of three molecular phenotypes indicating codominance.	1/2

Exercise 33 (5 pts) Hemochromatosis**Session 2016-2**

1	The origin of hemochromatosis is a mutation by substitution at the level of the HFE gene. Since the nucleotides of the normal allele HFE, presented in document 1, are identical to those of the mutated allele except for the nucleotide 274 where G in the normal allele is replaced by A in the mutated one. This mutation leads to the synthesis of an abnormal protein.	3/4
2	<p>When treated by the restriction enzyme Rsa1, the normal allele which presents only one recognition site GTAC at the level of nucleotides 243 – 246 is cut once between T in position 244 and A in position 245, thus we obtain 2 fragments the first is of 244 bp length and the second of $387 - 244 = 143$ pb length (3/4 pt)</p> <p>When treated by the restriction enzyme Rsa1, the normal allele which presents 2 recognition sites GTAC at the level of nucleotides 243-246 and at the level of nucleotides 272-275 is cut twice:</p> <ul style="list-style-type: none"> - between T in position 244 and A in position 245, giving the first fragment of 244 pb length, - between T in position 273 and A in position 274 which gives the second fragment $273 - 244 = 29$ bp length and the third fragment of $387 - 273 = 114$ bp length. <p>Therefore three fragments are obtained (3/4 pt)</p>	11/2
3	<p>Since each of the two parents has no family history for hemochromatosis, the frequency for each of them to be heterozygous is 1/10 (frequency in the considered population). Thus the risk for both of them to be heterozygotes is $1/10 \times 1/10 = 1/100$</p> <p>Since the allele responsible for the disease is recessive, the risk for a heterozygous couple to have an affected child is 1/4.</p> <p>Hence the risk for this couple to have an affected child is $1/100 \times 1/4 = 1/400$</p>	1/2
4	The electrophoregram shows only the fragments to which the radioactive molecular probe is hybridized. Since the recognized sequence to which the MP gets fixed is localized only at the level of nucleotide 273, thus the 244 bp fragment is not hybridized and doesn't appear in the electrophoregram.	3/4
5	<p>The electrophoregram shows 3 bands: band 143 bp characterizing the normal allele and bands 29bp and 114 bp characterizing the mutated one.</p> <p>The electrophoregram of child IV15 shows one thick band at the level of 143 bp corresponding to the normal allele. Hence he is healthy homozygote. (1/2 pt)</p> <p>The electrophoregram of child IV16 shows the 3 bands. Thus he is heterozygote and since the allele of the disease is recessive, he is healthy. (1/2pt)</p> <p>The electrophoregram of child IV17 shows two thick bands, 29 bp and 114 bp corresponding to the mutated allele. Thus she is recessive homozygote. She will be sick after the age of 40 years. Hence, among the three children, only the girl 17 will be sick after the age of 40 years. (1/2pt)</p>	11/2

Exercise 34 (5 pts) Cystic Fibrosis

Session 2017-1

1.1	The origin of the disease is a mutation of gene coding for the protein CFTR leading to the modification of amino acid 508.	1/4
1.2	The alteration of this protein blocks the passage of the Cl^- ions and water leading to an increase in the viscosity of the mucus, particularly at the level of the lungs and the digestive tract.	1/4
2	The allele responsible for the disease is recessive with respect to the normal allele. Since individuals II-6 and II-8 who are affected, have healthy parent's I-1 and I-2 which means that this allele was masked by the parents. N be the symbol of the "normal" allele and m the symbol of the allele responsible for cystic fibrosis.	1/2
3	If the gene is located of the non-homologous segment of chromosome Y, the transmission is transmitted from father to son (any affected boy would necessarily have an affected father) but this is not the case since the son II-6 who is affected by cystic fibrosis has a healthy father. Then the gene is not located of the non-homologous segment of chromosome Y. (1/4 pt) If the gene is located of the non-homologous segment of chromosome X, the daughter II-8 being of the recessive phenotype should have $Xm // Xm$ as genotype and should inherit an Xm chromosome from her father I-2 whose genotype should be then $Xm // Y$ which means he should be affected but this is not the case because he is healthy. Then the gene is not located of the non-homologous segment of chromosome X. If the gene is located on the homologous segment of X and Y, the boy II-6 would be of genotype $Xm // Ym$, Ym is inherited from his father I-1, and the daughter II-8 would have as genotype $Xm // Xm$, one of these two chromosomes is inherited from her father. Then the father would have as genotype $Xm // Ym$ and would be suffering from cystic fibrosis but this is not the case. (1/4 pt) Thus the gene in question is not carried by the sex chromosomes, it can only be autosomal.	1
4	II-8, having a recessive phenotype which manifests only in the homozygous state; Their genotype is m/m . Therefore she should be homozygous. III-3 he is normal in phenotype and the normal allele is dominant but gave birth for disease children IV-1 and IV-2 where one allele is inherited from the father III-3. Therefore, he is heterozygous in genotype. IV-2 is Nm since he is diseased of recessive phenotype to appear phenotypically it should be in the homozygous state. The genotype of IV-3 may be heterozygous $N // m$ or homozygous $N // N$. Since she has dominant phenotype which manifests when the allele is present in a single copy or two copies.	1
5	The risk for the child to be affected by an autosomal recessive disease: The risk of the father to be normal heterozygous \times the risk of the mother to be normal heterozygous \times the risk of the infant to carry the mutant alleles in two copies Or the female IV-3 is born from a heterozygous parent Nm that have sick children of genotype mm . The risk of this female IV-3 to carry the mutant allele is $2/3$. Father IV-4 of no family history, the risk to carry the mutant allele in the studied population is $1/20$. In the case of heterozygous parents, the risk of the infant to inherit the allele m from both parents is $1/4$.	1/2

Exercise 35 (5 pts) Genetics and cancer**Session 2017-2**

1-1	The dysfunction of this system of regulation can produce a clone of cells, thus forming a tumor.	1/4
1-2	The cancerous cells lose their contact with their neighboring cells; they tend to migrate and colonize in other tissues: this is metastasis.	1/4
2	In lot A of genotype p53+//p53+, the percentage of mice that don't develop cancer stays constant at 100% during 500 days. However, the percentage decreases from 100% to 70% in mice of lot B of genotype p53+//p53- between day 280 and day 500. This shows that the allele p53- favors the development of cancer when it's present in one copy. However, in lot C having the genotype p53-//p53-, the percentage of mice not developing cancer begins to diminish from 100% on day 50 to null on day 250 days which is less than day 280 corresponding to the appearance of cancer in lot B. Therefore, the allele p53- accelerates the appearance of cancer and its action is amplified when it exists in 2 copies.	1
3	The origin of cancer is a mutation by substitution of gene p53. Since the nucleotides of alleles are identical except at the level of the 3rd nucleotide of codon 249 where the G nucleotide in the allele p53+ is substituted by the nucleotide T in the allele p53-.	1
4	The mutation by substitution at the level of codon 249 leads to an amino acid different than that translated by the normal allele. This modification of amino acid has as a consequence the synthesis of an abnormal and non-functional protein. As a consequence, the regulatory system of cellular divisions becomes nonfunctional and the cells divide in an uncontrollable manner, producing thus a clone of cells forming tumors.	1
5	Document 3 shows that in the smokers, the number of mutation by substitution is 5 and the number of individuals affected by cancer is moderate; whereas, the non-smokers present 3 ($3 < 5$) mutations by substitution that limits the development of cancer in them. This shows that tobacco is a risk factor for cancer.	3/4
6	In the smokers and consumers of alcohol, the number of mutation is 7, a value greater than 5, which is the number of mutation in the smokers (and also greater than 3, in non-smokers and non-alcohol consumers). In addition to the increase in number, the mutations exist in different types: deletions and insertions in addition to substitutions, the only type mutations revealed in the two groups 1 and 2. Since the mutations at the level of gene p53 is at the origin of tumors, the increase in the number of mutations as well as the occurrence of new types of mutations, favor the appearance of cancer and therefore justifies the high number in individuals affected by cancer which are smokers and alcohol consumers.	3/4

Exercise 36 (5 pts) Diagnostic of galactosemia**Session 2018-1**

1. The allele of the disease is recessive. Parents I1 and I2 are normal but have two affected children II-3 and II-6. This shows that the allele of the disease is carried at least by one of the parents who phenotypically doesn't express the disease, so the allele is masked, thus it is recessive (g) with respect to the normal allele (N). N normal dominant allele m: mutated recessive allele.
2. If the gene is carried by the non-homologous part of Y First argument: there shouldn't be affected girls since girls do not have the gonosome Y. This is not the case since the girl II-3 is affected. Second argument: The father of each affected boy should be necessarily affected since the boy inherit gonosome Y from his father. This is not the case since the father I-2 of boy II-6 is healthy. Thus, the gene is not carried by chromosome Y.

If the gene is carried by the non-homologous part of X, the diseased girl II-3 of genotype $X^m//X^m$ should inherit X^m from her father whose genotype should be $X^m//Y$ and should be diseased. This is not the case.

If the gene of the disease is localized on the homologous part of X and Y, the boy II6 will have the genotype $X^m//Y^m$ and his sister II-3 will have the genotype $X^m//X^m$. The boy will inherit Y^m from his father and the girl will inherit X^m from her father. So, the genotype of the father should be $X^m//Y^m$ and he will be phenotypically affected. The gene is not localized on a gonosome. Thus, the gene responsible for galactosemia is autosomal.

3. The genotype of Mrs.G is N/N or N/m since the dominant homozygous and heterozygous states. The genotype of IV4 is m/m because the recessive allele is expressed only in homozygous state.
4. The risk for the child IV-5 to be diseased is:

The risk of the mother Mrs.G to be heterozygous for the gene is 2/3 since she has heterozygous parents knowing that her brother III-5 is affected.

The risk of the father Mr. G to be heterozygous for the gene 1/100 since he has no family history for the disease, his probability to be carrier is equal to the proportion of heterozygous in the population.

The risk of the child to have both recessive alleles of the disease if the parents are heterozygous is $\frac{1}{4}$.

Thus, the risk for the fetus to be diseased (genotype m/m) is: $2/3 \times 1/100 \times 1/4 = 1/600$.

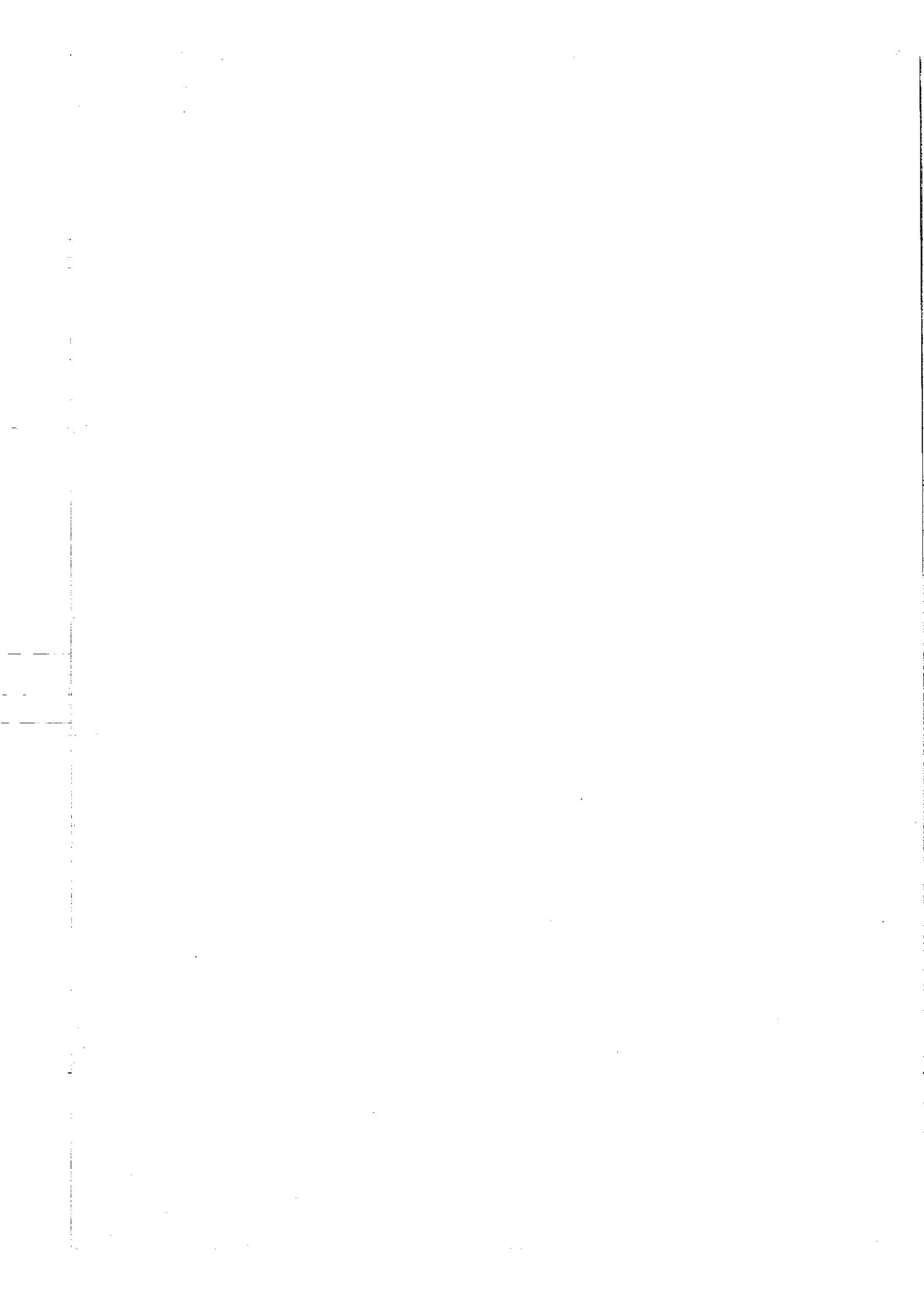
5. The fragments of allele 1: 2 fragments of sizes 111 and 114 bp.
- The fragments of allele 2: 3 fragments of sizes 111, 73 and 41 bp.
6. Document 2 shows the restriction sites of the two alleles of the gene (one of them is the mutant allele responsible for galactosemia), these restriction sites result from the combined actions of two enzymes: only the allele 2 is cut by HpaII: after the action of these two enzymes, we obtain two restriction fragments from the allele 1 (from 111 bp to 114 bp). From the allele 2 we obtain 3 restriction fragments (111bp, 73 bp and 41 bp).
- Document 3 shows that infant IV4 is diseased and have an obligatory genotype m/m, since he has in his electropherogram 3 DNA fragments of allele m, (111 bp; 73 bp, 41 bp); corresponding to the same allele 2, so the allele 2 is the mutated allele.
7. The electropherogram of the DNA of the fetus shows two fragments of sizes 114 bp and 111 bp, resulting, in a unique action of enzyme SacI corresponding to the normal allele. So, the genotype of this fetus is N/N and it not be galactosemic.

Exercise 37 (4.5 pts) Patau syndrome

Session 2018-2

1	Hypothesis: - The excess of genetic material might be due trisomy 13 (linked or free). - The excess of genetic material might be due to a translocation of part of chromosome 13. - The excess of genetic material might be due to a certain mutation (duplication of a fragment of a chromosome).	0.5						
2	Document 1 shows that the healthy individual and the fetus possess two fluorescent probes A which correspond to the two chromosomes 10. The fetus presents three fluorescent probes B which correspond to the two fluorescent probes B. Additionally, one of the three probe B is attached to probe A. Opposite to the healthy individual who presents two probes B. Since any excess of genetic material of chromosome 13 in cells causes Patau syndrome, therefore, the doctor's affirmation that the fetus will be affected by Patau syndrome is justified.	0.75						
3	Since one of the pair of chromosome 13 of the mother shows a lost part and one of chromosomes 10 has an excess of the same part, then the mother presents neither gain nor loss in the genetic material and her DNA mass is conserved. As a result, the mother has the normal phenotype.	0.75						
4	Document 2 shows that the fetus possesses a pair of chromosomes 10 and a pair of chromosomes 13; the exact total number of chromosomes is normal. Therefore, the fetus' abnormality is not in number. However, one chromosome 10 of the fetus is longer than the pair of chromosomes 10 of the healthy individual but the other copy of chromosome 10 and both copies of chromosome 13 have equal length as those of the healthy individual. It is therefore the structure of the chromosomes that is abnormal.	1						
5.1	<p>Scheme showing the types of the parental gametes</p> <table style="width: 100%; text-align: center;"> <thead> <tr> <th style="width: 40%;">Types of gametes of the mother</th> <th style="width: 20%;">gamete of the father</th> <th style="width: 40%;"></th> </tr> </thead> <tbody> <tr> <td> Gametes: mother 10+13 10+13- 10 13 </td> <td> father 10 13 </td> <td></td> </tr> </tbody> </table>	Types of gametes of the mother	gamete of the father		Gametes: mother 10+13 10+13- 10 13	 father 10 13		1
Types of gametes of the mother	gamete of the father							
Gametes: mother 10+13 10+13- 10 13	 father 10 13							
5.2	<p>The gametes at the origin of the fetus karyotype are:</p> <table style="width: 100%; text-align: center;"> <tr> <td style="width: 33%;">mother 10+13</td> <td style="width: 33%;">father 10+13</td> <td style="width: 33%;"></td> </tr> <tr> <td></td> <td></td> <td></td> </tr> </table>	mother 10+13	father 10+13					0.5
mother 10+13	father 10+13							

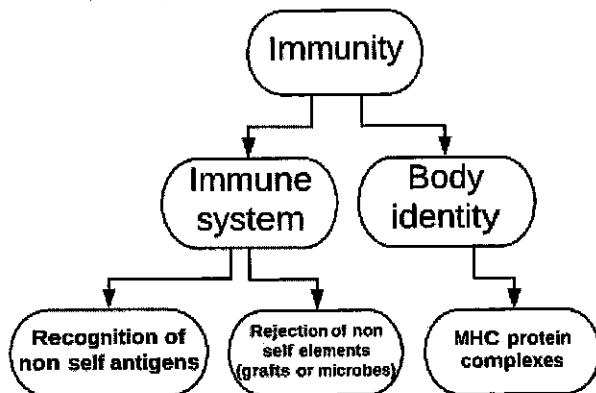
Ch. 4 Immunity



Immunity Course abstract

Our environment is full of pathogens, but it is very rare that we fall ill, this is due to the presence of natural barriers (skin, saliva, tears, acid of the stomach...) that prevents partially the penetration of these pathogens; and if one of these barriers is passed, there is intervention of a well-organized immune system with cells and molecules able to distinguish between self and non self, and to neutralize and/or eliminate non-self-antigens recognized as foreign.

The activity of the immune system necessitates the presence of an identity of the body (self-markers) that permits to distinguish self from non-self. This identity is determined by protein complexes called MHC.



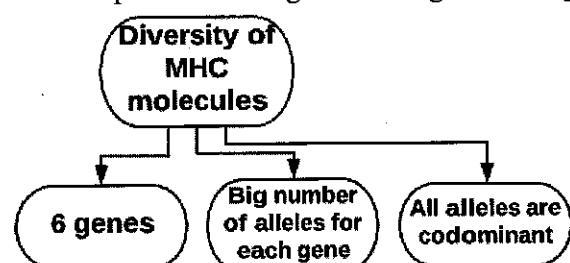
1. The identity of the body is determined by a complex of protein molecules called MHC molecules (called HLA in humans) and by the antigens of blood groups called agglutinogens.

1.1. MHC molecules are of two classes:

- Class I (molecules A, B and C) found on the cell membranes of all nucleated cells.
- Class II (D_P , D_Q and D_R) found only on the cell membranes of some immune cells.

The diversity obtained in the MHC molecules is due to the presence of 6 genes having each a big number of alleles in the population that are all codominant.

The genes of MHC are linked on chromosome 6. They are transmitted from the parents to the children generally "en bloc", that's why the probability for two brothers or sisters to have same MHC is 1/4.



1.2. The antigens of blood types or agglutinogens are located on some body cells like red blood cells. They are of two types: A and B and they are determined by a gene located on the chromosome 9. Agglutinins are antibody molecules that cause the agglutination of red blood cells, they are found in the plasma of the blood of the individuals of some blood types.

The agglutinogens and the agglutinins of the different blood types are given in the table below:

Blood type	A	B	AB	O
Agglutinogens	A	B	A and B	None
Agglutinins	Anti-B	Anti-A	None	Anti-A and Anti-B

In addition, ABO blood types, a person might have rhesus antigens and be rhesus positive; in their absence the person is considered as rhesus negative.

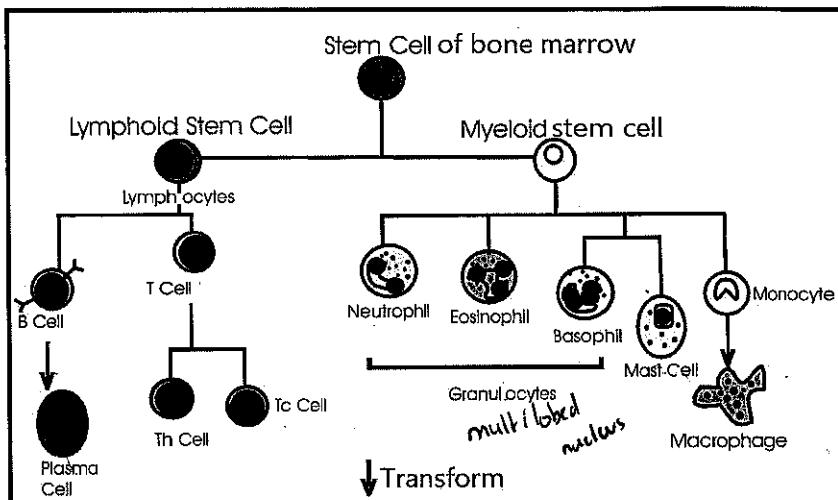
The reject of the blood transfusion is caused by the agglutination of the red blood cells of the donor carrying a given agglutinogen with the corresponding agglutinins of the recipient.

2. The immune system.

2.1. Cells of the immune system (white blood cells or leukocytes)

Remarks:

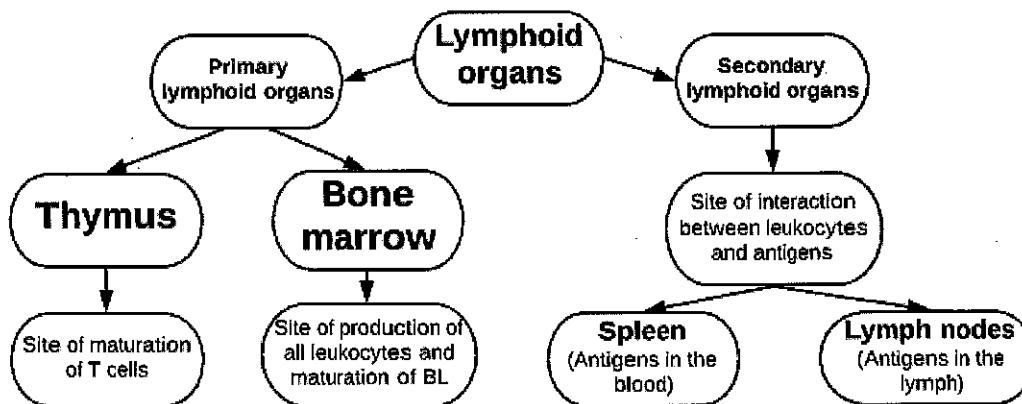
- Y B lymphocyte has membrane antibody receptors.
- Y Plasma cell has developed cytoplasmic organelles (RER and Golgi body) that secrete antibodies.
- Y TH or T4 has CD4 receptors.
- Y TC or T8 has CD8 receptors.



Note the shape of the nucleus of each type of ~~red~~ blood cells.
wrote

2.2. The organs of the immune system (lymphoid organs)

The lymphocytes circulate continuously between the blood and the lymph through the lymph nodes and the spleen in order to maintain a continuous surveillance of the entire body against any non-self-element.



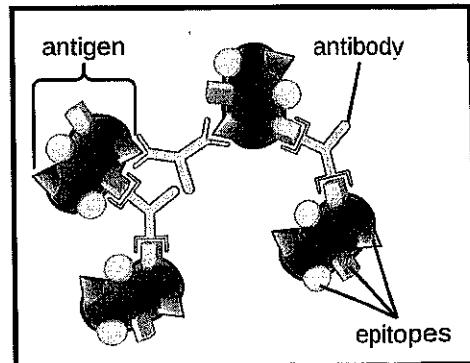
2.3. Characteristics of specific immune cells

2.3.1. B Lymphocytes

B cell recognizes cellular or soluble antigens due to membrane antibody receptors. It transforms into plasma cell and secretes antibodies, it is characterized by developed RER and Golgi body.

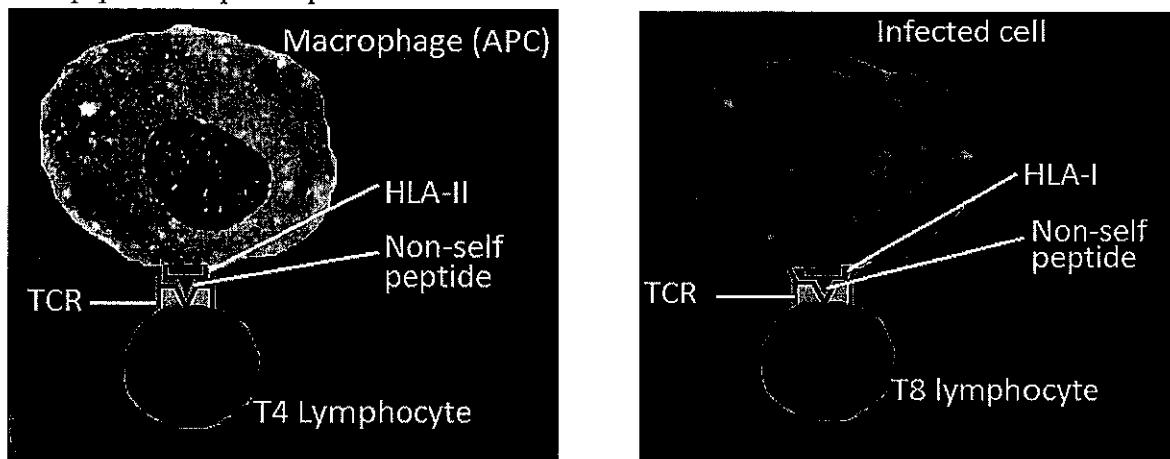
The antibody is made up of four chains (2 heavy and 2 light chains) it has a constant region and a variable region that can bind in a specific manner on a part of the antigen called epitope.

Agglutination is the binding of many antibodies on many antigens, it leads to the formation of the immune complex.



2.3.2. T lymphocytes

T lymphocytes have double specific membrane receptors (TCR) for self MHC and non-self-peptides associated with it. T4 is specific to MHC-II non-self-peptide complex expressed on the surface of the macrophage having transformed into antigen presenting cell (APC), while T8 is specific to MHC-I non-self-peptide complex expressed on the surface of the infected cell.

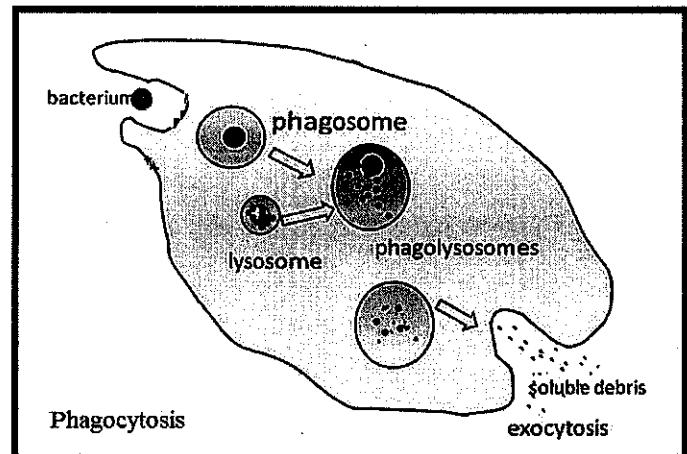


3. The immune response

3.1. Non-specific immune response

It is characterized by the inflammation reaction (redness, heat, swelling and pain) observed after infection. Infected cells and microbes as well as macrophages release cytokines that attract white blood cells to the inflamed tissue (chemotaxis) after enlarging the blood vessels to make more blood flow (redness and heat) and increase the vascular permeability causing plasma leakage to the tissue (swelling that causes pain).

Monocytes are attracted by the cytokines, they cross the blood capillaries walls (diapedesis) to reach the inflamed tissue where they transform into macrophages and make phagocytosis (approach, adhesion, engulf and digestion by lysosomes in a phagosome).



3.2. Specific immune response

3.2.1. Types of specific immune responses

There are two types of specific immune responses:

- ❖ Specific humoral immune response made by the specific antibodies secreted by plasma cells against either extracellular intruders, intracellular intruders or toxins. The antibodies directed against intracellular intruders are able to agglutinate them before their penetration in the target cells.
- ❖ Specific cell mediated immune response made by the Tc lymphocytes that kill the infected cells by a virus or an intracellular bacterium.

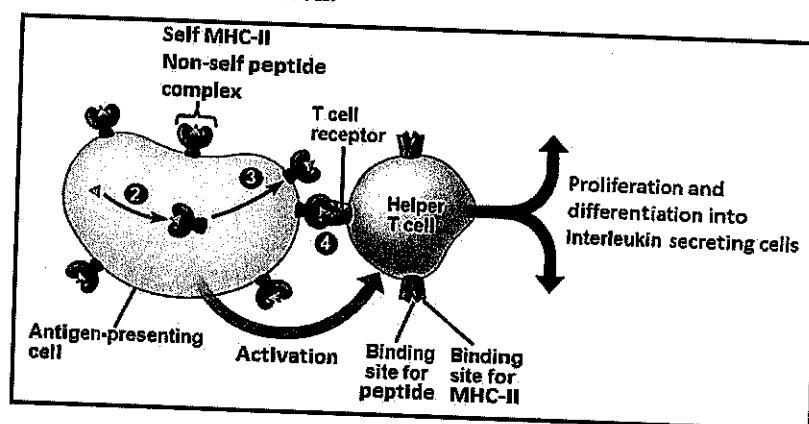
3.2.2. Mechanism of specific immune response

This response includes three phases:

First phase: The induction phase of the specific immune response

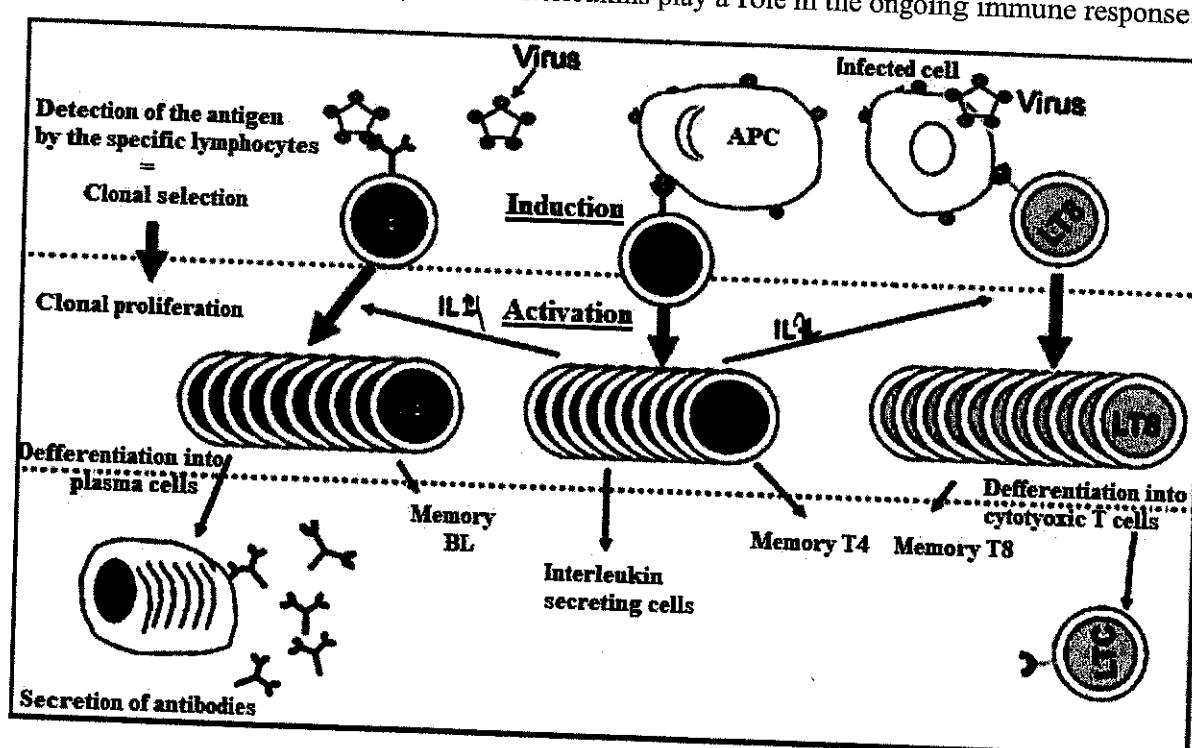
When the macrophage phagocytizes the non-self-antigen it digests it and associates its peptides to the HLA-II molecules on its membrane surface. Then the macrophage migrates to the closest lymph node, it becomes an antigen presenting cell or APC and waits for the T4 cells that circulate between the lymph and the blood through the lymph nodes.

When the specific T4 arrives, it binds on the HLA-II non-self-peptide complex by its TCR and it becomes activated, this is the clonal selection.



Second phase: The activation phase

The activated T4 cells proliferate, make a clone of identical cells having same TCR; some of them become memory cells, the others differentiate into interleukin secreting cells that secrete cytokines called interleukins (IL-2 and IL-4). These interleukins play a role in the ongoing immune response.



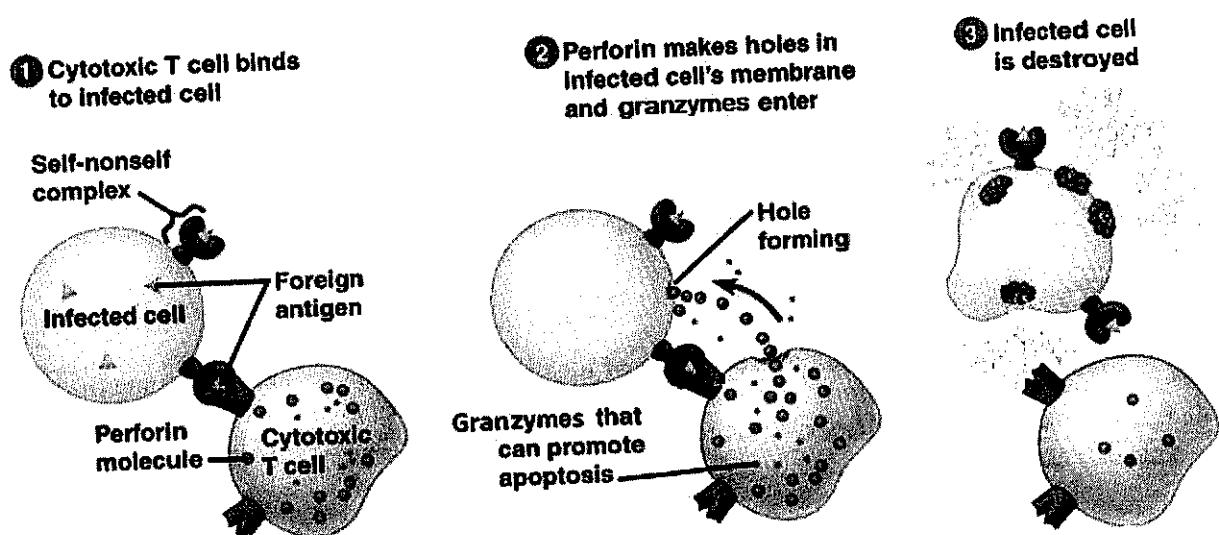
Third phase: The effector phase:

1- T8 cells:

Interleukin 2 activates T8 cells having encountered and recognized the non-self-peptide on the HLA-I of the infected cells by their TCR, they become active, proliferate and give memory cells, some differentiate into cytotoxic Tc cells that are able to kill the infected cells.

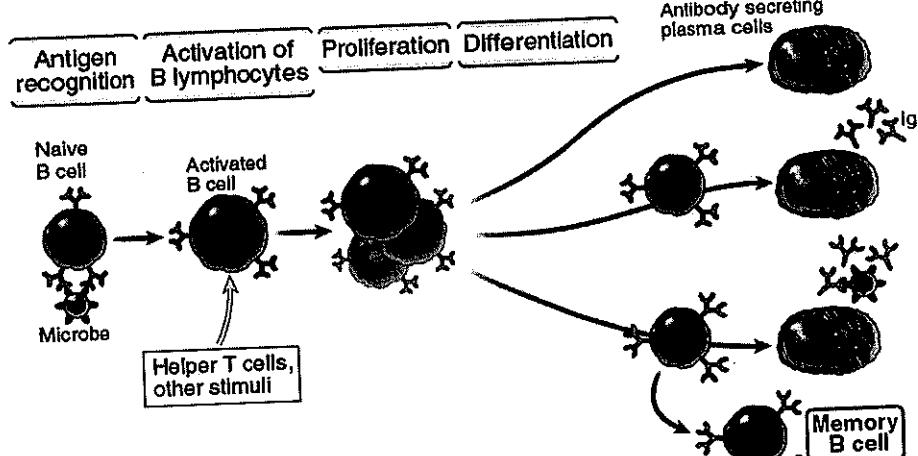
Mechanism of cytotoxicity:

The Tc recognizes the target cell by double recognition and binds by its TCR on HLA-I, non-self-peptide complex on its surface, then it releases perforin that makes polyperforin channels through the cell membrane of the infected cell, then it releases granzymes that enter by the polyperforin channels and trigger inside the infected cell an enzymatic chain reaction that lead to the degradation of the DNA of the infected cell and leads to its death.



2- B cells:

Interleukin 4 activates BL cells having encountered the free non-self-antigen by membrane antibody receptors, they become active, proliferate and give memory cells; some differentiate into plasma cells rich in RER and GA and they secrete antibodies that are specific to the encountered antigen.



Roles of the antibodies (effectors of the humoral response):

A- Neutralization of toxicity of the toxins: the toxins harm the target cells by binding on specific membrane receptors, when the antibodies bind on the toxins by their variable regions they cover their site of attachment to the target cells; and prevents the toxin from harming them.

Neutralization of the infectivity of viruses and intracellular bacteria: The virus infects the target cells by binding by their antigens on some specific membrane receptors on the target cells. The binding of the antibodies by their binding sites on the virus's antigens prevents them from binding on the specific receptors of the target cells and infecting them.

B- The opsonization: Once the antibodies bind by their binding sites of the variable regions on an antigen, their constant region become able to bind on membrane receptors on the macrophages. Thus, the antibodies create molecular bridges between the antigen and the phagocyte to facilitate the adherence, this is the opsonization, then the macrophage phagocytizes the antigen in order to eliminate it.

C- The activation of the complement: The binding of the antibodies by their variable regions on bacteria antigens activate the complement components C1 that bind on the constant regions of the antibodies, this activation leads to the complement cascade activation from C1 to C9 that forms at the end membrane attack complexes on the surface of the bacteria that perforate them and lead to their destruction.

3.3. Immune memory

After the proliferation of the immune cells, some of them become memory cells, they play a role in the protection during the next infection by the same microbe by making the secondary immune response faster, more amplified and more persistent than the primary immune response.

The immune memory is the basic of vaccination.

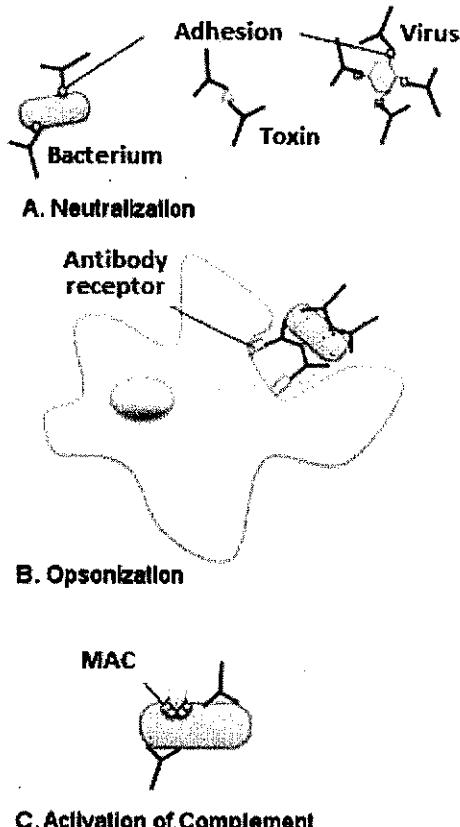
3.4. Immunological medical treatments

Vaccination: It consists of injecting attenuated microbes or attenuated toxins (toxoid) in the body in order to induce immune memory able to protect the body from the subsequent possible infections.

Serotherapy: It consists of injecting specific antibody molecules to neutralize some microbes or toxins (snake venom) that might or have been entered the body.

Immunotherapy: injection of immune cells specific against cancer that are cultured in vitro after being taken from the patient.

Remark: Immunotherapy is more efficient than the chemotherapy. This latter destroys cancer cells but destroys also leukocytes and leads to immunodeficiency.



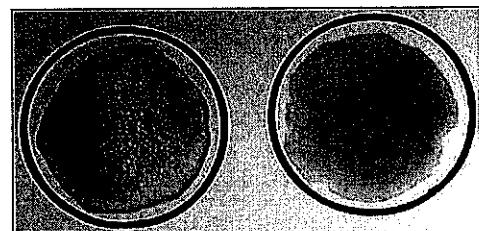
3.5. Diagnostic applications of the antibodies

Antibodies are used in order to test whether an individual is infected or not by a microbe.

3.5.1. Serological tests

Agglutination reaction.

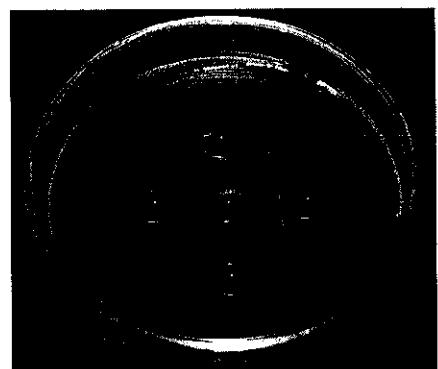
The agglutination of a microbe by the plasma of an individual indicates that this individual has specific antibodies against this microbe, thus he is affected.



Positive result Negative result
Infected not infected

Immunodiffusion in gel

The agglutination of a microbe by the antibodies of the plasma can be observed on a gel when the tested antigens are small and not able to be seen in the test tube. This agglutination is revealed by the presence of a precipitation arc. In the opposite figure a precipitation arc observed between the serum S and the antigen D indicates that the tested person has anti-D antibodies. He is infected by the microbe having the antigens D.



3.5.2. Immunomarking tests

ELISA

It is used to quantify the amount of antibodies against an intruder (HIV, Hepatitis), it is more sensitive than agglutination test and immunodiffusion in gel. Moreover, it is able to determine the concentration of antibodies and thus the duration of the infection by comparison with control cases.

Immunofluorescence

It is used to test for cellular antigens as tumor antigens. It consists on adding specific fluorescent antibodies on a sample of cells then washing it before observation under microscope. The detection of fluorescence indicates the presence of tumor antigens or other antigens in question.

4. Immune disorders

4.1. Immunodeficiency

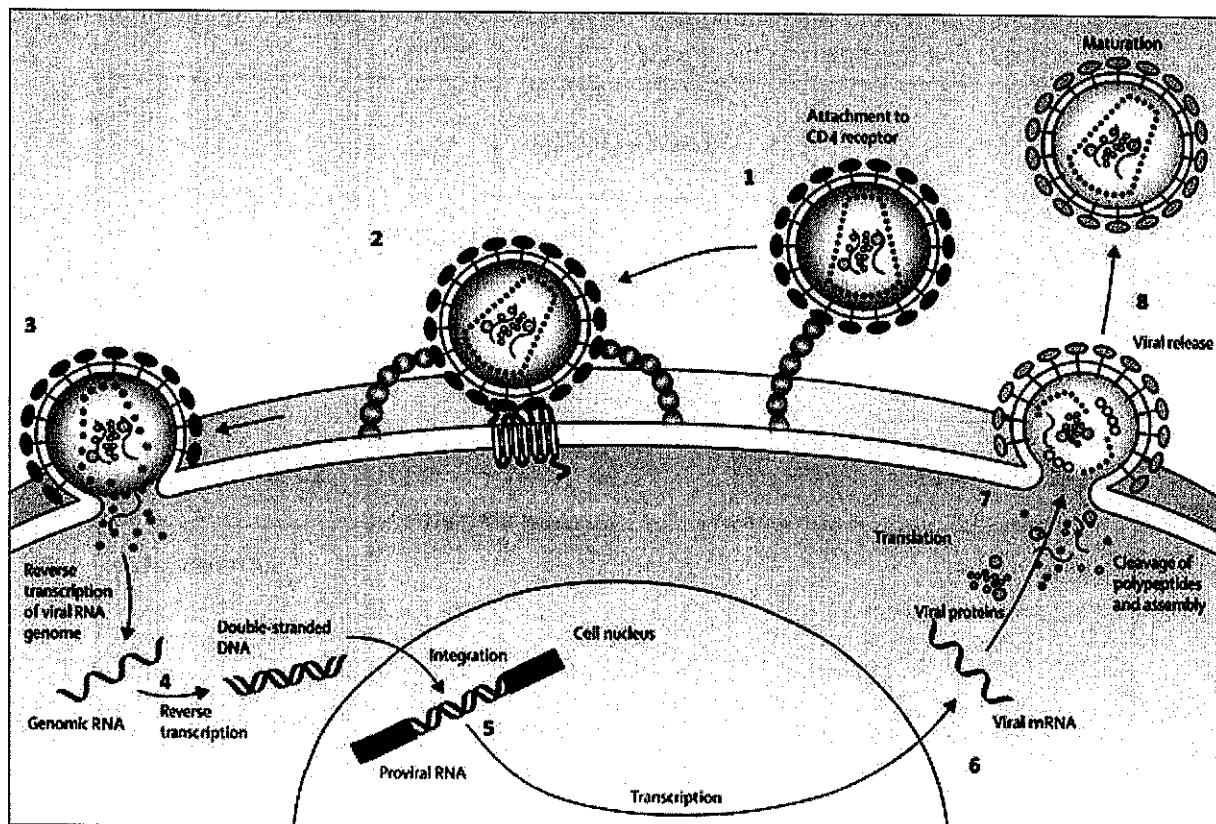
- 4.1.1. Congenital: caused by a deficiency in some immune cells or processes. A deficiency in BL, in complement or in macrophages leads to infections by extracellular intruders. A deficiency in T8 cells leads to infections by intracellular intruders. A deficiency in T4 cells leads to infections by intra and extracellular intruders.
- 4.1.2. Acquired: caused by immunosuppressors, cancer, stress, malnutrition or HIV infection.

4.2. HIV infection

HIV infects the cells having CD4 receptors: T4 and macrophages.

4.2.1. Multiplication of HIV

After binding on CD4 molecules by the membrane proteins of HIV, it inserts its RNA into the cell, where it is transcribed into DNA by the help of the enzyme reverse transcriptase. Then the viral DNA is inserted into the cell genome where it remains dormant or is activated to be transcribed into RNA that encode the synthesis of viral proteins. The viral proteins make with the RNA of the virus new viruses that bud at the surface of the cell and infect new cells.



4.2.2. Progression of the infection by HIV

After the infection, the HIV multiplies, its amount increases in the body. It starts infecting T4 cells that drop. This phase is seronegative.

The body starts to fight the virus and produce anti-HIV antibodies that neutralize and eliminate the virus from the plasma but it persists inside the infected cells, this is the seropositive asymptomatic phase.

The decrease of T4 cells leads to a state of slight immunodeficiency where the body encounters repeated infections.

A severe immunodeficiency AIDS is attained when the number of T4 cells become very low (less than 200/ml of blood), the person is infected by opportunistic diseases, microbes that are normally benign. These infections lead to the death of the individual.

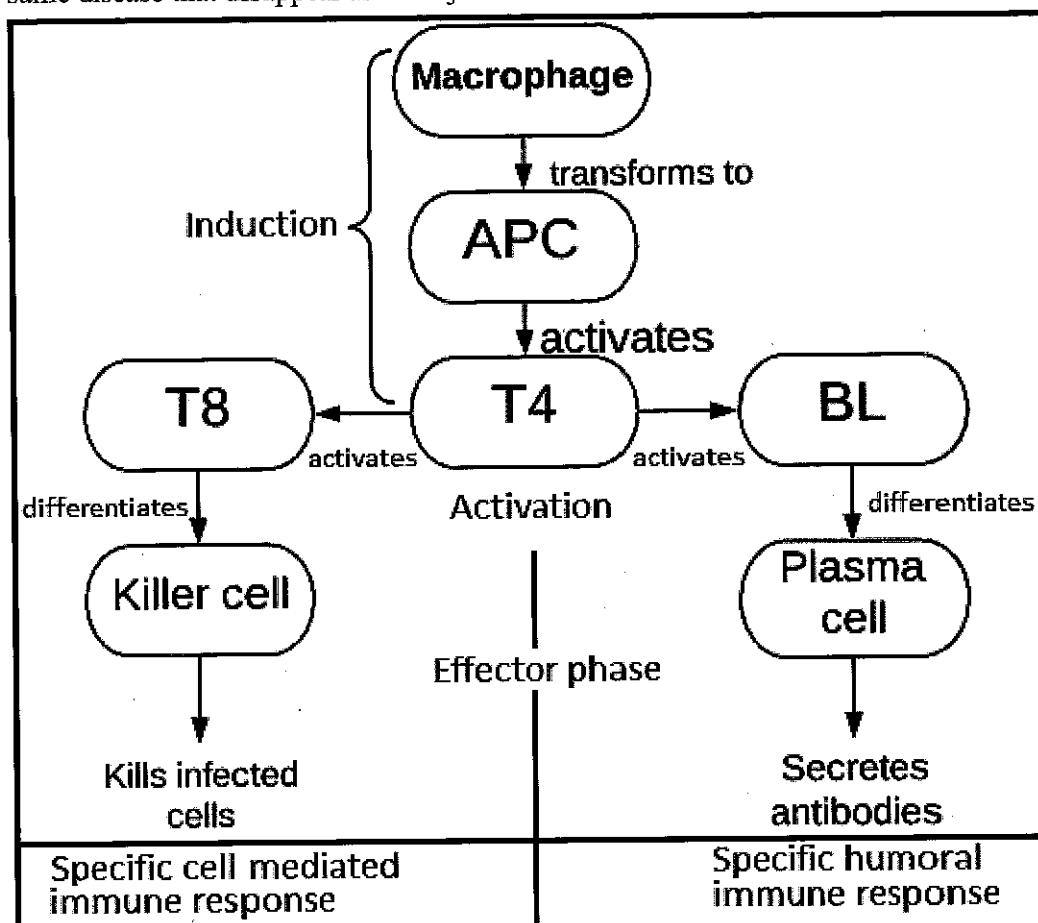
4.3. Autoimmunity

Is caused by autoreactive T and B lymphocytes that are directed against self-antigens. It can be:

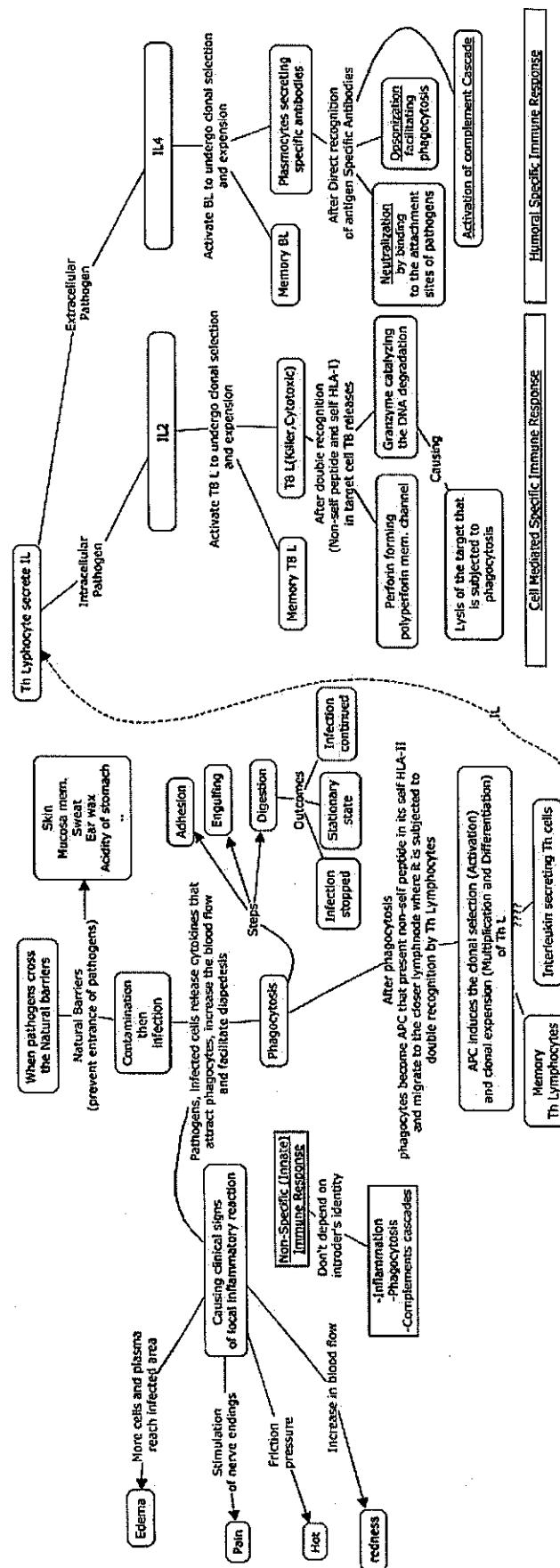
- ❖ Organ specific leading for the destruction of some organs as the pancreas, the thyroid or the nervous system.
- ❖ Non-organ specific leading for the formation of immune complexes with some antigens that precipitate in the kidneys or the joints or under the skin leading for inflammations.

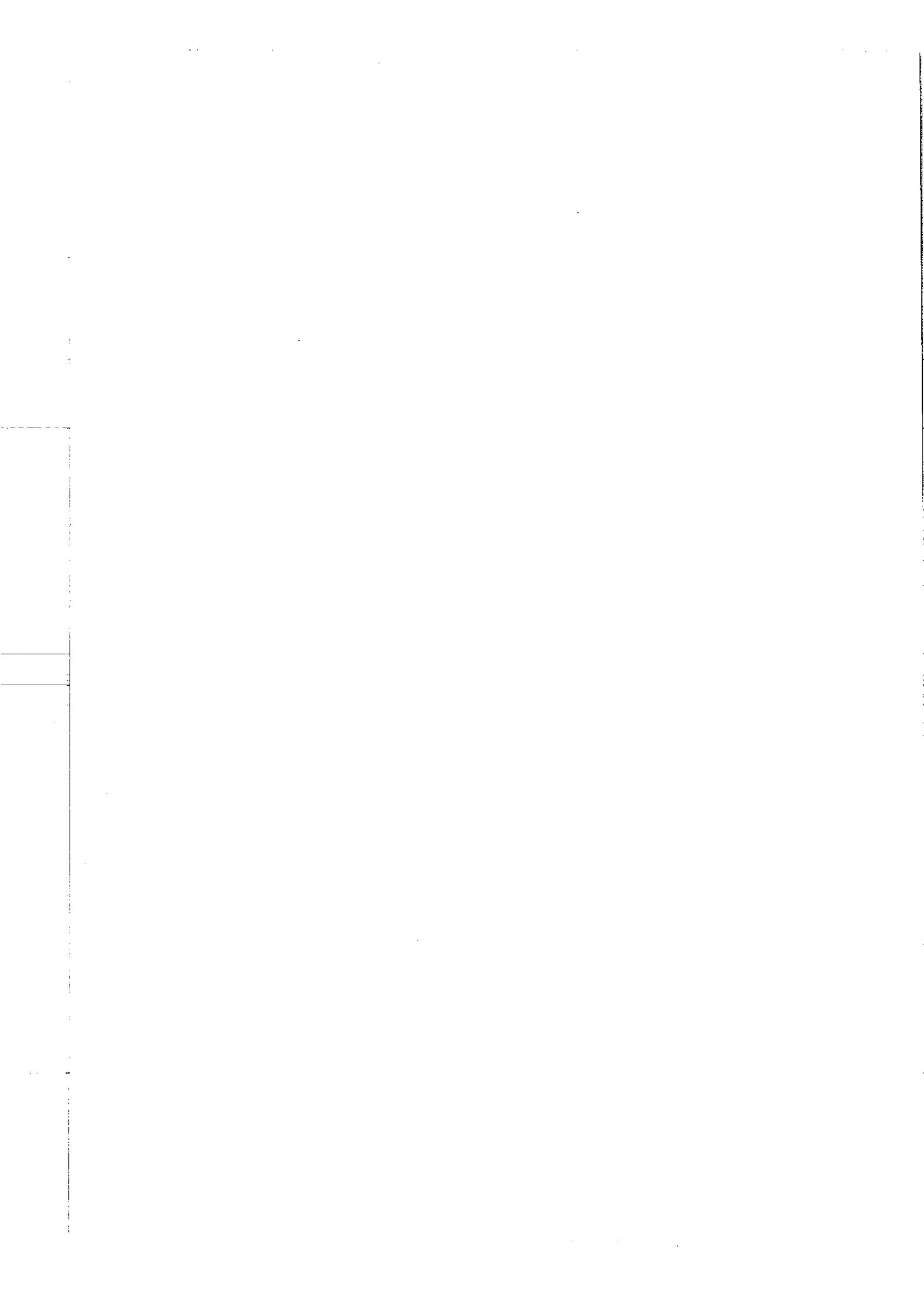
These diseases are known by:

- ❖ Injection of immunosuppressors that attenuate the symptoms of the disease.
- ❖ The injection of the plasma (antibodies) of an affected individual into a normal one lead to same disease that disappear as the injected antibodies decline.



Induction and activation of the specific immune responses

Concept map



Immunity

Training exercises

EXERCISE 1 The immunological identity

In order to study the components of the immunological identity of bodies; some studies are made concerning the HLA molecules named also the molecules of the MHC. The study concerns a group of persons having received kidney grafts from other sibling or non-siblings but always having different HLA. Then after one year the percentage of rejections is determined as a function of the number of incompatibilities of HLA molecules. The results are represented in the table in document 1.

Number of incompatibilities of HLA molecules A and B	0	1	2	3	4
Percentage of rejections during one year %	30	32	35	40	45

Document 1

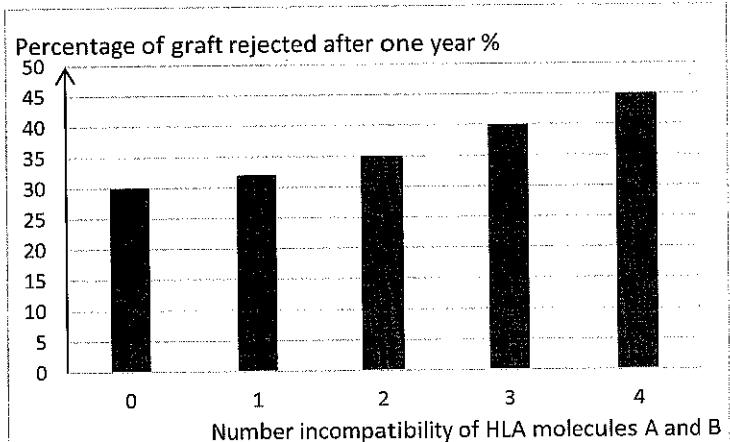
1. Identify the type of the graft studied by this experiment.
2. Represent the experimental results in a histogram.
3. Interpret the obtained results.
4. Indicate the factors at the origin of the diversity of HLA molecules.

A graft of kidney is realized between two brothers Karim and Salim of same HLA, after 2 days the grafted kidney starts making redness, presents an edema and it stops functioning totally.

5. Indicate if this graft is rejected or accepted.
6. Formulate an explanatory hypothesis of the results of this graft.

Solution:

1. The allograft and the type of transplant carried out between two genetically different individuals belonging to the same species. In this case, transplants are performed between people with different HLA therefore genetically different and the same species. Then these grafts are allografts.
2. Histogram showing percent rejection of grafts after one year as a function of the number of incompatibilities of HLA molecules A and B.



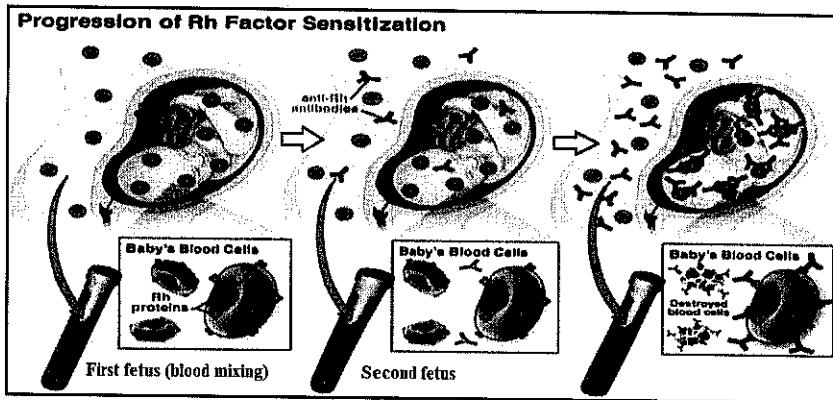
3. The percentage rejection of the graft after one-year increases by 30 % to 45% with the increase in the number of incompatibilities of HLA A and B from 0 to 4. This shows that the graft rejection is amplified by the incompatibility of the HLA molecules A and B.
4. Existence of 6 genes, a large number of alleles of each gene in the population all codominant.
5. The transplant is rejected.
6. Hypothesis: The two brothers are of different blood groups.
Or
An infection of the kidney is the cause of rejection of the transplant.

EXERCISE 2 He's positive, she's negative: What will that do to baby?

Normally, maternal and fetal blood supplies don't mix during pregnancy, but during childbirth, some fetal blood may enter the mother's system. If the mother is Rh-negative and the fetus is Rh-positive, the woman's immune system responds by specific antibodies to the Rh factor. In a subsequent pregnancy these antibodies cross the placenta and enter fetal circulation. If the next fetus is also Rh-positive, the mother's antibodies destroy fetal red blood cells. The baby may be born anemic or yellowish, and in severe cases many fetuses have died.

On the other hand, the agglutinins anti-A and anti-B of the blood types of ABO system are innate, they are much bigger than the antibodies produced against rhesus factor. Anti-A and anti-B do not cross the placenta and reach the baby's blood as anti-rhesus antibodies.

Document 1 shows the steps of the reaction against rhesus factor mentioned in the text.



- 1 Indicate the non-self element that is rejected in this case.
- 2 Pick out from the text:
 - 2.1 The moment of the entry of rhesus antigens to the mother's blood.
 - 2.2 The consequence of the presence of anti-rhesus on the next pregnancy.
 - 3 Justify that the first baby with rhesus positive blood is not affected by anti-rhesus.
 - 4 Name the cells responsible for the production of anti-rhesus antibodies.
 - 5 Indicate the site of induction of the immunity against rhesus antigens.
 - 6 Show that the problem of incompatibility of blood types is observed in the case of rhesus system but not in the case of ABO system.

Solution:

- 1 Rhesus factor carried by red blood cells.
- 2.1 During delivery, fetal blood may enter the mother's system.
- 2.2 If the next fetus is also Rh-positive, the mother's antibodies destroy fetal red blood cells. The baby may be born anemic or yellowish, and in severe cases many fetuses have died.
- 3 The maternal and fetal blood mix only during childbirth so it is only during a subsequent pregnancy that these antibodies cross the placenta and enter the fetal circulation, during the first pregnancy no anti-Rh antibodies in the maternal blood since these antibodies are not innate, which justifies that the first baby with rhesus positive blood is not affected by the anti-rhesus.
- 4 The plasma cells.
- 5 The spleen.
- 6 The anti-A and anti-B agglutinins are much larger than the antibodies produced against the rhesus factor, so they do not cross the placenta and do not reach the baby's blood so the problem of the incompatibility of the groups blood is not observed.

EXERCISE 3 An infection by a virus

The mononucleosis virus is a virus that infects man as well as horse. After the infection of this latter the antigens of the virus are presented on the surface of the red blood cells. Mr. X achieved under his physician's request, a medical analysis in order to detect the infection by the mononucleosis, the test is performed as follows.

First stage:

On a slide with three slots, we deposit a drop of serum in each of these slots: slot 1: serum of horse infected by mononucleosis.
slot 2: serum of non-infected animal.
slot 3: serum of M. X.

Second stage:

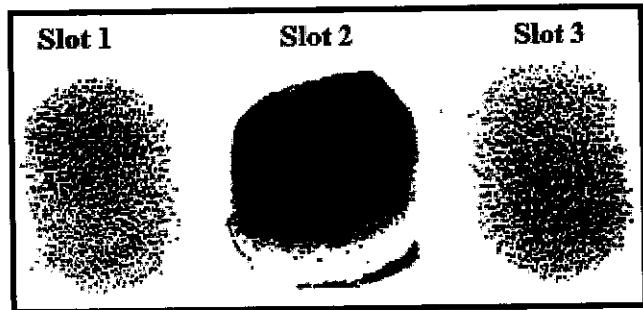
We add to each of these serum drops some small quantity red blood cells of horse infected by the virus of the mononucleosis.

Third stage:

We observe the slide in order to search if the red blood cells of horse had been agglutinated by the serum. The photographs of the document 1 present the obtained results.

Fourth stage:

After some time of their agglutination and incubation at 37°C, the three slots take the aspect of the slot 2, this is caused by the lyses of the agglutinated red blood cells by the action of the complement.

**Document 1**

1. Indicate the factor that makes the same virus infect man and horses at the same time.
2. Indicate if the virus of mononucleosis is an intracellular or extracellular intruder.
3. Determine the state of M. X.
4. Explain the role of the antibodies in the defense against the virus of mononucleosis.
5. Explain the mechanism of lysis of the red blood cells in the two slots 1 and 3 after 24 h of their agglutination.
6. Justify that the immune response revealed by document 1 is unable alone to eliminate the virus of mononucleosis from the body.

Solution:

1. To infect an animal, viruses must have attachment receptors on the cells of that animal, so to infect humans and horses the mononucleosis viruses have similar receptors on human cells and horse cells.
2. The mononucleosis virus is an intracellular agent.
3. As only agglutination of the red blood cells is observed in the presence of the serum of the man similar to that observed with the serum of the infected horse, then the serum of the man X contains anti-mononucleosis virus antibodies. So, he is infected.
4. Viruses enter their target cell through the binding of their surface molecules to a specific receptor expressed by the membrane of the target cell. By binding to these surface molecules, the antibodies can prevent entry of the virus into the host cell and thus neutralize its infectivity.
5. When antibodies bind to antigens expressed on the surface of red blood cells, their constant regions may bind the complement component C1 that activates other components of the complement, it is the cascade of the complement which results in the formation of a membrane attack complex which perforates the target cell coated with antibodies and causes its lysis.
6. The immune response revealed by document 1 is unable to eliminate the body's mononucleosis virus alone because the antibodies neutralize efficiently the viruses but are unable to eliminate the infected body cells by this virus, the body needs a specific cell-mediated immune response to remove these infected cells and subsequently eliminate the virus from the body.

EXERCISE 4 The lymphoid organs

We realize, on mice, two series of experiments, in which the mice are submitted to the ablation of the bone marrow or of the thymus followed by the treatments indicated in the table below.

Experiments	1 st series		2 nd series	
	treatment	results	treatment	results
Control mice	Graft of an organ of mouse A of different MHC	Rejection of the graft after 10 days	Injection of antigen X	Agglutination of the serum with antigen X
Ablation of thymus		Acceptation		No agglutination
Ablation of the bone marrow		Acceptation		No agglutination

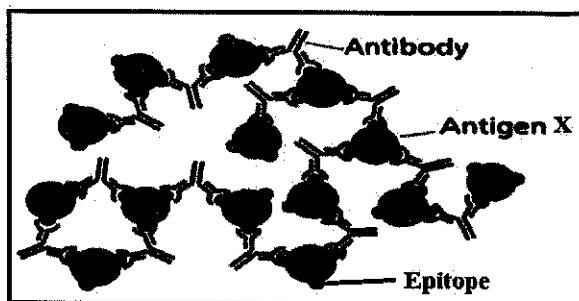
1. Determine, based on the first series of experiments; the organs responsible for the rejection of the graft.
2. Explain the results of the second series of experiments.
3. Indicate the type of the immune response induced against the antigen X.
4. Schematize the agglutination made by the serum with the antigen X.

Concerning the 1st series of experiments, a graft of an organ of a mouse B of MHC different than that of the mouse A leads to a rejection after 6 days.

5. Formulate a hypothesis explaining the difference of the results of the grafts A and B.

Solution:

1. Since the graft is rejected after 10 days in the control mice but it is accepted after removal of the thymus or after the removal of the bone marrow. So, the thymus and bone marrow are responsible for rejection of the graft.
2. After phagocytosis of antigens X, macrophages are transformed into APCs that activate specific T4 cells. These secrete interleukin 4 necessary for the activation of B cells specific for antigen X that will agglutinate these antigens by secreting specific antibodies after transformation into plasma cells.
B lymphocytes and T4 lymphocytes originate in the bone marrow, so that in case of removal of the bone marrow no agglutination of antigens X is observed because there is absence of BL and LT4. On the other hand, the T4 cells mature in the thymus, this process is necessary so that the LT4 will be able to recognize the APC carrying the peptides of the antigen X, then in the absence of thymus, the T4 will not be able of recognizing APCs and being activated in order to activate in turn the LB, that is why we observe a lack of agglutination in the absence of thymus.
3. It is a specific humoral immune response.
4. Figure showing the agglutination of antigens X by the specific antibodies

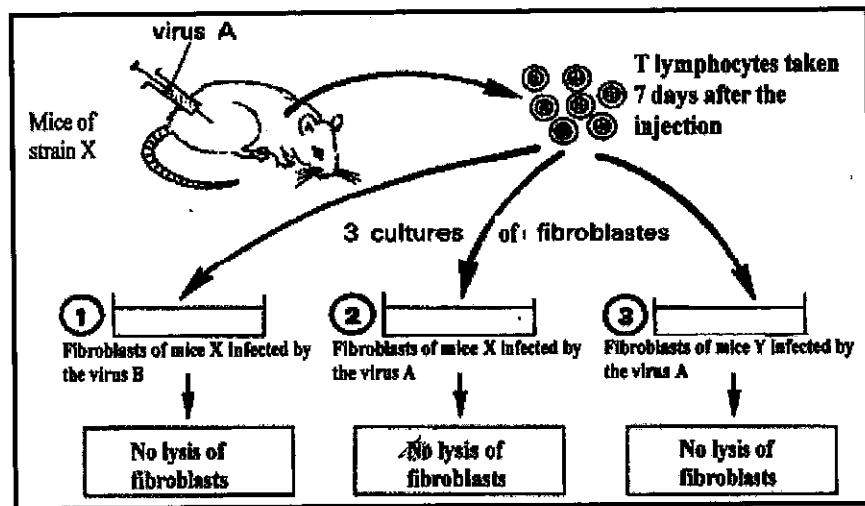


5. Hypothesis: Mice B have higher incompatibility with the grafted mice.

EXERCISE 5 A specific immune response

Document 1, schematized below, represents the conditions and the results of a performed experiment.

1. Name the lymphocytes used in this experiment. Justify.
2. Justify the necessity of a cell mediated immune response to eliminate the virus.
3. Explain, starting from the acquired knowledge, the steps of the induction of the specific immune response against the virus A.
4. Interpret the results observed in cultures 1, 2 and 3.
5. Explain the mechanism of the destruction of the fibroblasts by the T lymphocytes.



Document 1

Solution:

1. Tc because TL sensitized against virus A causes lysis of fibroblasts infected by virus A.
2. Viruses are intracellular pathogens can only multiply within the host cell, elimination of the virus from the body requires destruction of cells infected by this virus, which is performed by Tc lymphocytes, effectors of the specific cell mediated immune response.
3. When the macrophage phagocytizes the non-self antigen, digests it and associates its peptides with HLA-II on the surface of its membrane, the macrophage becomes an antigen presenting cell (APC) that then migrates to the lymph node and waits for T4 cells that circulate between the lymph nodes and the blood. When the specific T4 arrives, it binds to the HLA-II-non-self peptide complex by its TCR and becomes activated.
Activated T4 cells proliferate and differentiate: some of them become memory cells, others complete their differentiation into cells that secrete interleukin 2 which activates T8 cells that have encountered non-self peptide on HLA-I of the infected cell.
4. No lysis of the fibroblasts of a strain X mouse infected with a virus B cultured with lymphocytes immunized against virus A taken from a mouse of the same strain X, while a lysis of fibroblasts of the same strain but infected with the virus A is observed in the presence of these same lymphocytes. This shows that the lymphocytes only destroy the cells infected with the same antigen to which they are immunized, whereas these same lymphocytes do not cause lysis of the fibroblast of another strain Y infected by the same virus. This shows that lymphocytes immunized against a virus only destroy cells of the same strain infected by the same virus.
5. When a cytotoxic cell recognizes an infected cell and binds to the HLA-I-non-self peptide complex on the target cell membrane by its TCR, it releases its perforin content that perforates the membrane of the target cell forming polyperforin channel; then the Tc cell releases the granzymes that enter the polyperforin channel and trigger within the infected cell a chain of enzymatic reactions that lead to the degradation of the DNA of the infected cell and lead to its death.

EXERCISE 6 Specific humoral immune response

B lymphocytes are taken from a mouse previously injected by an antigen A. After incubation with radioactive amino acids in different conditions, we search in the medium for radioactive proteins. The conditions and the results are given in document 1.

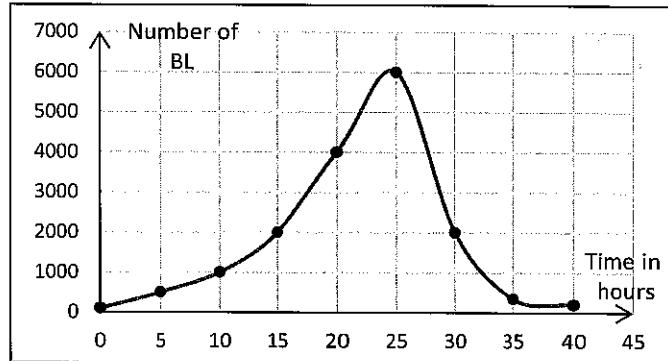
Experiment	Incubation with radioactive amino acids	Results (radioactive proteins)
A	B lymphocytes + antigen A	No
B	B lymphocytes + antigen B + interleukin 4	No
C	B lymphocytes + antigen A + interleukin 4	Yes

Document 1

1. Explain these results.

We measure the number of BL in the medium C. The results are represented by the graph in document 2.

2. Represent in a table the information of this graph.
 3.1 Analyze the results of the graph.
 3.2 What can you conclude?
 4. Indicate the importance of the BL that persisted at 40 hours.

**Document 2****Solution:**

1. The radioactive proteins that are the antibodies appear in the culture medium only in the presence of BL sensitized against antigen A and interleukin 4 but not in the absence of interleukin 4 or in the presence of another antigen B because the activation of BL and its transformation into plasma cells requires the recognition of the antigen by the specific BL through membrane receptors and interleukin 4, then the BL multiply and differentiate into plasma cells that secrete the antibodies.

Time in hours	0	5	10	15	20	25	30	35	40
Number of BL	50	500	1000	2000	4000	6000	2000	400	200

2. Table showing the variation of the number of BL during time.
 3.1 The number of BL increases from 50 to 6000 when the time passes from 0 to 25 hours, however, beyond this time the number of BL decreases from 6000 to 100 when the time passes from 25 to 40 hours.
 3.2 We conclude that IL4 stimulates the multiplication of BL for a limited time.
 4. B lymphocytes that persist at 40 hours are memory cells that protect the body against subsequent infection by the same antigen.

EXERCISE 7 Vaccination

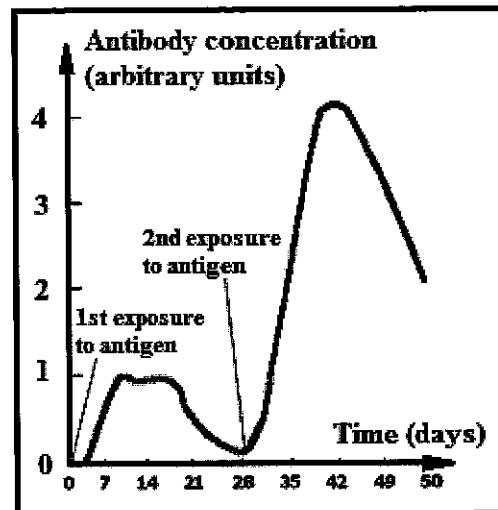
Hepatitis B virus has infected 25000 persons in 1985 in the United States. This number has declined progressively after the vaccine against hepatitis was licensed to become near to 2000 in 2012.

1. Explain the decrease in the number of infected persons by hepatitis after the license of the vaccine.

The gene for hepatitis B surface antigen (HBs Ag, the coat protein of the virus) has been isolated and expressed in yeast cells in order to produce specific antigens for vaccination.

The vaccinated persons are subjected to two exposures to the antigens of hepatitis, the concentration of antibodies is measured after the two exposures and the obtained results are shown in the graph in document 1. Note that the threshold of protection against hepatitis B virus is of 0.5 a.u.

2. Interpret the results shown in Document 1.
3. Explain the difference observed between the primary immune response and the secondary immune response.
4. Show that the two exposures to the antigens of hepatitis are not enough to keep a continuous protection against hepatitis virus.



Document 1

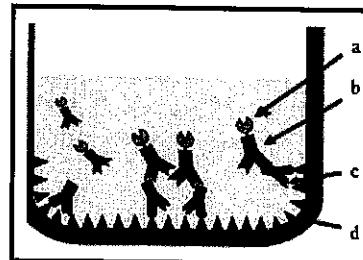
Solution:

1. The administration of hepatitis B vaccine induces specific immunological memory in vaccinated individuals which confers protection against infections by the same pathogen, which reduces the number of individuals affected by this virus, so the number of infected people reduced.
2. Three days after the first exposure of the individual to the antigen, the antibody concentration begins to increase from 0 to 1 a.u. from 10 days and stabilizes till 18 days, however it decreases to 0.2 a.u. at 28 days. This shows that the exposure of the individual to the antigen stimulates a weak temporary secretion of antibodies; after the second exposure to the antigen the antibody concentration increases from 0.2 a.u. to 4.3 a.u. towards 38 days while it decreases to 2.2 a.u. around 50 days. This shows that the second exposure to the antigen amplifies the secretion of antibodies for a longer time.
3. During the secondary immune response there are memory cells that are more numerous and more differentiated than naive B cells sensitized during the primary immune response, these memory cells are able to recognize the antigen faster and proliferate and differentiate to a larger number of plasma cells that secrete antibodies at a higher concentration that is able to persist for a longer time.
4. After 50 days of the first exposure to the hepatitis antigen, the antibody concentration is decreasing relatively fast, this decrease will reach a value below the threshold after a fairly short time, losing all protection against hepatitis B virus, then these two vaccinations are not sufficient for continued protection against the hepatitis B virus.

EXERCISE 8 HIV infection

In order to test an individual for the infection by the HIV, a test is performed. A schematic representation of such a test is represented in document 1.

1. Name the test shown in document 1.
2. Label the figure in document 1 using the letters from a to d.
3. Indicate if the represented test is negative or positive.
Justify.



Document 1

Document 2 shows the variation of the amount of HIV and anti-HIV in the serum of a person infected by the virus. The threshold for testing for HIV antibodies using document 1 test is 6 a.u. The so-called fourth-generation tests also detect the presence of an antigen (p24), which appears best 14 days after infection.

4. Explain the variation of the amount of viruses represented in document 2.
5. Pick out from the graph:
 - 5.1. The time at which the infection by HIV starts to be detected by the test in document 1.
 - 5.2. The minimal rate of HIV detected by the test of fourth generation.
6. Explain the effect HIV infection on the immune system.

Solution:

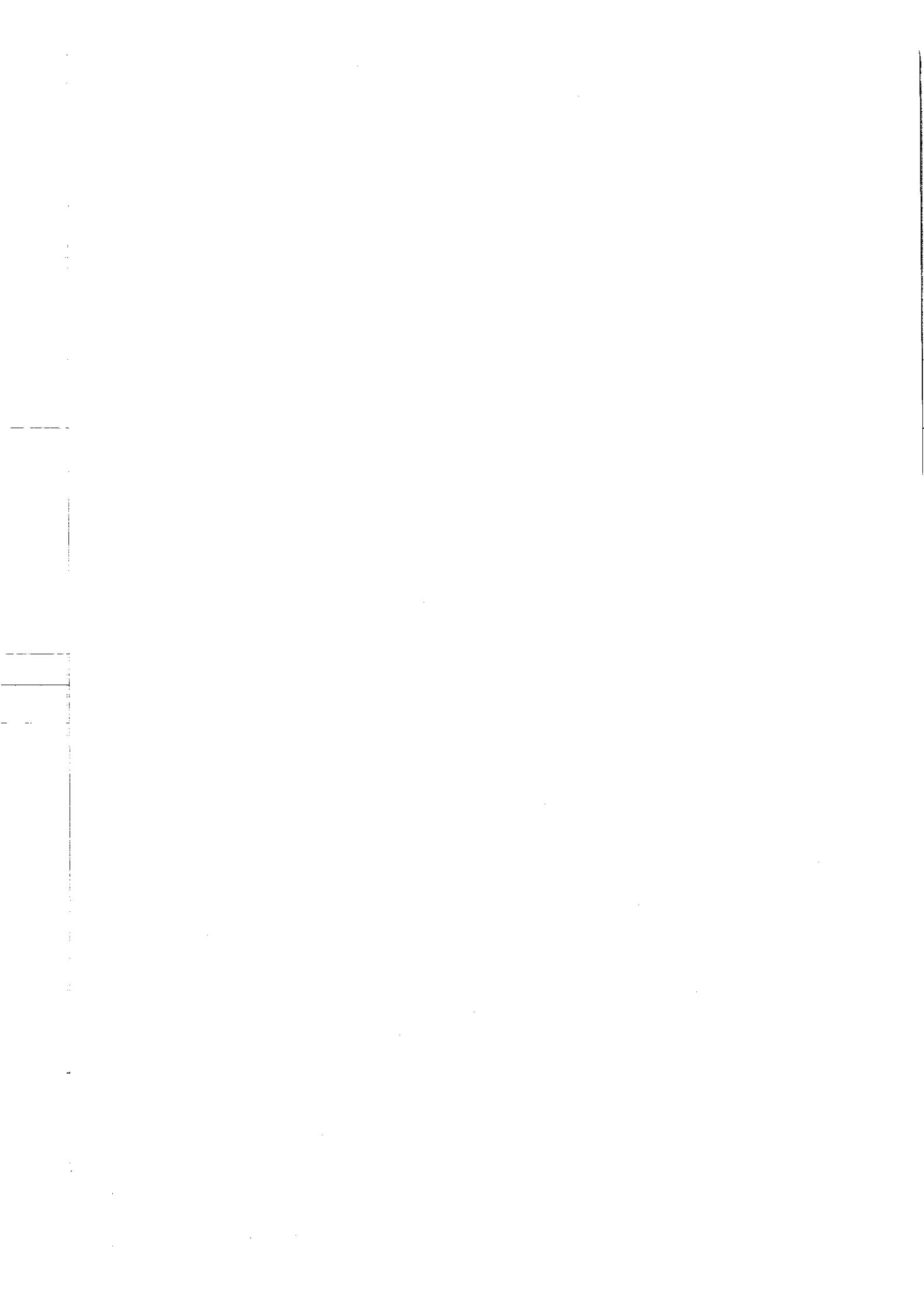
1. ELISA.
2. a. Enzyme that colors a specific substrate
b. Anti-human Ig antibody
c. Specific anti-HIV antibody, d. HIV antigen
3. This test is positive because this person has anti-HIV antibodies in his serum.
4. The rate of HIV increases rapidly between the second week and the tenth week from 0 a.u. to 8 a.u. because of the multiplication of the virus in the infected cells, this multiplication is achieved by the insertion of the viral RNA into the cells then this RNA is transcribed into DNA by the RT enzyme, then the resulting DNA is inserted into the nucleus of the infected cell, it is transcribed and translated to form new viruses that bud at the cell surface to increase the level of virus in the blood.

The rate of HIV decreases from 8 a.u. to be constant at 0.5 a.u. from 18 weeks, this decrease is caused by anti-HIV antibodies that appear as early as 3 weeks and increase progressively. These antibodies are able to neutralize the sites of attachment of the viruses to the target cells thus neutralizing the infectivity of the viruses and thus stops the multiplication of the virus. In addition, these antibodies are capable of agglutinating by their variable regions the free viruses to be fixed by their constant regions on macrophages by opsonization which facilitates the adhesion and the phagocytosis of the viruses, this causes the decrease in their rate.

- 5.1. 12 weeks.
- 5.2. 2 a.u.
6. HIV infection destroys the T4 lymphocytes required for the induction of humoral and cell specific immune responses so HIV infection results in immunodeficiency.



Document 2



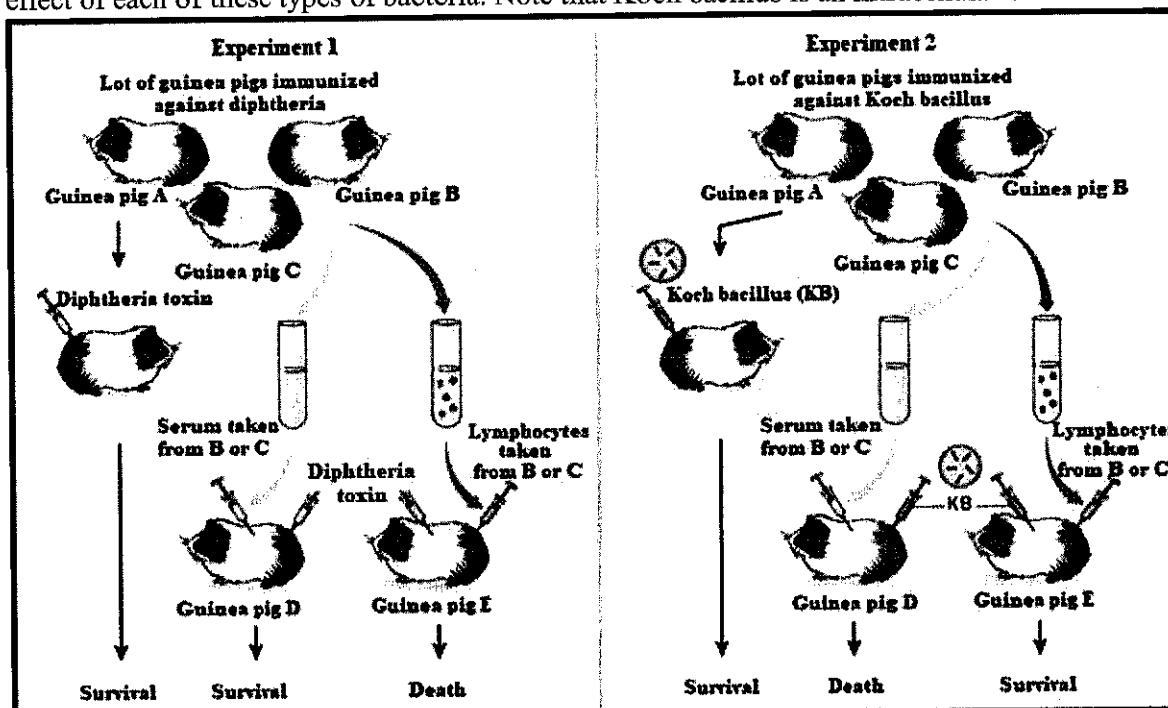
Immunity

Solved exercises

EXERCISE 1 Specific immune response

You cannot escape bacteria, they are everywhere! The good thing is that very few bacteria are harmful. Some of them are so because they are intracellular, they are able to harm the body cells by multiplying inside them, others are extracellular but toxic, they produce toxins that perturbate the function of some body cells by binding on their membrane receptors.

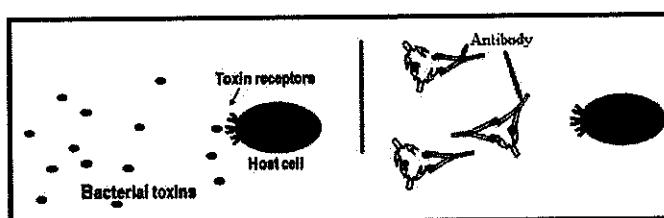
The following experiment is achieved in order to understand how the immune system neutralizes the effect of each of these types of bacteria. Note that Koch bacillus is an intracellular bacterium.

**Document 1**

1. Pick out the problem studied by this experiment.
2. Compare these two experiments.
3. Deduce the elements of blood implicated in the immune response against each type of bacteria.
4. Specify the type of immune response involved against each bacterium.

Documents 2 and 3 show two different mechanisms of the specific immune response.

5. Match each of the two documents 2 and 3 to the corresponding experiment of the two experiments 1 and 2 in document 1. Justify your answer.
6. Explain the mechanism of action of the immunity in each of the two cases in documents 2 and 3.

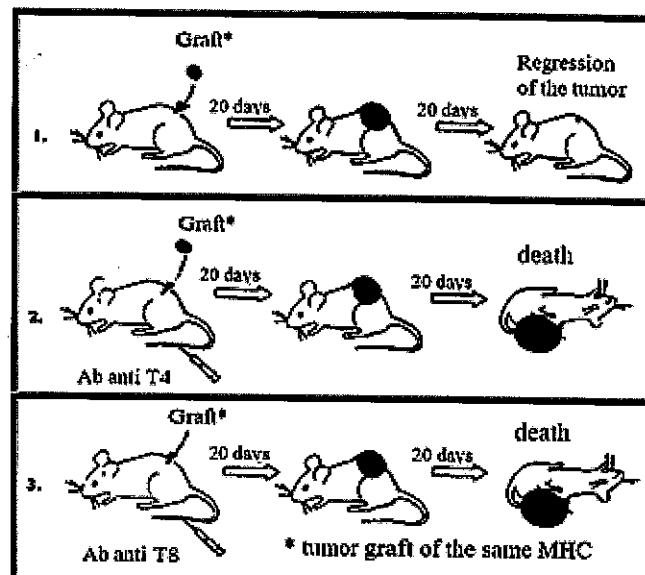
**Document 2****Document 3**

EXERCISE 2 Immunity and treatment of tumor

Much has changed in our understanding of cancer since Hippocrates, in around 400BC, described a tumor, most likely of breast tissue, as resembling a crab and named it a 'cancer' (which is Latin for crab).

In the aim to study the immune response induced against the tumors (cancers) several experiments are achieved. These experiments aim to determine the elements of the body responsible for the immune responses against cancer. Document 1 shows one of these experiments.

1. Formulate, by referring to the text, the problem studied by this experiment.
2. Describe this experiment.
3. Explain the necessity for the transplant to be of the same MHC of the recipient mouse to permit for the experiment to answer the posed problem.
4. Interpret the results of this experiment.
5. Explain the results of mouse 2.



Document 1

Document 2 shows an experiment of transfer of T8 of a normal mouse, after the regression of a tumor, to a mouse having received a tumor graft at the same time of injection of T8.

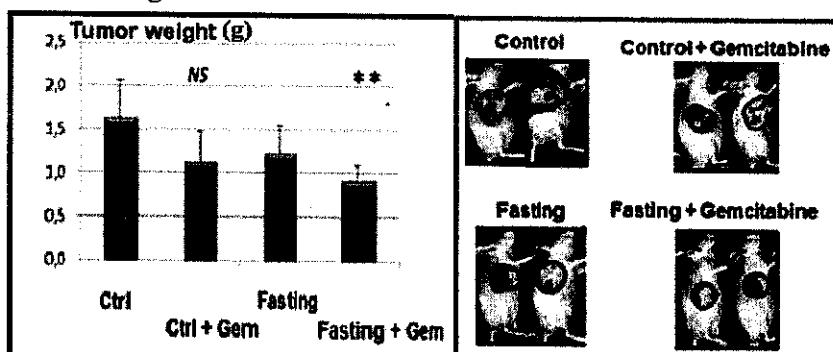
6. Explain the results of this experiment.

The results of these two experiments require the lysis of the tumor cells by some immune cells. These tumor cells express new peptides that are considered as non self.

7. Explain the mechanism of lysis of the tumor cells by the suitable immune cells.

Aiming to study the effect of fasting combined or not with a drug called gemcitabine on tumor, four groups of NOD mice totally immunodeficient are manipulated differently. The performed manipulations and the obtained results are given in document 3.

8. Show starting from these experiments that fasting and gemcitabine are two factors of inhibition of tumor independent from the action of T4 and T8 shown above.



Document 3

EXERCISE 3 The secretion of antibodies

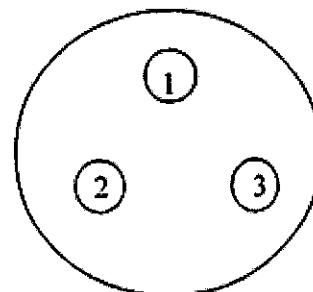
In order to study the effect of a secondary immune response in the elimination of an antigen from the body, two series of experiments are done.

1st series of experiments: We inject in mice A by intravenous route a suitable amount of an antigen, the bovine serum albumin (BSA), which causes the appearance of antibodies that we measure during the 7 weeks which follow this injection. Mice B also receive an injection of BSA; this is followed, eight weeks later by a second injection of another antigen, teta to mice A and BSA to mouse B. Two weeks after the injection either of BSA to the mice A or of antigen teta to the mice B, the serum is taken and we test its capacity to cause the agglutination of antigens BSA and teta by a test of immunodiffusion on gel. The results of measurements of the rates of antibodies of the mice A and B are shown in document 1.

		Injection of BSA (mice A and B)						Injection of antigen teta to mice A and BSA to mice B					
		Time (weeks)	2	3	4	5	7	8	9	12	13	14	15
Rate of antibodies a.u.	mice A	1	10	15	105	2	1	1	80	10	3	2	
	mice B	1	9	12	100	2	1	60	300	250	200	150	

Document 1

1. Draw the graph showing the evolution of the rate of antibodies in the mice A and B shown by document 1.
2. Analyze the results shown by the table.
3. Draw out the differences between the primary and the secondary immune responses.
4. Explain the origin of the secondary immune response's characteristics.
5. Draw the results of the test of immunodiffusion on gel for the mice A at times 5 and 12 weeks while using Document 2 where:
1: BSA, 2: teta, 3: serum of mice A.



Document 2

2nd Series of experiments:

The complement cascade is activated directly by the bacteria, but it is highly activated by the constant regions of the antibodies bound on them; this activation will lead to the cleavage of the components of the complement among which the component C3 that is cut into two fragments C3a and C3b, we decide to study the effect of C3b fragment on the process of phagocytosis made by the macrophage. The figure in document 3 summarizes the experimental setup and the obtained results.

6. What can you deduce from document 3?
7. Explain, based on all what preceded, the fast elimination of antigens during the secondary immune response.

Content of the medium	Adhesion and phagocytosis
1 Macrophage Bacteria	±
2 Macrophage Bacteria Fragment C3b	++
3 Macrophage Bacteria Specific antibodies	++
4 Macrophage Bacteria Fragment C3b Specific antibodies	++++

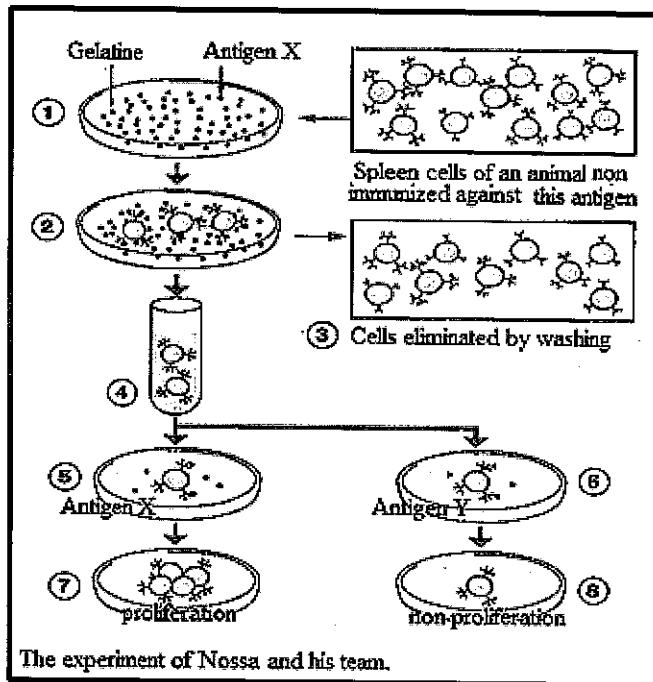
+ : Arbitrary speed of phagocytosis
- : Decrease of the speed with time

Document 3

EXERCISE 4 Role of T4 in the induction of the immune response

The immune system is able to recognize and defend all the types of non self antigens that are introduced to the body, this means by its ability to differentiate all the types of lymphocytes that are directed against these antigens. In order to search if a given animal is able to produce specific lymphocytes against an antigen before encountering it (making contact with it), Nossal and his team executed the experiment schematized in document 1.

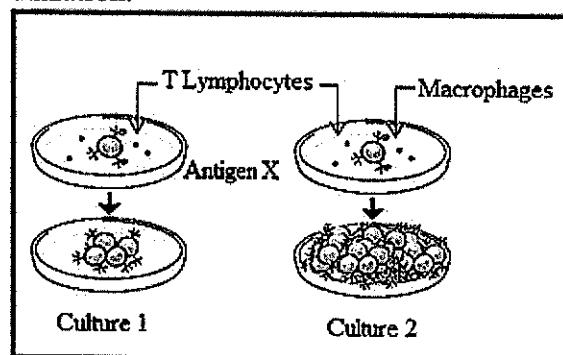
1. Describe this experiment in few lines.
- 2.1 Pose the problem studied by this experiment.
- 2.2 Formulate a hypothesis that aims to answer it.
3. Justify that the lymphocytes used in this experiment are BL.
4. Show that the tested hypothesis is validated.
5. Explain the mechanism of the production of specific lymphocytes before immunization.



Document 1

After the proliferation made in the medium (7) of this experiment, macrophages and T lymphocytes are used in order to study some characteristics of the activation of B lymphocytes. The experiments made are represented in the document 2.

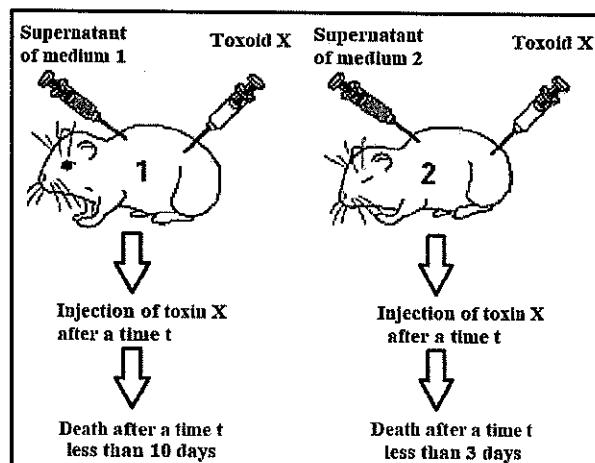
6. Interpret the results of this experiment.



Document 2

The antigen X is a toxin; this toxin is used with the supernatant (the serum taken from the culture) of the culture media 1 and 2 before the formation of plasma cells, in the experiment schematized in document 3.

7. Explain the obtained results to show the role of T4 cells in the induction of the immune response against the toxin X.

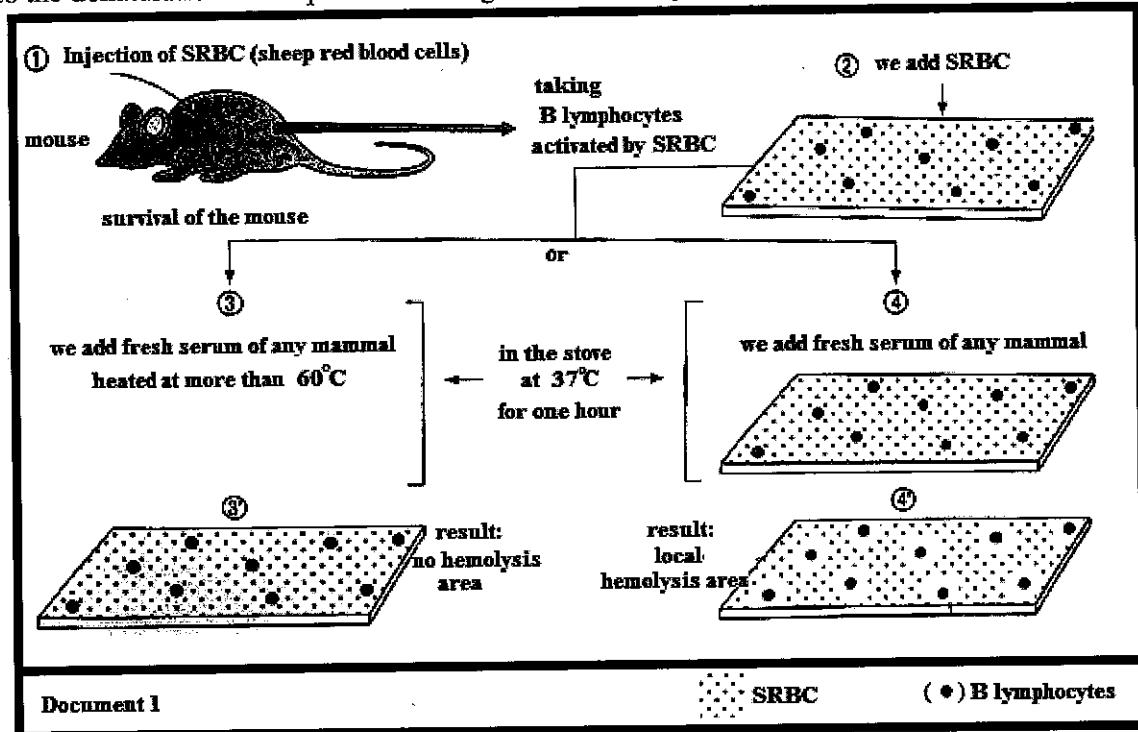


Document 3

EXERCISE 5 Specific humoral immune response

To study some characteristics of the immune response, sheep red blood cells are usually used in experiments performed on mice. The red blood cells of the sheep are not harmful for the mice, moreover, the agglutination of these red blood cells is easily detected at the naked eye and their lysis (destruction) is simply observed by the appearance in the medium of empty spaces called hemolysis areas.

In order to study the factors that are responsible for the lysis of the red blood cells (hemolysis), an experiment is made and schematized in the document below. We note that the temperature of 60°C leads to the denaturation of the proteins among which the enzymes.



1. Pick out, starting from text and by referring to the document 1, the reason of using SRBC in this experiment.
2. Interpret the results of this experiment.
- 3.1 Name the component of the serum in relation with the results obtained in this experiment.
- 3.2 Justify starting from the experiment if this component is specific for the species or not.
4. Explain the appearance of the hemolysis areas only around the B lymphocytes.

This same experiment is repeated in the cases summarized in the table in document 2.

Time	Mice A	Mice B	Mice C
T1	Irradiation	Ablation of thymus	Irradiation and ablation of thymus
T2	-	-	Graft of bone marrow and thymus
T3	Injection of SRBC		
T4	Taking BL		
Results	No hemolysis	No hemolysis	Hemolysis

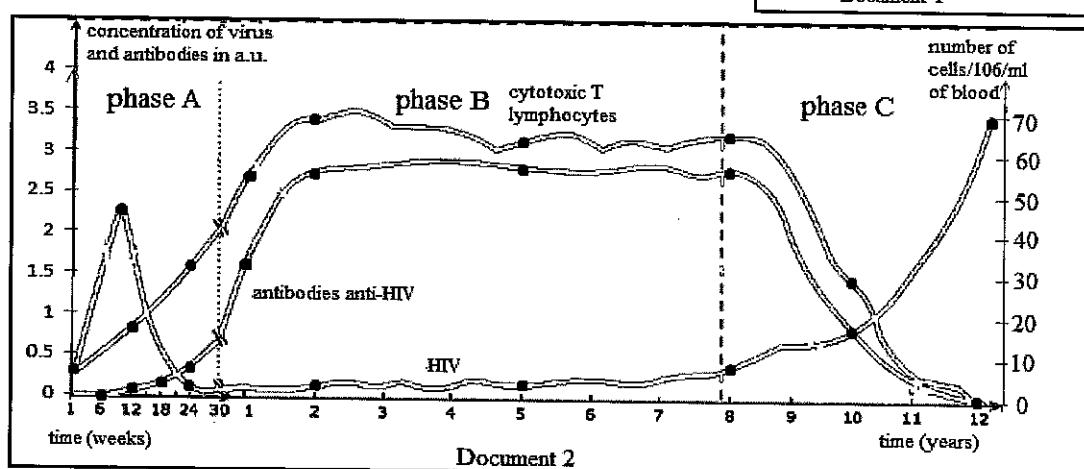
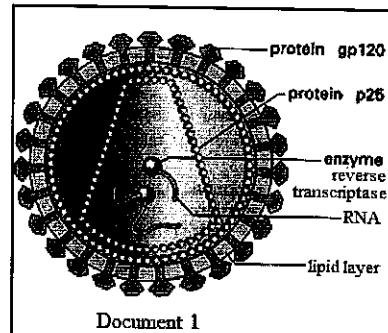
Document 2

5. What can you deduce starting from these experiments?

EXERCISE 6 Screening for the infection of HIV

The envelope of the virus of AIDS (or HIV) presents a protein that can bind to CD4 carried by the membranes of many types of immune cells (monocytes, macrophages, and mainly the lymphocytes T4). This bonding permits the penetration in the immune cell of the viral capsids and its content.

We follow up in certain individuals infected by the HIV the evolution of many elements of the blood. The results of the measurements are represented by the graph of the document 2.



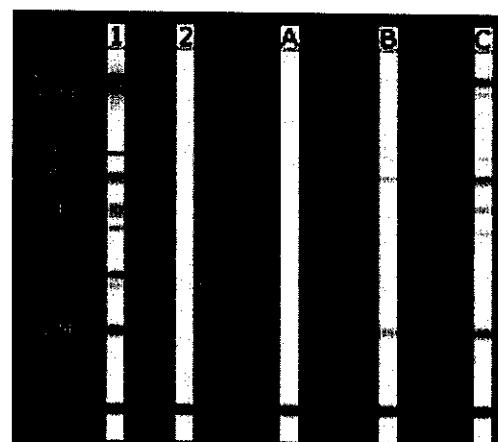
1. Represent in a table the variation of the number of cytotoxic T cells as a function of time.
2. Interpret the curves showing the variation of the concentrations of the virus and anti-HIV antibodies till 30 weeks.
3. Explain, by referring to the given and to acquired knowledge, the evolution of the quantity of HIV during the phase A.

In order to screen out the infection by the HIV, two tests are manipulated for three persons A, B and C with two control tests for two persons: (1) is infected while (2) is healthy. The first test consists on the determination of the concentration anti-HIV antibodies by ELISA test. The second test consist of the search in the blood of the individual for viral antigens by the technique southern blot. In this technique the individual that presents all the antigenic varieties of the HIV is considered infected.

The results are represented in documents 3 and 4.

Individual	1	2	A	B	C
Concentration of anti-HIV antibodies in a.u.	1.5	0	0.4	0.7	1.8

Document 3



Document 4

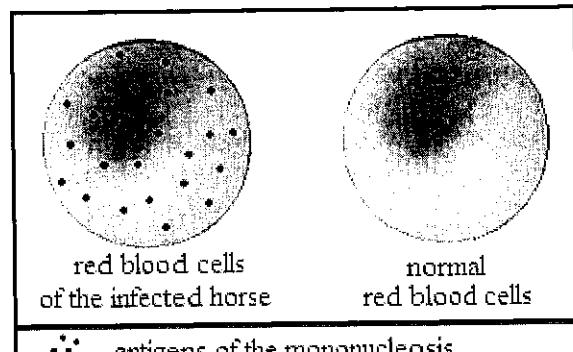
4. Specify starting from document 2 the duration of the infection of the individual (1).
5. Show that the results of the two realized tests confirm the infection by the HIV for the individual C.
6. Indicate the antigenic component of the HIV that is able to be used as vaccine for the HIV. Justify.

EXERCISE 7 Infection by the mononucleosis

Mononucleosis virus is a virus that infects human as well as horse. After the infection of the latter (horse), the red blood cells are subjected to hemolysis leading to the decrease in the number of red blood cells that provokes severe anemia. A modification observed at the level of the red blood cells is represented by document 1.

On the other hand, after around 7 days from the infection by the virus of the mononucleosis, the serum of the horse will contain antibodies directed against the antigens of mononucleosis.

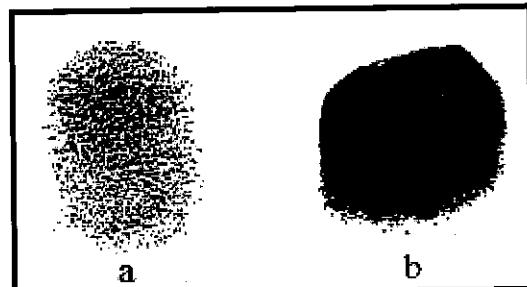
- Explain the necessity of the 7 days after the infection to obtain anti-mononucleosis in the serum of the infected horse.



Document 1

A man performed under the request of his doctor a medical analysis in order to test the infection by the mononucleosis. The realized test consists in mixing on a slide a drop of blood of the tested man with the serum of the horse infected by mononucleosis. The results of the test show an agglutination of the red blood cells of the man by the serum of the horse (doc 2-a).

After 24 hours of their agglutination and incubation at 37°C with the serum of any mammal, the drop of blood of Mr. X takes the aspect represented by document 2-b.

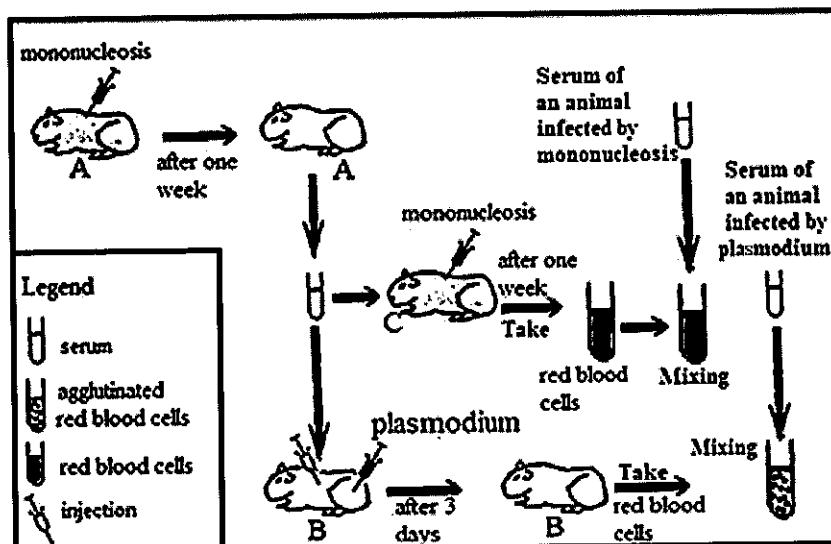


Document 2

- Schematize the phenomenon leading to the result of the document 2-a.
- Explain, by referring to the acquired knowledge and to the given information above, the state of severe anemia provoked by the virus of the mononucleosis.

The experiment in document 3 is realized in order to study one of the characteristics of the immune response. The plasmodium is a pathogen that leads like the virus of mononucleosis to the expression of non self antigens on the surface of the red blood cells.

- Interpret the results of this experiment.
- Explain, based on all what preceded, how the body prevents the mononucleosis virus from causing anemia.



Document 3

EXERCISE 8 A kind of diabetes affecting mice

NOD mice discovered in Japan in 1980, often show diabetes identical to the individuals of the human species affected by the juvenile insulin dependent diabetes. Two lots of NOD mice are considered in the experiment: a lot is treated starting from birth with cyclosporine, a medicine that decreases the intensity of the immune response (immunosuppressor); the other lot doesn't receive this substance.

The graph in document 1 indicates the percentage of mice affected by diabetes as a function of time for the two lots.

1. Represent the data of the graph in a table.
2. Interpret the obtained results.

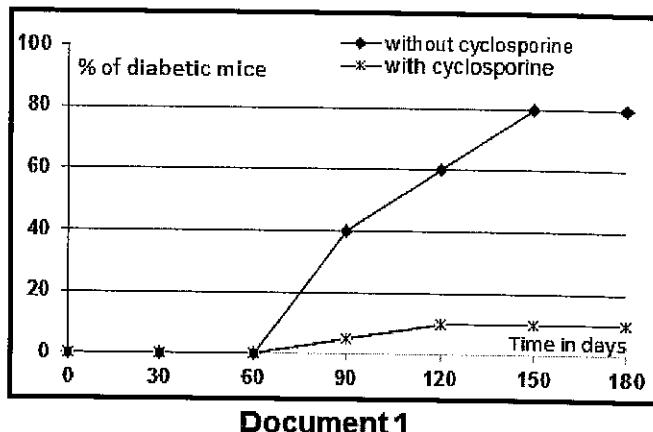


Figure (a) in document 2 shows in a diabetic mouse a Langerhans islet, while the figure (b) shows an islet in mice treated with cyclosporine.

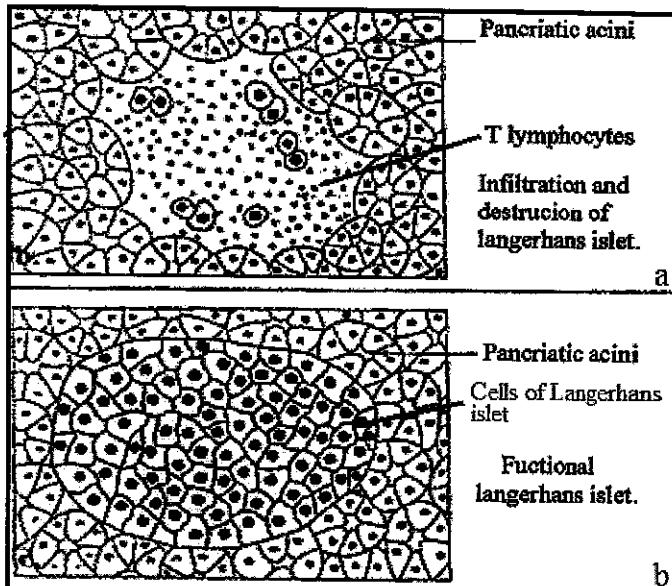
3. Draw out the cause of diabetes in NOD mice.
4. Explain the mechanism of cytotoxicity leading to the destruction of the cells of Langerhans islets.

In order to determine the mechanism of the activation of the auto reactive T lymphocytes, an experiment is made on a lot of healthy and another lot of NOD mice.

The amount of interleukin IL-2 is measured in both lots of mice after being injected by a vaccine V.

5. Formulate the tested hypothesis by this experiment about the origin of the diabetes in the NOD mice.

The results of the measurement of the amount of IL-2 mentioned above are represented in document 3.



Time in hours	0	24	48	72	96	120	144
Interleukins secreted at the level of the normal mice (pg.mL^{-1})	0	0	0	5	20	57	73
Interleukins secreted at the level of the NOD mice (pg.mL^{-1})	0	0	15	75	123	168	190

Document 3

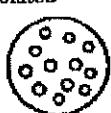
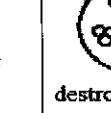
6. Explain the delay of secretion of the interleukin in the normal lot of mice.
7. Validate the tested hypothesis formulated in the part 4.

EXERCISE 9 Blood transfusion

A person lost a big quantity of blood after a car accident. The normal treatment in such case is a blood transfusion of similar blood type, but in its absence, a transfusion can be made starting from different blood types having compatible red blood cells. We consider in this case that the patient has blood type AB.

1. Specify, by referring to the text and to the acquired knowledge, the blood types that can substitute the type AB in a blood transfusion to this person.

This person had received blood from another person of blood type O. During the few hours after a complete transfusion of blood of type O, samples of blood of the patient were taken. These samples show a slight agglutination of blood that starts decreasing and disappears after 3 hours. In order to determine the cause of the appearance and then of the disappearance of this agglutination, an experiment was made. This experiment is schematized in the table in document 1. Note that boiling the serum destroys its proteins.

Tube	1	2	3	4
Isotonic solution of red blood cells of type AB in ml	2	2	2	2
Serum test anti-A in ml	-	1	1	1
Fresh serum of blood type AB in ml	1	-	0.5	-
Boiled serum of blood type AB in ml	-	-	-	1
Results: Aspect of red blood cells observed under microscope in the first hour.	Red blood cells intact and isolated	Agglutinated red blood cells (not destroyed)	Agglutinated red blood cells (not destroyed)	Agglutinated red blood cells (not destroyed)
				
Results: Aspect of the red blood cells observed under microscope after 3 hours	Red blood cells intact and isolated	Agglutinated red blood cells (not destroyed)	Lysed red blood cells (Hemolysis)	Agglutinated red blood cells (not destroyed)
				

Document 1

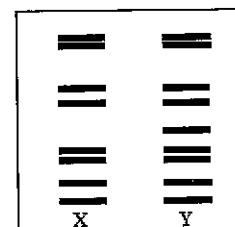
2. Interpret the obtained results in this experiment.
3. Explain the results obtained in the tube 3.

The manipulation of tube 3 is repeated by using the fresh serum of a person X of blood type AB that has an immunodeficiency (weak immunity) against extracellular bacteria. The results show the absence of the lysis of the red blood cells.

4. Formulate a hypothesis that explains the immunodeficiency of this person.

An electrophoresis of a group of proteins of the serum of the person X and of another normal person Y is made in order to validate the hypothesis mad above. The results are shown in document 2.

5. Starting from the analysis of the document 2, validate your hypothesis.



Document 2

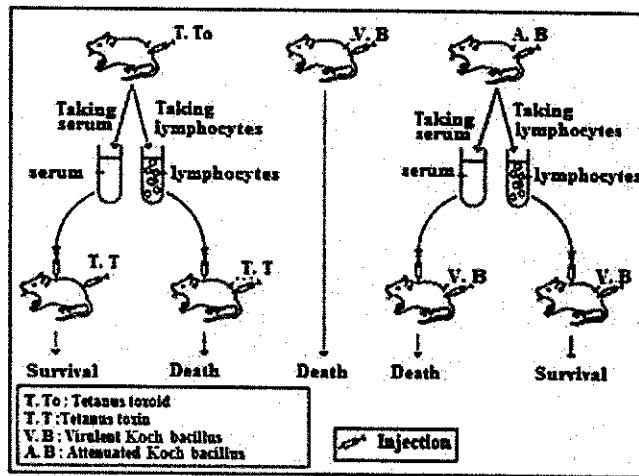
EXERCISE 10 Infection by a toxic bacterium

C1. Tetanus is a common component of the intestinal flora of herbivores. Daily, these animals excrete large amounts of spores that will contaminate the external environment. In case of accidental injuries that are protruding or containing a foreign body (such as a nail), the spores can develop and trigger tetanus.

In its acute form, tetanus infection results in generalized and intense muscle contractions, accompanied by sweating, causing respiratory distress and rapid death within 12 to 48 hours.

In order to study the immune response triggered against tetanus toxin in comparison with the response triggered against another bacterium (Koch bacillus), the experiment shown in document 1 was performed.

1. Describe the experiment shown by Document 1.
2. Interpret the obtained results.
3. Specify the type of immune response triggered against tetanus toxin.

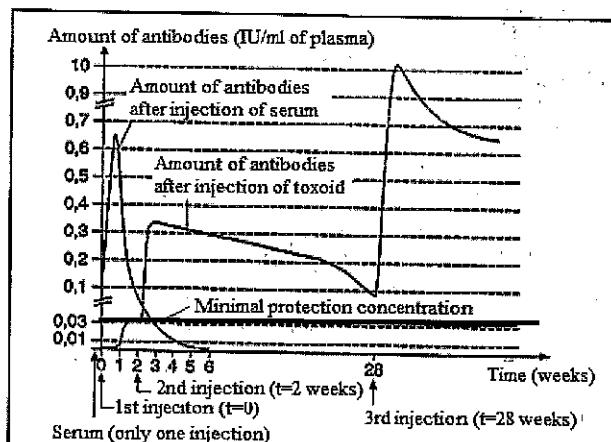
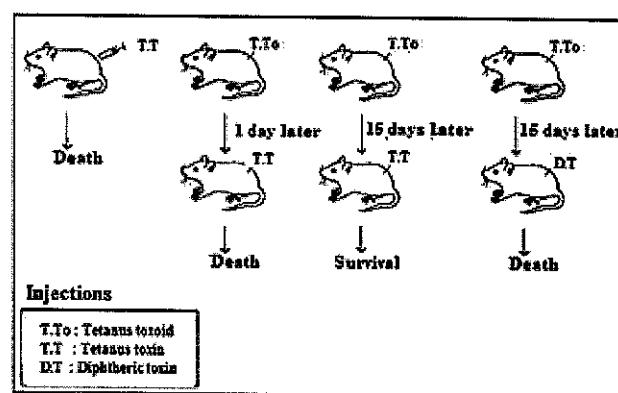


In order to study the characteristics of this immune response, another experiment was carried out, it is represented in document 2.

4. Specify, with reference to document 2, two characteristics of the immune response triggered against tetanus toxin.

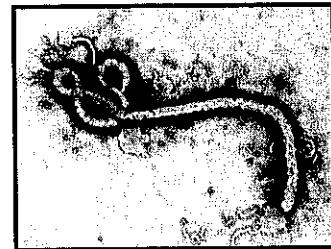
In a person who may have been contaminated following an injury, the doctor makes an injection of anti-tetanus serum (specific antibody of human origin), coupled with tetanus toxoid. Document 3 shows the evolution of the amount of antibodies in the blood of this person that result from the injection of serum and those resulting from the injection of the toxoid (1st, 2nd and 3rd injection).

5. Identify each of the two immunological techniques listed above.
6. Explain how the injection of tetanus toxin specific antibodies helps protect and eliminate toxin from the body.
7. Interpret the results obtained after the injection of serum.
8. Explain why the amount of antibodies obtained after the second injection of the toxoid is higher than that obtained after the first injection.



EXERCISE II Immunity against Ebola

Ebola virus disease (formerly known as Ebola haemorrhagic fever) is a serious, often fatal disease with a lethality rate of up to 90%. As the name suggests, it is due to the virus called Ebola EBOV (Document 1). The human being is infected by contact with infected animals or with biological fluids of infected persons. Most cases occur as a result of human-to-human transmission that occurs when blood, body fluids, or secretions from infected individuals enter the body of a healthy person through a skin lesion or mucous membrane.



Document 1

In order to understand the high lethality of EBOV, scientists have created a mutant EBOV version unable to produce a VP35 protein, this version does not cause the infection when injected to a person.

1. Derive from the text the role of the VP35 protein.

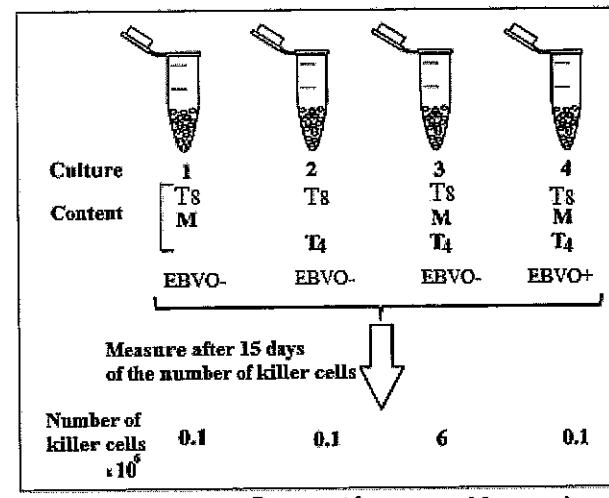
In order to determine the mode of action of the VP35 protein in the multiplication of EBOV; a series of experiments were performed as follows.

1st experiment: The number of killer cells specific to EBOV is measured under different experimental conditions in the presence of the virulent version EBOV + or mutant EBOV-. The results are shown in document 2.

2. Explain the role of killer cells in eliminating EBVO-infected cells.
3. Interpret the experimental results shown in Document 2.

Other experiments show the same results for plasma cells derived from B cell differentiation.

4. Indicate the role of plasma cells.
5. Explain the origin of the high lethality of EBOV.



Document 2

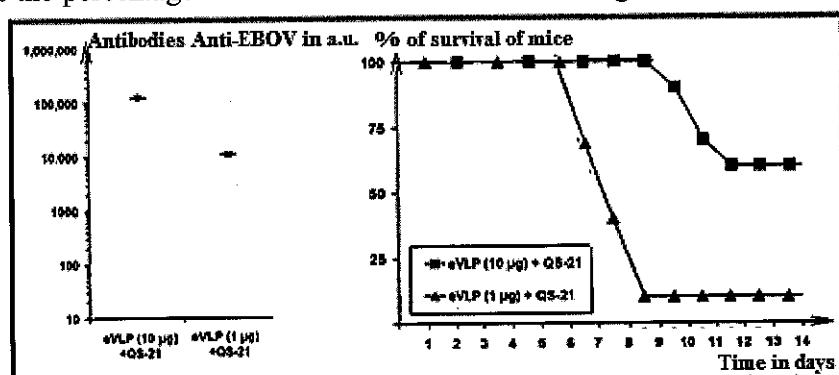
2nd experiment: As part of the EBOV vaccine efficacy study, two lots of mice were vaccinated with an EBOV viral antigen (evlp) extract injected at two different doses of 1 μ g and 10 μ g with adjuvant QS-21.

The antibody levels as well as the percentage of survival of the mice after being infected with the virulent virus are measured.

The results are shown in Document 3.

6. Specify the role of the specific humoral immune response in the defense against EBOV.
7. Analyze the results shown in Document 3.

- 7.2. What can you conclude?



Document 3

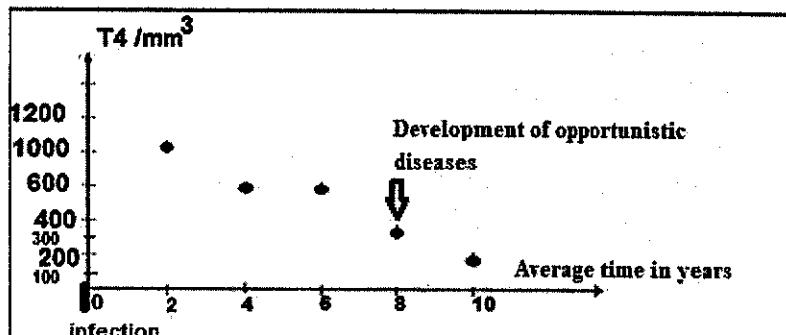
EXERCISE 12 AIDS and immunity

A.I.D.S. (Acquired Immunodeficiency Syndrome) is due to infection of the body with H.I.V. Every organism is, from a certain evolutionary stage, sensitive to a number of "opportunistic" microbial and viral diseases that lead to death. Document 1 shows the variation in the number of LT4 in an individual with HIV.

1.1. Analyze the results in document 1.

1.2. Conclude the cause of the development of opportunistic diseases.

The decrease in the number of T4 is caused by the destruction of these lymphocytes due to the multiplication of HIV.



Document 1

2. Explain the mechanism of multiplication of HIV inside the T4 cells leading to its destruction.

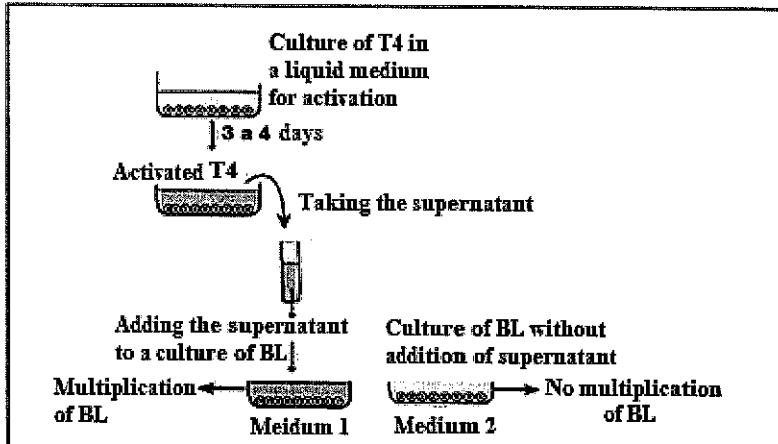
In order to determine the cause of opportunistic disease development after the destruction of LT4, we decide to study the role of T4 in the immune response. We realized the experiment represented by document 2.

3. Interpret the results of this experiment.

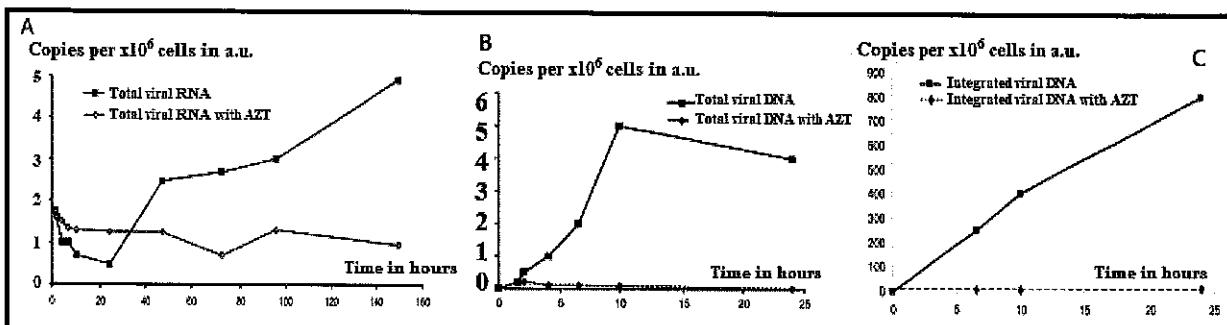
4. Indicate the contents of the supernatant.

5. Explain, from documents 1 and 2, the development of opportunistic diseases in an individual with HIV.

A drug called AZT is given to treat people infected with HIV. Document 3 shows the results of some experiments aimed to study the mode of action of AZT.



Document 2



Document 3

6. Deduce starting from document 3 the mode of action of AZT.

7. Explain how AZT prevents the development of opportunistic diseases.

EXERCISE 13 Immunity and cancer

Leukemia does not have any causes identified so far. Several risk factors may nevertheless favor the occurrence of the disease, including family history, a genetic anomaly, genetic disorders, exposure to high doses of radiation or to benzene, or chemotherapy.

The symptoms may differ depending on the type of leukemia, but most often, fatigue, enlargement of the lymph nodes, fever, episodes of depression, weight loss, bone or joint pain, anemia, frequent infections and unusual bleeding.

The diagnosis is made by performing a blood count, CBC, showing according to the type of leukemia a decrease or an increase in the number of white cells, and a drop in red blood cells.

For acute leukemias, intensive chemotherapy, bone marrow transplantation, anti-mitotics and corticosteroids drugs can be prescribed.

- 1.** Pick out from the text:

- 1.1.** Possible causes of leukemia.
- 1.2.** The name of the test that reveals the presence of "leukemia".
- 1.3.** The treatments used

A cancer results from the intense and anarchic proliferation of a cell of the body, following an alteration of its genetic program that allows it to express new peptides and change the rate of the expression of some proteins like PD-L1 that has an ability to inactivate Tc cells.

These cancer cells are recognized and destroyed by Tc but it is difficult for the body to completely eliminate these cancer cells.

- 2.1.** Explain how these cancer cells are recognized and destroyed by Tc cells.

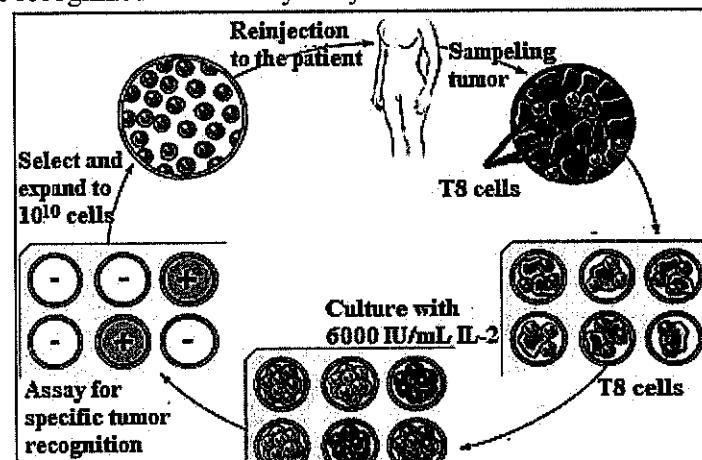
- 2.2.** Formulate a hypothesis to explain the inability of the body to completely eliminate these cancer cells.

Considering the high speed of multiplication of cancer cells, important progress has been made in cancer immunotherapy which is based on the technique in document 1.

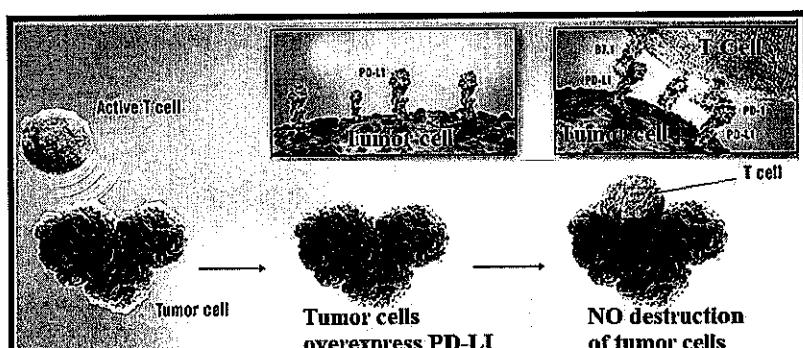
- 3.** Justify the use of IL-2 in this technique.
- 4.** Formulate a hypothesis explaining the limited effectiveness of this technique.

Many studies were done in order to understand the limited effectiveness of some immunotherapy techniques. Document 2 represents the summary of one of these studies.

- 5.** Explain, based on document 2 and on the preceding information, the limited effectiveness of the technique in document 1.



Document 1

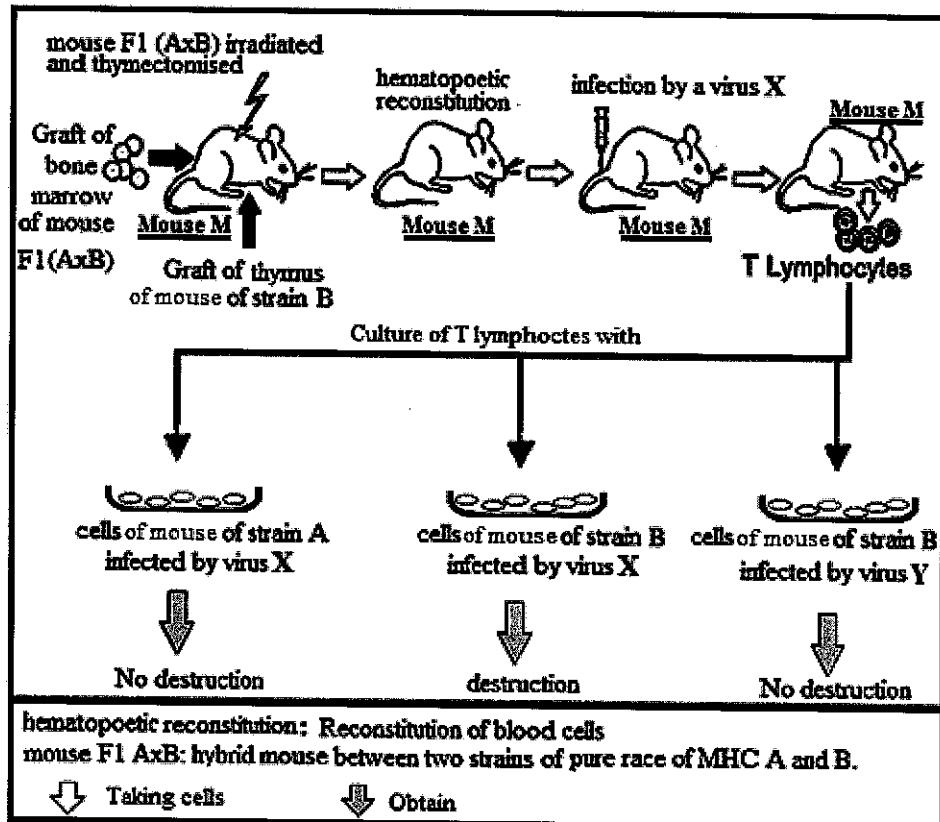


Document 2

EXERCISE 14 The acquisition of immune competence

All the immune cells of mammals and birds are produced in the bone marrow. However, in the mammals, a cellular population of leukocytes do not become immunocompetent unless it is kept in a small organ called the thymus while the other population acquires this immunocompetence in the bone marrow.

In the framework of studying some characteristics of the acquisition of immunocompetence in mammals, the experiment schematized in Document 1 were carried out.



Document 1

1. Describe the experiment schematized in document 1.
2. Interpret the results of this experiment.
3. Explain the mechanism of the destruction of the cells of strain B infected by virus X.

In the framework of the comparison between the thymus and another organ in the birds called bursa of Fabricius, an experiment is realized and represented in the table in document 2.

Experiments		Results
Ablation of the thymus of a mouse	Skin graft of other mice	Skin graft is not rejected
	Injection of antigens	Generally, no antibodies
Ablation of the bursa of Fabricius of a chick	Skin graft of another bird	Fast rejection of the graft
	Injection of antigen	No production of antibodies

Document 2

1. Explain the results obtained by the experiment done on the mouse.
2. Show that the role of the bursa of Fabricius is different than that of the thymus.
3. Specify if the deficiency of the thymus is more dangerous on the life of a mammal than the deficiency of the bursa of Fabricius on the life of a bird.



Immunity

Solved exercises solutions

Exercise 1 Specific immune response

1. How does the immune system neutralize the effect of different types of bacteria?
2. In both experiments, a lot of guinea pigs is immunized against a pathogen, which is the agent of diphtheria in experiment 1 but it is the Koch bacillus in experiment 2.
In both experiments, the serum and the lymphocytes are taken from the immunized guinea pigs and each injected into a different guinea pig which is then injected by the same active agent of the one that has immunized the initial lot, also in both cases an immunized guinea pig is injected again by the same active agent as the immunized one agent.
In experiment 1, the guinea-pig receiving the lymphocytes dies whereas the other two guinea-pigs survive, whereas in experiment 2 the guinea-pig receiving the serum dies, whereas the other two guinea pigs survive.
3. The guinea pig A immunized against diphtheria or KB survives following its injection respectively by diphtheria toxin or KB. But as only guinea pig D injected by the serum of B or C immunized against diphtheria survives following its injection by fatal diphtheria toxin, whereas the guinea pig E which has received B or C lymphocytes dies. Thus, a chemical substance of the serum is involved in the immune response against diphtheria toxin.
Whereas in experiment 2 it is only the guinea pig E which received the lymphocytes of B or C immunized against KB followed by an injection by the KB survives, but the guinea pig D injected by the serum of B or C followed by an injection by the KB dies. Thus, the lymphocytes are involved in the immune response against KB.
4. It is a specific humoral immune response against diphtheria toxin because it is the serum antibodies that protect the guinea pig and not the lymphocytes, the antibodies of the serum are the effectors of the specific humoral immune response.
It is a specific cell-mediated immune response against BK because it is the lymphocytes that protect the guinea pig and not the serum antibodies, some lymphocytes are the effectors of the specific cell mediated immune response.
5. Document 2 corresponds to the neutralization of an antigen by the antibodies whereas it is a specific humoral mediated immune response corresponding to experiment 1.
Document 3 corresponds to the lysis of an LTc-infected cell, whereas it is a specific cell-mediated immune response corresponding to experiment 2.
6. The mechanism of immune action in doc 2:
the toxins attack the target cells by binding to membrane receptors. When the antibodies bind to the toxins they cover their attachment sites on the target cells; and prevent the toxin from acting, this is the neutralization.
The mechanism of immune action in doc 3:
The cytotoxic cells recognize the infected cell and bind to the HLA-I- non-self peptide by the TCRs and release perforin that make polyperforine channels through the plasma membrane. then the Tc cell releases the granzymes that enter the polyperforine channel and triggers within the infected cell a chain of enzymatic reactions that lead to the degradation of the infected cell's DNA and leads to its death.

Exercise 2 Immunity against tumor

1. What are the body elements responsible for immune responses against cancer?
2. A tumor transplant of identical MHC develops 20 days after grafting in a mouse 1, the next 20 days shows a regression of the tumor. The same transplant carried out in a mouse 2 injected by the anti-LT4 Abs and in a mouse 3 injected by the anti-LT8 Abs, show the same development of the tumor after 20 days and develops in the following 20 days to end by the death of these mice.
3. If the tumor is of different MHC, it can be rejected due to the different MHC marker but not because of its tumor character. On the other hand, a grafted tumor from a different MHC mouse presents for the experiment two variable factors that do not allow us to draw the conclusion proper to the problem.
4. Tumor grafts identical MHC are grafted in three mice of the same strain, these grafts take the same aspects developed after 20 days but at the end of the following 20 days a regression of the tumor is observed only in the first mouse showing that the normal mouse is able to fight against the tumor, but the second injected by the anti-T4 Ab as well as the third mouse injected by the anti T8 Ab shows a significant development of the tumor after 20 days, to end in the death of two mice. This shows that the presence and cooperation between LT4 and LT8 is essential to fight against tumors.
5. Antibodies are proteins that have specific binding sites on another molecule. The anti-LT4 antibodies will therefore bind to a molecule present on the surface of T4 lymphocytes and thus agglutinate them. They will be neutralized and can't accomplish their role. It is a specific cell-mediated immune response whose effectors are LT8s that attack and destroy cancer cells but the LT4 activated by the macrophages (APC) must secrete IL2 to activate LT8, they remain inactive and no lysis of the cancer cells is obtained.
6. This experiment confirms the results of the first experiment by showing that LT8 are responsible for tumor regression and that LT8 should be activated by LT4 already activated by APC, hence in the first experiment the regression of the tumor is observed only after 20 days, time necessary for the activation of the LT8 whereas in the second experiment the injected LT8 contain the selected and activated clone. This is why the regression of the tumor is faster 10 days instead of 20.
7. The cytotoxic cell recognizes the cancer cell and binds with the TCR on the HLA-I - non-self peptide complex to its membrane, then it releases perforin that make a polyperforin channel across the plasma membrane; then the Tc cell releases the granzymes that enter the polyperforin channel and triggers within the infected cell a chain of enzymatic reactions that lead to the degradation of the infected cell's DNA and leads to its death.
8. The tumor weight is 1.6 g in the control mouse, it decreases to 1.2 g with fasting and decreases more to 1.1 g with treatment by gemcitabine, however this weight decreases much more with the combination of fasting with treatment by gemcitabine to 0.9 g. This indicates that fasting and gemcitabine help in the regression of the tumor. But since the experimented mice are totally immunodeficient so the action of fasting and gemcitabine is not in relation with the activity of the immune system, then it is independent from the action of T4 and T8.

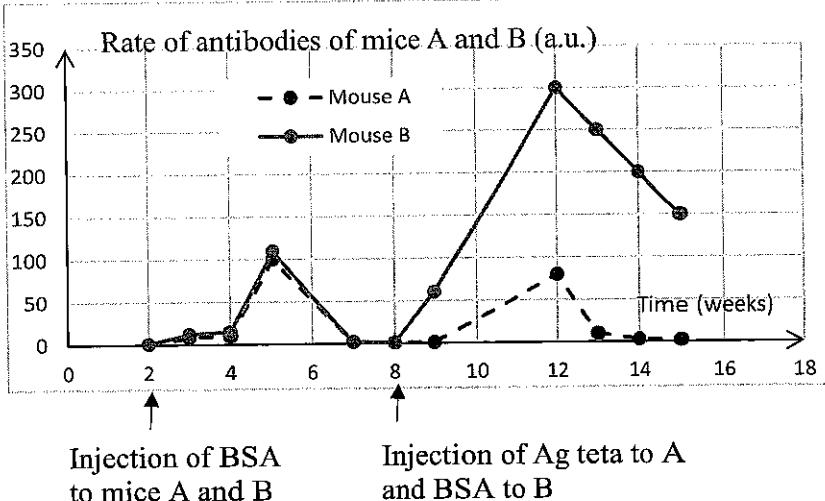
Exercise 3 The secretion of antibodies

1. Graph showing the variation of the rate of antibodies of the mice A and B after injection of antigens BSA and teta during time.

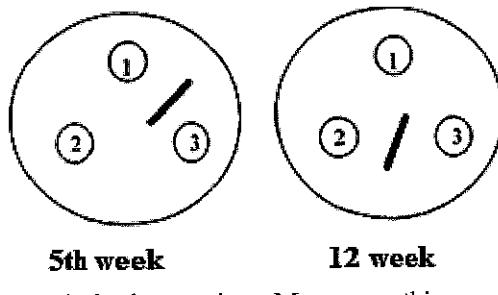
Scale:

50 a.u.

→ 2 weeks



2. Following the Injection of two Mice A and B by BSA the antibody rate increases in the two mice from 1 to 100 a.u. in the mouse A, and from 1 to 105 a.u. in the B mouse when the time passes from 2 at 5 weeks but beyond this time the antibody level decreases in the two mice, to become 1 a.u. at the 8th week, whereas when the mouse A is injected by the antigen teta the antibody level increases up to 80 at the 12th week but decreases rapidly until 2 at the 15th week but in the mouse B injected for a second time with the BSA antigen it increases rapidly up to 300 a.u. at the twelfth week a value 3.75 times higher than A but beyond this time the antibody level decreases to 150 a.u. and remains 75 times higher than that of A at the fifteenth week.
3. The secondary immune response is faster, more amplified and more persistent.
4. When an antigen enters the body, it causes a clonal expansion of the B cells specific to it. Each clone gives, on the one hand, short-lived plasma cells and, on the other hand, long-lasting memory cells that will be activated during the secondary response. The partial differentiation of these cells, prior to the second introduction of antigen, explains the short latency (faster). The high number of effector cells obtained from the activation of memory cells, results in an amplification of the immune response and a longer persistence of the response.
5. Results of the gel immunodiffusion test for mouse A at times 5 and 12 weeks.
6. Since adhesion and phagocytosis of bacteria are observed in low speed in the presence of macrophage only with a decrease with time, while they are with higher speed (double) in the presence of C3b fragment of the complement with no decrease with time, the same in the presence of the specific antibodies this means that C3b and antibodies amplify and keep adhesion and phagocytosis for longer time. Moreover, this speed increases again to the double when specific antibodies and C3b fragment are present together with no decrease with time. Thus, there is a cooperation between C3b fragment and specific antibodies in the amplification and the persistence of the speed of adhesion and phagocytosis.
7. The binding of antibodies to a bacterium activates the component C1 of the complement, which in turn activates the enzymatic cascade of complement, this cascade leads to the cleavage of C3 to give C3b fragment that amplify and keep adhesion and phagocytosis persistence. Since in the secondary immune response amount of antibodies is much rapid and higher than that of the primary, then the secondary immune response accelerates and amplifies the activation of the complement and the process of phagocytosis that permit a very fast elimination of the non self antigens.



Exercise 4 Role of T4 in the induction of the immune response

1. Antigens X placed in a medium containing gelatin are mixed with spleen cells of an animal not immunized with this antigen and then a number of cells are removed by washing, then the remaining cells are brought into contact with antigen X or in the presence of an antigen Y. With X there is a proliferation, with Y no proliferation.
2. 2-1. Is a given animal able to produce specific lymphocytes against an antigen before contact with it?
2-2. Hypothesis: The body is able to produce specific lymphocytes against an antigen before contact with it.
3. The lymphocytes bound by the antigens X are B lymphocytes because they are bound by free antigens since only B lymphocytes have membrane antibodies which are receptors capable of binding to free antigens.
4. Since some immune cells, of the non-immunized animal against antigen X, bind to this antigen, these immune cells proliferate in the presence of this antigen and do not proliferate in the presence of another antigen, this shows that the antigens bind cells that are specific to it. This validates the hypothesis tested that before the immunization against a given antigen there are immune cells that are specific to it.
5. During the production of lymphocytes, the bone marrow produces all the varieties of B lymphocytes each having membrane antibodies specific for a given antigen (rather for an epitope), these lymphocytes comprise autoreactive BL. During the maturation process, the bone marrow removes auto-reactive B-cells by recognizing them by self antigens, while the other lymphocytes directed against non-self antigens are preserved.
6. The culture of TL with BL in a medium containing antigens X gives very slight proliferation of BL, whereas this proliferation is very high in a medium containing BL, TL and macrophages. This means that the cooperation between TL and macrophages leads to high proliferation of BL.
7. Macrophages are responsible for the induction of the humoral immune response by activation of T lymphocytes which in turn activate B lymphocytes by the secretion of interleukins; in fact, macrophages (APC) present non-self peptides after their association with HLA-II to T4 lymphocytes which recognize them by TCR, then proliferate and differentiate into interleukin-secreting cells, interleukin-4 is able to activate B lymphocytes having recognized the antigen by their membrane receptors which are specific antibodies for the antigen. This will happen in both mice 1 and 2 in document 3, but this process needs 10 days to be accomplished in mouse 1 where the culture medium 1 supernatant is injected having no IL-4; whereas, after injection of supernatant of medium 2 containing IL-4, the process of activation of BL is induced directly and leads to a fast secretion of antibodies. These antibodies are able to protect the mouse 2, starting from day 3 earlier than mouse 1, by neutralizing the toxins.

Exercise 5 Specific humoral immune response

1. The red blood cells of the sheep are not harmful for the mice, moreover, the agglutination of these red blood cells is easily detected at the naked eye and their lysis (destruction) is simply observed by the appearance in the medium of empty spaces called hemolysis areas.
2. The lysis areas appear in the case of placing B lymphocytes with fresh serum, but when this serum is heated to more than 60°C the lysis areas do not appear. This shows that the serum contains a factor necessary (protein substances destroyed by heat) for the lysis of SRBC by B lymphocytes.
- 3.1. The complement.
- 3.2. This factor is not species-specific since any serum of a mammal is able to give the GRM lysis results.
4. The antibodies secreted by the B lymphocytes agglutinate the RBC, the component C1 of the complement gets activated by the constant region of the antibody bound on the RBC, this leads to the activation of the complement cascade resulting in the formation of a membrane attack complex by polymerization of the components C9 of the complement which results in the lysis of the red blood cell, the lysis of RBCs leads to the formation of areas.
5. Since the absence of bone marrow or thymus does not give rise to lysis, then the secretion of antibodies by B lymphocytes requires the intervention of bone marrow cells and thymus cells.

Exercise 6: Screening for the infection of HIV

1. A table showing the variation of the number of Tc lymphocytes in a person infected by the HIV as a function of time.

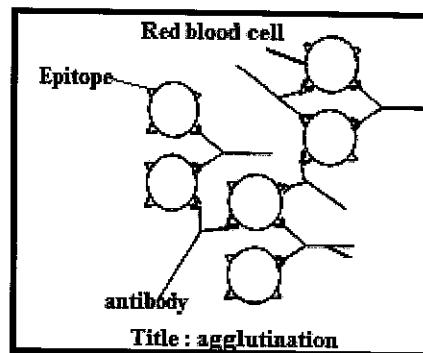
Time	In weeks			In years					
	1	12	24	1	2	5	8	10	12
Number of Tc lymphocytes in $10^6/\text{ml}$	7	17	32	53	68	63	63	30	0

2. The concentration of the virus increases from 0.3 a.u. to 2.4 a.u between the week 1 and 9 in the case where the quantity of the antibodies is null, then with the increase of the level of antibodies to 0.3 between 9 and 27 weeks the amount of the virus decreases to be null, this means that the virus multiplies in the absence of antibodies then the antibodies eliminate it.
3. During the phase A, the quantity of the virus increases during the first 9 weeks to 2.4 a.u. because of the multiplication of the virus in the cells of the body, then this quantity decreases with the increase of the antibodies because the antibodies neutralize the virus and prevent it from infecting new cells that will stop its multiplication, moreover the free HIV are phagocytized by the help of opsonization then they decrease.
4. Starting from the graph the amount of antibodies of 1.5 a.u. corresponds for a duration of infection of 24 weeks.
5. The amount of antibodies in C is greater than the infected one, and he has all the antigenic verities.
6. Gp 160 or Gp 120, since they are detected only in the person infected by HIV.

Exercise 7: Infection by the mononucleosis

- During the days following infection, the viruses are phagocytosed, digested and their peptides are associated with HLA class II molecules on the surface of macrophages that become antigen-presenting cells; these cells migrate to the nearest lymphatic ganglion where they come into contact with circulating T4 cells. The T4 cell, possessing a specific TCR for the complex HLA-II non-self peptide, will be activated, it will proliferate into a large number of identical cells, this is the clonal selection. Some cells become memory cells and the others differentiate into secretory cells of interleukin 4 which will activate the B cells that have recognized the non-self antigen by the membrane receptor. Activated B cells proliferate and differentiate after leaving memories to give antibody secreting plasma cells. These mechanisms necessitate around 7 days.

2.



- After the red blood cells are agglutinated and incubated with the serum of any mammal, the drop of blood takes on the appearance of the document 2-b because of the hemolysis of these red blood cells by activation of the complement by the antibodies fixed on the red blood cells, indeed the activation of the complement causes the formation by the complement cascade of a membrane attack complex resulting in the lysis of the red cell. This explains the severe anemia in patients who lose their red blood cells following their complement hemolysis.
- The injection of serum from an animal affected by mononucleosis with an injection of mononucleosis does not show agglutination of red blood cells by the same serum while the injection of this serum with plasmodium to an animal shows an agglutination of its red blood cells by the serum of an animal infected with plasmodium. This shows that the injection of serum from an animal infected with mononucleosis prevents the infection of another by this virus but does not prevent it from infection by another virus. It can be concluded that the serum of an affected animal is capable of specifically neutralizing the viruses that caused its infection.
- The body acts against the mononucleosis virus by secreting antibodies in the serum, these antibodies attach to the attachment sites of viruses to the cells to neutralize them and prevent them from infecting new cells, which prevents the antigens of the virus from being expressed on the surface of red blood cells. Then the red blood cells are protected from agglutination by anti-mononucleosis and lysis under the action of complement which prevents anemia.

Exercise 8 A kind of diabetes affecting mice

1.

Time in days		0	30	60	90	120	150	180
% of diabetic mice	with cyclosporine	0	0	0	5	10	10	10
	without cyclosporine	0	0	0	40	60	80	80

Table showing the percentage of diabetic rats as a function of time

2. The percentage of the diabetic mice in the two lots with and without cyclosporine is null between 0 and 60 days, it begins to increase to attain 10 % at 120 days in the mice with cyclosporine less than the mice without cyclosporine that is equal to 60 %, this latter continues its increase to 80 % at 150 days where it stays higher than that of the mice with cyclosporine that remain constant at 10 %, then both remain constant.
This means that the diabetes needs time to appear starting from birth and the cyclosporine attenuates diabetes.
3. The cause of diabetes of NOD mice is the T lymphocytes (auto reactive).
4. When a cytotoxic T cell recognizes one of the insulin-secreting cells of a Langerhans island and binds by the TCR on the HLA-I-peptide-self complex to its membrane, then it releases perforins that make a polyperforin channel across the plasma membrane; then the Tc cell releases the granzymes that enter through the polyperforin channel and triggers within the infected cell a chain of enzymatic reactions that lead to the degradation of the insulin-secreting cell's DNA and leads to its death.
5. Hypothesis: the activation of autoreactive T lymphocytes is caused by a high secretion of interleukin.
6. When the macrophage phagocytizes the antigen, it digests it and associates its peptides with HLA-II on the surface of its membrane, the macrophage becomes an antigen presenting cell or APC. The macrophage then migrates to the nearest lymph node and waits for T4 cells that circulate between the lymph nodes and the blood.
When the specific T4 arrives, it binds to the HLA-II-peptide complex by its TCR and becomes activated. Activated T4 cells proliferate and differentiate; some of them become memory cells, others complete their differentiation into secretory cells of interleukin 2 which intervenes in the activation of TC cells, hence the delay of 72 hours before the secretion of interleukin.
7. Between 0 and 24 hours the rate of interleukin remains null in the two lots of mice, it increases till 15 pg.mL^{-1} between 24 and 48 hours in the NOD mice higher than that of the normal mice that remains null, then it increases in the normal mice to 5 pg. mL^{-1} at 72 hours but remains lower than the NOD mice having 75 pg.mL^{-1} , then the two rates continue their increase to attain 73 pg.mL^{-1} in the normal mice that remains lower than the NOD mice that attain 190 pg.mL^{-1} .
Since in the normal mice the rate of interleukin is lower than that of the NOD mice then the hypothesis is validated.

Exercise 9 Blood transfusion

1. As the text indicates, in the absence of the same blood group, blood transfusion can be performed from blood groups with compatible red blood cells. In this case the patient has an AB blood group, so in his serum he does not have any agglutinin, all types of red blood cells of the other blood groups will be compatible for him, so groups A, B or O can substitute the group AB in a blood transfusion.
2. AB red blood cells mixed with 1 ml of fresh blood serum AB remain intact and isolated for three hours, while they are agglutinated after the addition of 1 ml of anti-A serum during the same period. This shows that the anti-A serum is responsible for the agglutination of red blood cells AB. On the other hand, these agglutinated red blood cells are destroyed after 3 hours if fresh serum of group AB is added but if this serum is boiled the red blood cells remain agglutinated without any lysis. This shows that the serum of group AB contains proteins which are responsible for the destruction of agglutinated red blood cells.
3. The red blood cells are agglutinated by the binding of specific antibodies to the agglutinogens present on the surface of red blood cells. The antibodies fixed by their variable regions on the agglutinogens are capable of activating the complement component C1 of the type AB serum which in turn activates the other components of the complement. Activation of the complement cascade results in the formation of a membrane attack complex by the polymerization of C9 components that results in the lysis of red blood cells.
4. Hypothesis: One (or more) of the complement components are inactive.
Or
One (or more) of the complement components is missing.
5. The electrophoresis shows in the person Y 9 bands that correspond to 9 different proteins, but in X there are only 8, which indicates that one of the components of this group of proteins is absent, which shows that one of the complement components is missing. (the hypothesis is validated for the hypothesis 1 and not for the hypothesis 2).

Exercise 10 Infection by a toxic bacterium

- 1 Tetanus toxoid is injected into a mouse A1, after 15 days, the serum and the lymphocytes are taken from this mouse, the serum is injected with tetanus toxin into a mouse A2 which remains alive, the lymphocytes are injected into a mouse A3 with tetanus toxin, it dies.
Another mouse B is injected by virulent bacilli, it dies.
A third mouse C1 is injected by attenuated bacilli, after 15 days, the serum and the lymphocytes are taken from this mouse, the serum is injected with virulent bacilli into a C2 mouse which dies, and the lymphocytes are injected with virulent bacilli to a mouse C3 that remains alive.
- 2 The mouse A2 survives after being injected with tetanus toxin and with the serum taken from a mouse previously injected with tetanus toxoid, on the contrary the mouse A3 dies after being injected with tetanus toxin and with the lymphocytes taken from the same mouse injected by tetanus toxoid. This shows that injection with tetanus toxoid induces immunity in the serum against tetanus toxin.
Mouse B dies after being injected with virulent bacilli. Similarly, the mouse C2 dies after being injected with virulent bacilli and with the serum taken from a mouse previously injected with attenuated bacilli, whereas the mouse C3 dies after being injected with virulent bacilli and with the lymphocytes taken from the same injected mouse by the attenuated bacilli. This shows that the injection by the attenuated bacilli induces an immunity by the lymphocytes against the virulent bacilli.
- 3 The immune response triggered against tetanus toxin is a specific humoral mediated immune response because it is the serum of the mouse injected with tetanus toxoid that protects the mouse injected with tetanus toxin, and the serum in this case contains specific antibodies against tetanus toxin that are the effectors of the specific humoral immune response.
- 4 The immune response triggered against the tetanus toxin is delayed (not immediate) because after a single day, it is not able to protect the mouse, but it protects after 15 days. And it is specific because it cannot protect against the diphtheria toxin injected into another mouse.
- 5 The injection of specific antibodies is called serotherapy, that of antigens is called vaccination
- 6 The elimination of the toxin from the body happens in two steps:
Neutralization of toxicity of toxin: toxins attack target cells by binding to membrane receptors. When antibodies attach to toxins by their variable regions, they cover their sites of attachment to the target cells; and prevent the toxin from acting.
Opsonization: the binding of antibodies to the toxin molecules by the variable regions, helps the phagocytes to identify these antigens by binding to the constant regions of the antibodies that have bound the toxin, then the antibodies create molecular bridges between the antigen and the macrophage thus facilitating its adhesion, this is opsonization then the macrophage will be able to phagocytose the antigens to eliminate them.
- 7 The amount of injected antibodies increases rapidly between 0.75 day from 0 to 0.65 IU / ml above the protection threshold, but it gradually decreases to reach the protection threshold which is 0.03 IU / ml towards the day 3, the rate continues to decrease to zero around 6 days. This shows that the injection of antibodies provides immediate and temporary protection.
- 8 The amount of antibodies obtained after the second injection of the toxoid is higher than that obtained after the first injection because the secondary immune response triggered after the second injection of the toxoid by the memory cells is faster, more amplified and more persistent than the primary response, because the memory cells are more numerous and more differentiated than the cells stimulated during the primary response.

Exercise 11 Immunity against Ebola

1. The role of the VP35 protein is to allow the virus to infect the body.
2. The cytotoxic cells recognize the infected cell and bind to the HLA-I - non-self peptide by the TCRs and release perforin that make polyperforin channels through the plasma membrane, then the Tc cell releases the granzymes that enter through polyperforin channel and trigger within the infected cell an enzymatic chain reaction that lead to the degradation of the infected cell's DNA and leads to its death.
3. T8 lymphocytes in the presence of EBVO-, put with macrophages or with T4 gives us a number of killer cells equal to 0.1×10^6 , this number will be much larger in the presence of T4 and macrophages at 6×10^6 , which indicates that there is cooperation of T8 with T4 and macrophages in order produce killer cells.
In contrast, T8 put with T4 and macrophages in the presence of EBVO + virus gives us a number of killer cells equal to that obtained in the first two cultures (1×10^6).
This shows that the EBOV + virus (the VP35 protein) inhibits the formation of killer cells.
4. The role of plasma cells is to secrete specific antibodies.
5. EBOV inhibits the formation of plasma cells and killer cells, which results in the inhibition of humoral and cell mediated specific immune responses, which allows the virus to infect new cells because it is not neutralized by antibodies and to multiply in these infected cells in the absence of killer cells that must lyse them. This explains the high lethality of EBOV.
6. The humoral mediated immune response is characterized by the production of anti-EBOV specific antibodies that are responsible for virus neutralization by attachment by their variable regions to their attachment sites on target cell receptors. This will prevent viruses from infecting new cells and multiplying inside.
- 7.1. The anti-EBOV antibody level is 100,000 a.u. in the case of the injection of $10 \mu\text{g}$ of eVLP with QS-21, it is 10 times greater than that obtained after the injection of $1 \mu\text{g}$ of eVLP with QS-21 (10,000 a.u.)
The% survival of the mice injected with $10 \mu\text{g}$ and $1 \mu\text{g}$ of eVLP with QS-21 is the same equal to 100% at time 0 days, it remains constant in the two batches of mice up to 6 days, but between 6 and 8.5 days this rate remains constant 100% in the mice injected with $10 \mu\text{g}$ of eVLP with QS-21 while it decreases to 10% a value 10 times lower in the mice injected with $1 \mu\text{g}$, this value remains constant between 8.5 and 11.5 and will be 6 times less than that of mice injected with $10 \mu\text{g}$ which decreases to 60%, both continue constant after 11.5 days and up to 14 days.
- 7.2. We can conclude that the high dose of antigen injected improves the efficacy of the vaccine against EBVO.

Exercise 12 AIDS and immunity

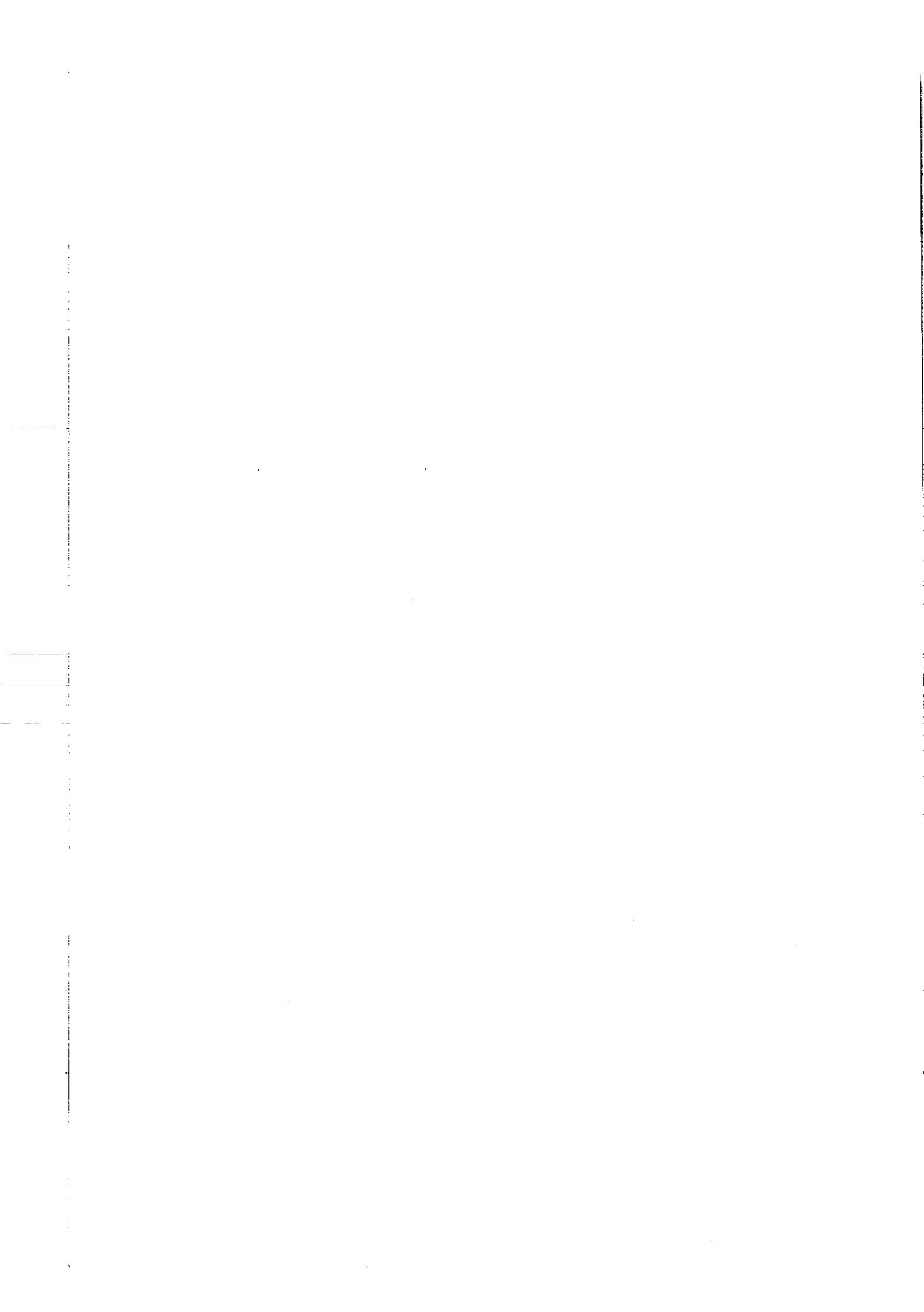
- 1.1. 2 years after HIV infection, the number of T4 begins to decrease progressively from $1000 / \text{mm}^3$ to $300 / \text{mm}^3$ after 8 years of infection, the time of emergence of opportunistic diseases. This same number continues its decrease to reach at 10 years of infection $200 / \text{mm}^3$ of blood.
- 1.2. Therefore, a decrease of the number of T4 less than $300 / \text{mm}^3$ is the cause of the development of opportunistic diseases.
2. HIV binds to the CD4 receptor on the surface of T4 cells, then it penetrates its RNA which is transcribed by the reverse transcriptase enzyme into DNA which is then integrated into the genome of the host cell. The transcription and translation of the viral DNA results in the formation of new copies of the viral RNA and proteins of its envelope. These two components are assembled to form new viruses that bud on the cell surface. This rapid multiplication of virus results in the death of the T4 cell.
3. A multiplication of the BL in the first medium that contains BL with the supernatant of the LT4 activated in contrast to the BL that does not multiply in the second medium containing BL only. This shows that the multiplication of BL requires the presence of a substance secreted by active T4 in the supernatant.
4. Interleukin 4
5. According to doc1: a number of T4 greater than $300 / \text{mm}^3$ of blood is essential to prevent the development of opportunistic diseases.
According to Doc 2 multiplication of BL requires the secretion of a substance by T4. T4 secretes interleukin 4, which stimulates the proliferation of BL, which leads to efficient antibody production.
In an individual with HIV the virus destroys T4 cells; the number of T4 decreases to less than $300 / \text{mm}^3$ of blood, the triggered immune response becomes ineffective allowing the development of opportunistic diseases.
6. The number of copies of viral RNA obtained without AZT reaches $5 \text{ a.u.} / 10^6 \text{ cells}$ but decreases to 1, 5 times less with AZT after 160 hours, and the viral DNA reaches $5 \text{ a.u.} / 10^6 \text{ cells}$ without AZT and decreases to be almost zero after 25 hours, similarly, integrated viral DNA increases without AZT to reach $800 \text{ a.u.} / 10^6 \text{ cells}$ in 25 hours, unlike the case with AZT where the integrated DNA remains zero. Therefore, AZT attenuates the duplication of viral RNA by inhibiting the synthesis of viral DNA and consequently its integration in the host genome.
7. AZT prevents the duplication of viral RNA by preventing the transcription of this RNA into DNA and consequently the integration of the viral DNA necessary for the transcription of the viral RNA, which prevents the multiplication of the virus in the T4 cells and protects them from destruction by HIV, this will keep the number of T4 higher than $300 / \text{mm}^3$ of blood, which will prevent the development of opportunistic diseases.

Exercise 13 Immunity and cancer

- 1.1. Several risk factors may nevertheless favor the occurrence of the disease, including family history, a genetic anomaly, genetic disorders (Down's syndrome and Franconi's disease), exposure to high doses of radiation or benzene, side effects of chemotherapy.
- 1.2. CBC.
- 1.3. For acute leukemias, intensive chemotherapy, bone marrow transplantation, antimitotics and corticosteroids may be prescribed.
- 2.1. By the double recognition the Tc recognizes by its TCR the new non-self peptide carried by the HLA class 1, it secretes the perforin to form polyperforin channels through the membranes of the infected cells, then the granzymes are secreted, they penetrate through the channels of polyperforin inside the cell to trigger an enzymatic reaction cascade leading to the destruction of the DNA which causes the death of the cancer cell.
- 2.2. Hypothesis: The cancer cells proliferate rapidly so that the immune system is quickly overwhelmed.
3. IL-2 is secreted by T4 cells to induce the proliferation of T8 cells, it is used in this technique to promote the invitro selective proliferation of specific T8 cells in order to balance the rapid multiplication of cancer cells.
4. Hypothesis: Cancer cells change peptides which makes the Tc already activated ineffective.
5. Cancer cells change the rate of expression of some proteins like PD-L1 that is able to inactivate Tc cells. The technique in document 1 aims to increase the number of Tc cells; document 2 shows that the tumor cells overexpress PD-L1 protein, this protein binds on the Tc cell receptors causing the inactivation of these cells and the inhibition of the destruction of tumor cells that explain the low effectiveness of this technique.

Exercise 14 The acquisition of immune competence

1. A hybrid F1 AxB mouse (M) between two pure races of MHC A and B, irradiated and thymectomized, underwent a bone marrow transplant of an F1 hybrid mouse AxB and a thymus of a mouse B, then a hematopoietic reconstitution is submitted in this mouse then it is infected with a virus X. After that T cells are removed; these lymphocytes are cultured with cells of line A infected with the virus X and cells of line B infected with the virus Y, we do not obtain any destruction of the cells, on the other hand, these T lymphocytes are cultivated with cells of line B infected with the virus X, we obtain a destruction of these cells.
2. No destruction of the cells of line A is obtained after culture with T lymphocytes of mice AxB with bone marrow AxB and thymus of line B, similarly when the cells are of the same thymus but infected by a virus Y, on the other hand, these cells are destroyed when they are of the same line as the thymus and are infected by the same virus X. This shows that the thymus induces an immune response against the cells of its line which are infected by the same virus used in immunization.
3. By the double recognition the LTc recognizes by its TCR the non-self peptides of the virus X carried by the MHC B class I, it secretes the perforin to form polyperforin channels through the membranes of the infected cells, then the granzymes are secreted, they penetrate through the polyperforin channels inside the cell to trigger an enzymatic reaction cascade leading to the destruction of the DNA which causes the death of the infected cell.
4. The thymus is the place of maturation of T4 and T8 lymphocytes. T4 cells are necessary for the induction of the humoral and cellular immune response after recognition of the MHC-II non-self peptide on the surface of an APC. LT4 secretes interleukin-2 to activate Tc, effectors of graft rejection, and interleukin-4 to activate B that secrete antibodies, so, in the absence of LT4, there is no induction of humoral and cellular immune responses. So, no graft rejection or antibody secretion.
5. In the absence of the bursa of Fabricius, there is a rapid rejection of the graft but an absence of antibody secretion, which differs from the results in the absence of the thymus, the Fabricius does not play a role in the rejection of the graft unlike the thymus. Then the bursa of Fabricius has a role different from that of the thymus.
6. The thymus deficiency is more dangerous than the bursa of Fabricius deficiency because the first suppresses the specific humoral and cell mediated immune responses, which causes infection by extra and intracellular agents. But the bursa of Fabricius only suppresses the specific humoral response which causes infection by extracellular agents only.



Immunity

Non-solved exercises

EXERCISE I Immunity against hepatitis

Hepatitis A is due to a virus that infects the liver cells. The patients show a liver necrosis by lysis of hepatic cells. The immune system induces an immune response that is able to neutralize the multiplication of the virus and to eliminate it from the body.

In order to study the nature of the immune responses triggered against hepatitis virus; we make the electrophoresis of serum proteins of an affected person and a healthy one, the results of the measurements of the corresponding proteins are shown in the table below.

Person	Albumin	α globulins	β globulins	γ globulins
Healthy	50	10	12	15
Affected	51	11	13	30

Document 1

1. Indicate the nature of the immune response that is able to:
 - 1.1. Inhibit the multiplication of hepatitis virus.
 - 1.2. Eliminate hepatitis virus from the body.
2. Specify the nature of the immune response revealed by document 1.

To determine the cells of the immune system that are involved in the induction of the immunity shown above, another experiment was made where immune cells were taken as follows:

- Macrophages (M_1), B lymphocytes (BL), and T lymphocytes (TL) from a person (P_1) affected by hepatitis.
- Macrophages (M_2) from another person affected by hepatitis (P_2), having different HLA from that of P_1 .

We realize, starting from these cells many cultures, document 2, in which we search for plasma cells (antibody secreting cells). The conditions and the results are represented in document 2.

Culture	1	2	3	4	5
Adding	BL+M1	TL+M1	BL+TL	BL+TL+M1	BL+TL+M2
Plasma cells	Null	Null	Null	Many	Null

Document 2

3. Interpret the obtained results.
4. Explain the result of the culture 5.

Starting from the TL and the hepatic cells of the patients P_1 and P_2 , we make four cellular cultures. The table of the document 3 shows the results of the different cultures after three days.

Medium	Medium with LT of P_1	Medium with LT of P_2
healthy hepatic cells	No lysis of the hepatic cells	No lysis of the hepatic cells
infected hepatic cells of the patient P_1	Lysis of the hepatic cells	No lysis of the hepatic cells

Document 3

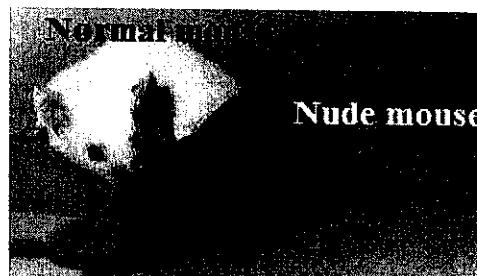
EXERCISE 2 Relation between different immune cells

Four lots of adult mice are subjected to many experiments; lot A is a control. Lots B, C, and D are submitted to an irradiation, a treatment that destroys the bone marrow stem cells, and to the ablation of the thymus. In addition, the lots B and D are injected with thymus cells and bone marrow cells respectively taken from a normal rat of the same strain, while the lot C is injected by both thymus cells and bone marrow cells. The four lots are then subjected to the injection of SRBC. Many days later the serum is taken from them and added to a suspension of SRBC in physiological water, an agglutination of SRBC is observed in the preparations of lots A and C only.

1. Tabulate the experimental conditions and results.
2. What will happen if the injected cells are from a mouse of different strain (different MHC)? Justify.
3. Interpret these experiments.
4. Specify if these experiments are sufficient to determine the cells that are responsible for the agglutination reaction.

A nude mouse is a mutant mouse deprived of its thymus from the birth.

5. Specify how the deprivation of the mouse from its thymus affect the populations of the leucocytes.
6. Show that the nude mouse is deprived of both humoral and cell mediated immunity.

**Document 1**

In order to study the mode of relation between the two types of lymphocytes used in the experiment described above; the experiment summarized in the table below was made.

Medium components	Medium 1	Medium 2
Spleen cells of nude mouse	+	+
SRBC	+	+
Supernatant of a spleen cells culture of normal mouse with SRBC	-	+
Results	No agglutination of SRBC	Agglutination of SRBC
Legend: (+) presence (-) absence		

Document 2

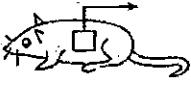
Taking into consideration the role of the thymus and B cells.

7. Determine the mode of communication between the TL and BL.
8. Explain the processes of immunity responsible for the activation of the lymphocytes leading to the agglutination.

EXERCISE 3 The rejection of a graft

Skin grafts are performed in mice according to the modalities on the document.

Notes: mice A, B, R, N * are not related. The mouse N *is devoid of the thymus starting from birth.

DONNEURS	LOTS RECEVEURS		
souris A	EXPÉRIENCE 1 souris R  greffon A1		 Rejet greffon A1 : cicatrice A1
	EXPÉRIENCE 2 souris R (expérience 1)  cicatrice A1 greffon A2		Rejet greffon A2 : cicatrices A1 + A2
souris B	EXPÉRIENCE 3 souris R (expérience 1)  cicatrice A1 greffon B		 Rejet greffon B : cicatrices A1 + B
	EXPÉRIENCE 4 souris N*  greffon B		 pas de rejet
	1er jour : GREFFE	6ème jour : APRÈS GREFFE	11ème jour : APRÈS GREFFE

Document 1

1. Describe the skin grafting experiments mentioned in document 1.
2. Explain, by referring to the document; why transplant rejection reveals the specificity of the immune response.
- 3.1 Compare experiments 1 and 2,
- 3.2. Conclude a characteristic of the secondary immune response.
4. Deduce, based on experiments 1 and 4, the cells responsible for graft rejection.

EXERCISE 4 Immune response against the graft

Some experiments of grafting are made on mice of different strains A, B and C. The strain is a group of mice that have the same MHC molecules. These experiments are represented in the table below.

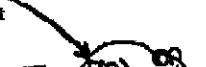
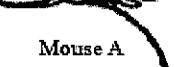
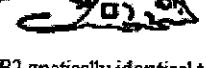
	Donor	Recipient	Results
Exp N°1	 Mouse A	 Mouse A' genetically identical to A	Acceptation of the graft
Exp N°2	 Mouse A	 1st graft of A Mouse B1	Rejection of the graft of A by B after 10 to 12 days
Exp N°3	 Mouse A  Mouse C	 2nd graft of A graft of C Mouse B1	Rejection of the 2nd graft of A by B1 after 2 to 3 days Rejection of the graft of C by B1 after 10 to 12 days
Exp. N°4	 Mouse A	 Exp. 4 a. Mouse B2 genetically identical to B1 and had receiving lymphocytes from B1 taken after the rejection of the first graft of A (see exp. 2)	Rejection of the graft of A by B ₂ after 2 to 3 days
		 Exp. 4 b. Mouse B3 genetically identical to B1 and had receiving serum from B1 taken after the rejection of the first graft of A	Rejection of the graft of A by B ₃ after 10 to 12 days
		 Exp. 4 c. Mouse B4 Mouse genetically identical to B1 and had submitted to the ablation of the thymus at birth	No rejection of the graft of A by the mouse B4

Table showing experiments of grafting made on mice of different strains.

1. Write two conclusion that can be taken from the experiments 1 and 2.
 2. Show, starting from the experiments 2 and 3; that the immune response is specific.
 3. Interpret the results of the experiment 4 by comparing it with the suitable control.
- After two weeks from the infection of the mouse A by a virus of hepatitis, we culture the lymphocytes of this mouse with the infected hepatic cells of the mouse B, no lysis had observed. On the other hand, these same lymphocytes are able of lyse the infected cells by the hepatitis of the mouse A.
4. Explain these results.
 5. Explain, by referring to the acquired knowledge, the mechanism of lysis of the infected cells by the lymphocytes.

EXERCISE 5 Induction of the immune response

Hepatitis B is due to a virus that infects the liver. The patients show a liver necrosis by lysis of hepatic cells.

An electrophoresis of serum proteins of an affected person and a healthy one was done. The results of the measurements of the corresponding proteins are shown in the table below.

Person		Albumin	α globulins	β globulins	γ -globulins
Rate of plasma proteins in a.u.	Healthy	50	10	12	15
	Infected	51	11	13	30

Document 1

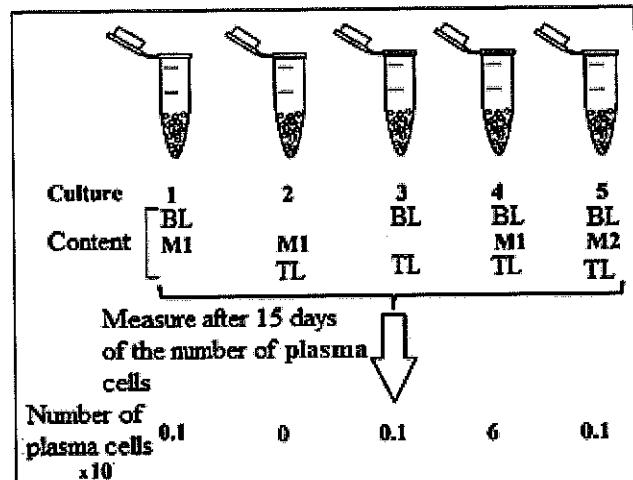
Taking into consideration the nature of γ globulins:

- 1.1.** Interpret the results of doc. 1.
- 1.2.** Conclude the type of immune response implicated in the defense against hepatitis virus.
- 2.** Explain the role of the antibodies in the protection of against the multiplication of the virus.

In order to determine the relation between the B lymphocytes and the other immune cells in the induction of the immune response, some experiments are carried out using:

- Macrophages (M₁), B lymphocytes (BL), and T lymphocytes (T L) from person (P₁).
- Macrophages (M₂) from another (P₂), having different HLA.

We realize several cultures in the presence of SRBC (sheep red blood cells). They are shown in the document 2, in which we search for plasma cells.

**Document 2**

- 3.1.** Pick out the problem studied by this experiment.
- 3.2.** Indicate one morphological characteristic able to identify the plasma cell.
- 3.3.** Interpret the results of this experiment.
- 4.2.** Conclude the relationship between B cells and the other immune cells in the induction of the immune response

From culture 4 of the experiment illustrated in Document 2, the antibodies are extracted and incubated with SRBC in 4 separate boxes for a suitable time. To these boxes macrophages of different origins or complement proteins of different origins are added.

Box	Added elements	Obtained results
1	No addition	Agglutination
2	Macrophages M1	Disappearance of SRBC
3	Macrophages M2	Disappearance of SRBC
4	Complement proteins of P1	Lysis of SRBC
5	Complement proteins of P2	Lysis of SRBC

- 5.** Explain the mechanism leading for the results obtained in the boxes 4 and 5.

6. Schematize the phenomenon leading for the disappearance of the SRBC in the boxes 2 and 3.

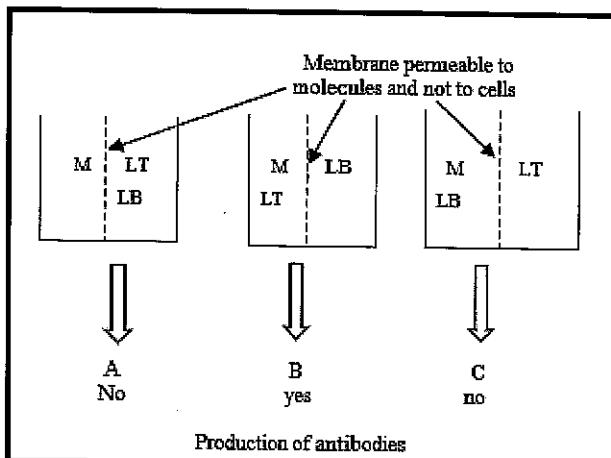
Document 3

EXERCISE 6 Production and characteristics of antibodies

We know that lymphocytes communicate between each other's to induce an immune response characterized by the production of antibodies.

In order to determine the mode of this communication, we carry out the experiment schematized in document 1.

1. Describe this experiment.
2. Determine, by referring to document 1, the mode of communication between these three cells.
3. Indicate the type of T lymphocytes that are behind the production of antibodies. Justify.
4. Explain, based on the results of this experiment, why the macrophage must migrate towards the nearest lymphatic node after phagocytosis.

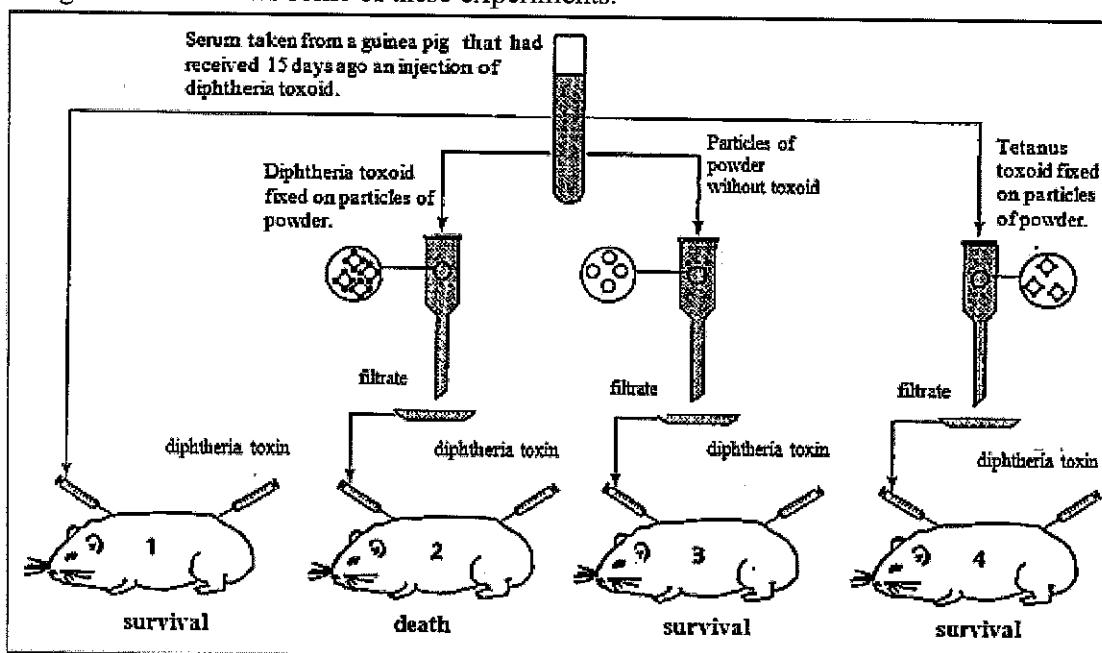


Document 1

During a vaccination against Diphtheria, the subject receives diphtheria toxoid. He then develops in few days an immune response characterized by the production of antibodies found in the internal medium. Experiments are made to determine the mode of action of these antibodies in the defense against diphtheria toxin.

5. Explain, by referring to the results of the experiment shown in document 1, the mechanism of the production of antibodies against diphtheria toxin.
6. Indicate the role of the antibodies in the defense against diphtheria toxin.

The figure below shows some of these experiments.



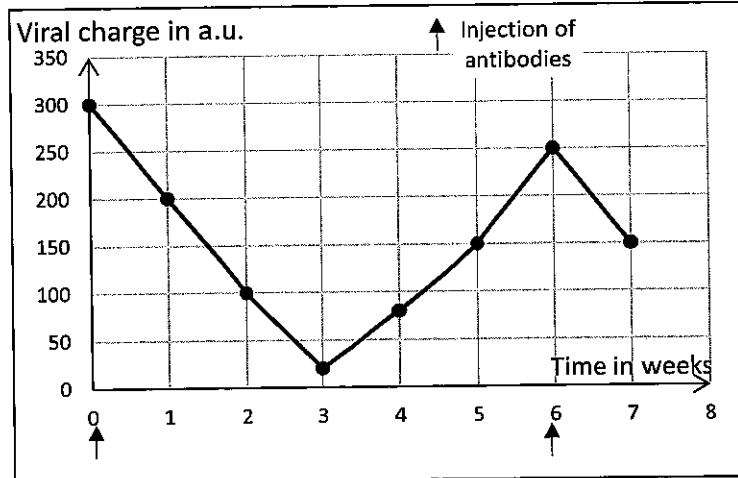
Document 2

7. Deduce, based on the experiment in document 2, two properties of the antibodies.

EXERCISE 7 Cooperation of immune responses against the intracellular agents

A patient undergoing an immunosuppressive treatment consults his doctor for repeated infections by a virus A. His doctor decides to make two kinds of treatment: the first consists of injecting him with amounts of antivirus antibody, prepared from individuals vaccinated against the same virus A. The second treatment consists of injecting him with T lymphocytes of his identical twin vaccinated against the same virus A. After each treatment the concentration of the viruses is measured in the serum of this individual at regular intervals of time. The results of measurements after the injection of the antibodies are represented in document 1.

- 1.** Determine, based on document 1, the physiological importance of the antibodies.



Document 1

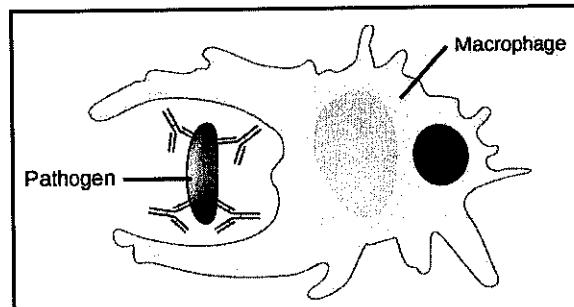
After the injection of T lymphocytes alone or with injection of antibodies the obtained results are shown in the table in document 2.

Time in days		0	1	2	3	4	5	6	7
Viral charge in a.u.	Injection of the T lymphocytes of the vaccinated twin at t=0	300	250	200	150	100	50	20	0
	Injection of antivirus A antibodies and T lymphocytes of the vaccinated twin.	300	200	100	20	0	0	0	0

Document 2

- 2.** Represent the data given by table in document 2 in a graph.
- 3.** Compare the results of two treatments shown by the table in document 2.
- 4.** Conclude the role of the antibodies in the elimination of the virus from the body.
- 5.** Justify why it is necessary for the lymphocytes T to be taken from the identical twin of the infected individual in order to be able to eliminate the virus from the body.
- 6.** Explain the mechanism of the elimination of the viruses by the injected T lymphocytes.

The figure in document 3 represents an important biological process made by the macrophages with the help of antibodies.



Document 2

- 7.** Explain the importance of this process in the elimination of the viruses from the body.

EXERCISE 8 Immunity against hepatitis

Hepatitis B (HBV) is caused by a virus (document 1) that gets into the cells of the liver.

To study the immune reactions of the body following an infection with this virus, the following experiments are carried out: Hepatitis B is caused by a virus (document 1) that gets into the cells of the liver.

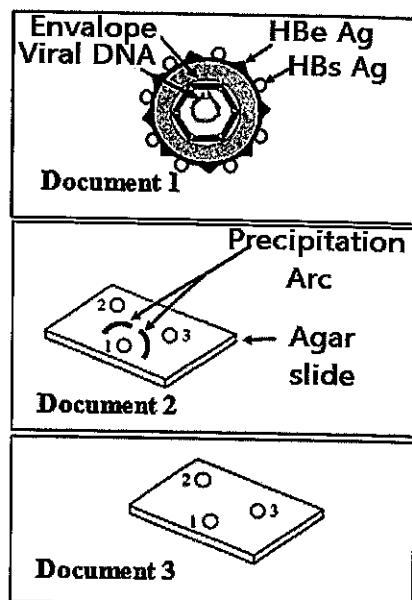
To study these immune reactions, the following experiments are carried out:

1st series of experiments

It is taken from an individual with hepatitis B (Patient X) of the blood serum and the HBs and HBe viral particles are purified.

In addition, a glass slide is covered with agar (nutrient medium for the diffusion of the substances) in which three wells are hollowed out:

- In well 1 of blood serum of patient X,
- In the well 2 a solution of HBs viral particles,
- In the well 3 a solution of HBe viral particles.



The results are represented in document 2.

The result of the same experiment carried out with the blood serum of a healthy individual is observed in document 3.

1. Justify that the infection with HBV induces the production of specific antibodies.
2. Explain, using schema, the action of the serum on the HBV naming the phenomenon at the origin of the formation of precipitation arcs.

2nd series of experiments

To study another aspect of the immune response against HBV, a second series of experiments is carried out. The experiments and their results are summarized in the following table:

Experiment	Results
Isolation of lymphocytes of patient X	Lymphocytes from patient X
Culture of hepatic cells infected by HBV from patient X and cells from patient Y in the presence of lymphocytes from patient X	1st case: Culture of cells X infected by HBV 2nd case: Culture of cells Y infected by HBV
Microscopic observation After 2 days	Two micrographs showing cellular changes over 2 days.

Document 4

3. Interpret these experiences.
4. Justify the duration of 3 days of culture.
5. Explain, from what preceded, the mechanism of the fight against the HBV.

EXERCISE 2 Fight against a virus

When a mouse is infected with an X virus, there is a hypertrophy of the lymph nodes. A sample in such a ganglion reveals the presence of a group of lymphocytes among which that represented by document 1. In an uninfected mouse, there is no hypertrophy of lymph nodes and a lymph node sample does not show accumulation of such lymphocytes.

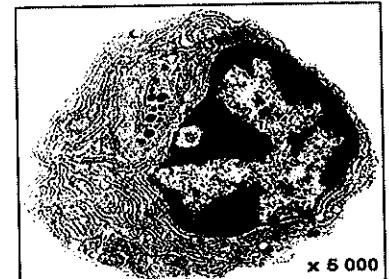
Document 2 shows the evolution of immunoglobulin levels in a mouse infected with the X virus, and in an uninfected mouse.

1. Identify the cell of document 1.
2. Interpret the experimental results in document 2.

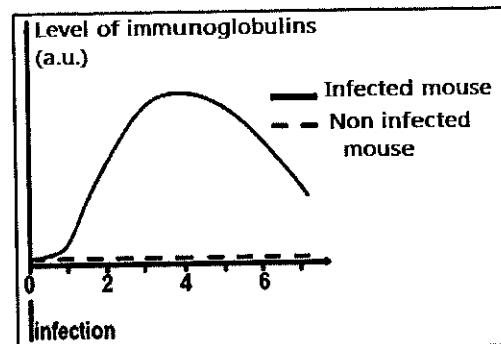
A virus X is inoculated with a mouse of strain A. 10 days later, the lymphocytes of this mouse are removed. These lymphocytes are then brought together as represented in document 3.

3. Show that the lymphocytes only destroy infected cells.
4. Verify that the lymphocytes perform double recognition to recognize infected cells.
5. Show that the lymphocytes are specific for a single antigen to which they are sensitized.

Document 4 is a photograph taken by electron microscopy from a sample in the second case of document 3.



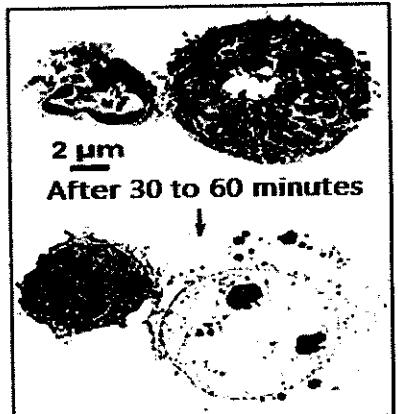
Document 1



Document 2

Injection of virus X to mouse of strain A	Virus X → Mouse of strain A			
After 10 days, lymphocytes are isolated	Lymphocytes of mouse of strain A			
Culturing lymphocytes with cells	1st case	2nd case	3rd case	4th case
	Non infected cells of strain A	Cells of strain A infected by virus X	Cells of strain B infected by virus X	Cells of strain A infected by virus Y
Results	No cell destruction	Destruction of infected cells	No destruction of infected cells	No destruction of infected cells

Document 3



Document 4

EXERCISE 10 The fight against cancer

In order to study the defense of the body against tumor cells (cancerous), for this, the following experiments and observations are realized.

Mouse S is immunized against foreign tumor cells and then the experiments carried out are summarized in document 1.

1. Determine the conditions necessary for the destruction of cancer cells.
2. Identify the type of immune response developed against the tumor cells.

In 1960 a theory made it possible to set up immunotherapy proposing two treatments to fight against this disease.

Treatment 1: Repeated intravenous injections of interleukin II are given to patient with cancer, and then the number of total lymphocytes is measured over time.

The results of the assay are represented by document 2.

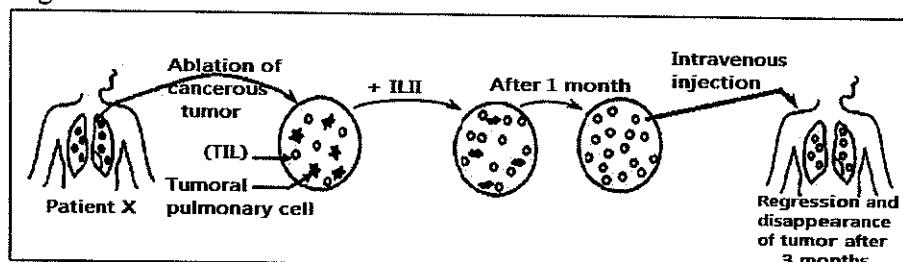
Observation1: A remarkable regression of the tumor in the sick person was noticed after treatment 1.

1. Specify the effect of ILII treatment.
2. Explain how treatment 1 leads to regression of the cancerous tumor in the sick subject.
3. Formulate a hypothesis to explain why this treatment causes the regression of the cancerous tumor but unable to make it disappear.

Treatment 2: To improve this therapy a second treatment, called adoptive immunotherapy was practiced in the sick subject. The stages of this treatment are represented in document 3.

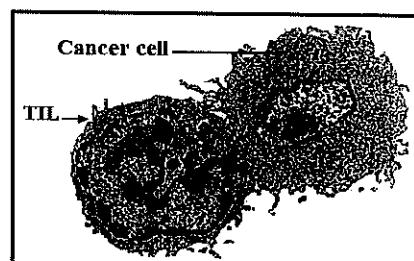
TIL: lymphocytes infiltrating the tumor

Ultramicroscopic observations of the pulmonary cancer cells in the presence of the TILs taken from the patient X having undergone the injection of the TIL alone during the second treatment are shown in document 4.

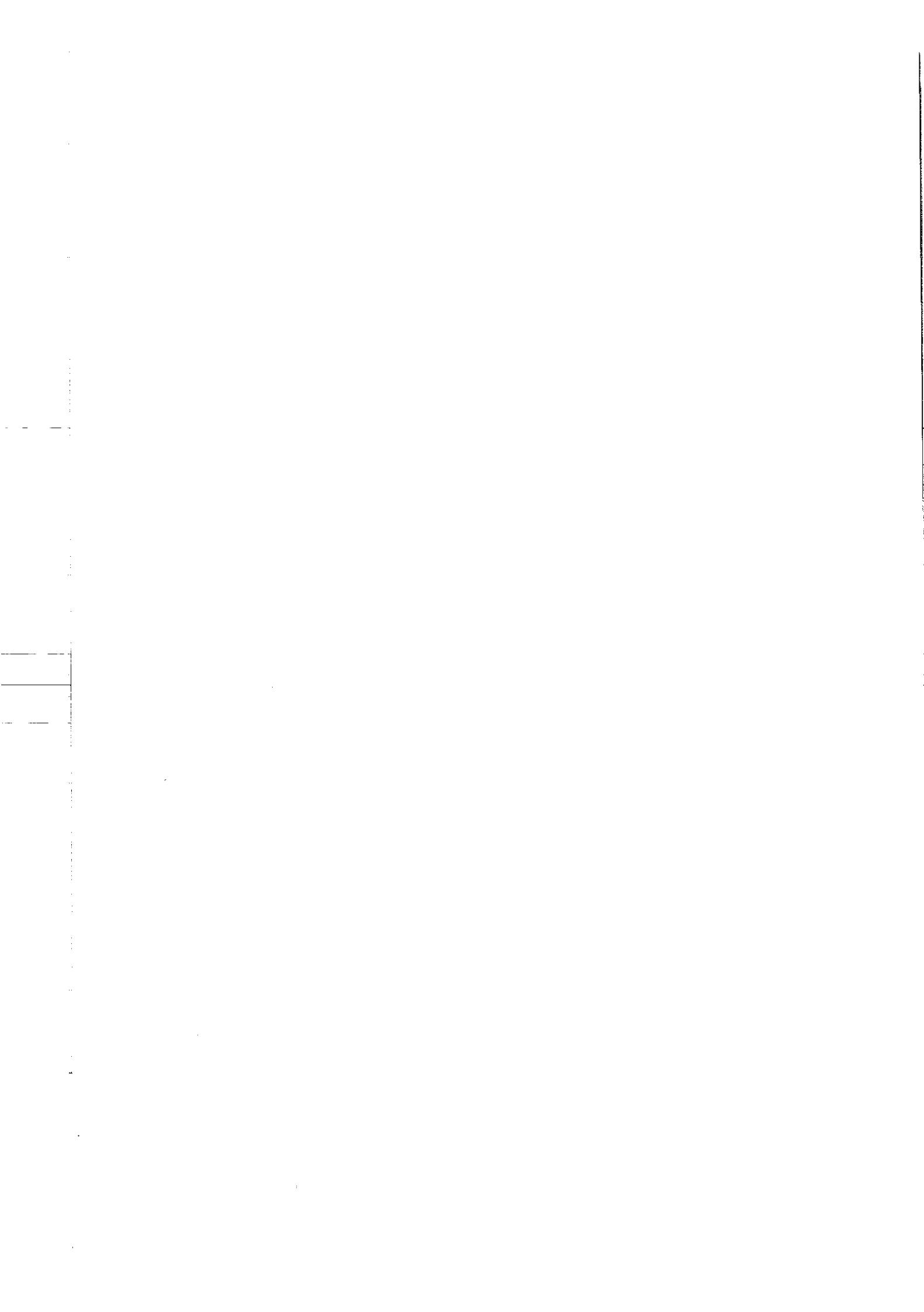


Document 3

4. Identify the type of lymphocytes that have infiltrated the tumor.
5. Explain how the second treatment led to the regression and then the disappearance of the cancerous tumor in the patient.
6. Explain, based on what preceded and your knowledge, the cellular and molecular mechanisms leading to the elimination of tumor cells.



Document 4



Immunity

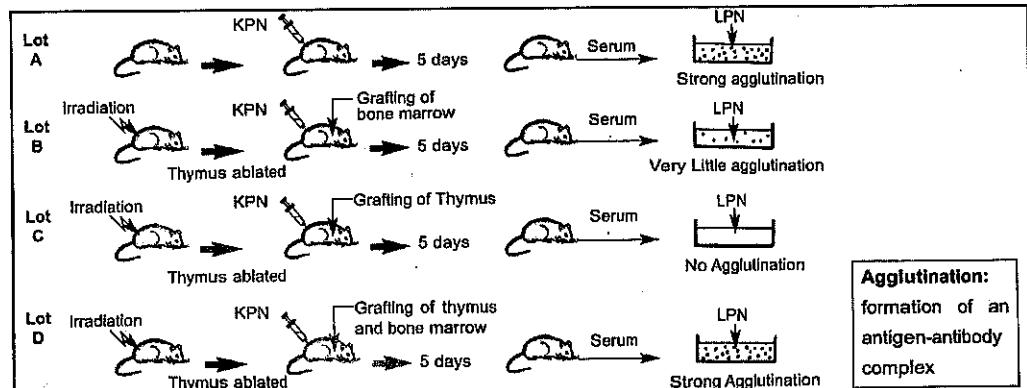
Immune official exercises

Exercise 1 (7.5 pts) Relation between immune cells

Session 2001-1

B and T-lymphocytes originate from stem cells in the bone marrow. B-lymphocytes undergo maturation in the bone marrow, while T-lymphocytes undergo maturation in the thymus.

A series of experiments was performed on four lots of mice three of which were subjected to ablation of the thymus then irradiation (irradiation kills the cells of the bone marrow). After grafting the thymus, the bone marrow, or both, they injected the mice with killed pneumococci (KPN). Five days later, they added living pneumococci (LPN) to their serum. Lot A was the control group.



Document 1

1. Interpret the results of the experiments in document 1.
In the attempt to specify the nature of the relation between Band T lymphocytes, they placed sensitized Band T lymphocytes (i.e. they had been previously in contact with a soluble antigen Z) in a Marbrook culture chamber (document 2). The results of the culture are shown in the table in document 3.

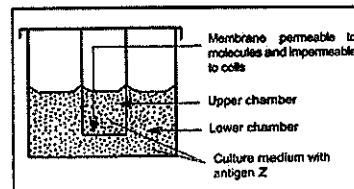
Marbrook chamber consists of two compartments (upper and lower) separated by a membrane permeable to molecules and impermeable to cells.

2. Deduce, starting from the results of the experiment shown in document 3, the mode of communication between B and T lymphocytes

Document (4a) summarizes schematically a new series of experiments whose results are provided in table (4b). The serum and the used blood cells originate from the same mouse, which was subjected, a few days before, to an injection of killed pneumococci (KPN).

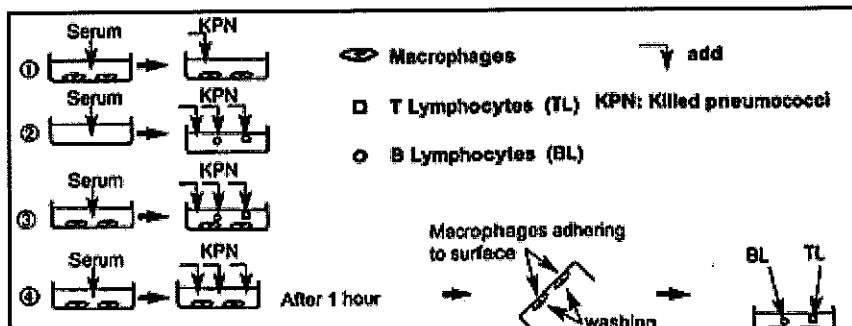
3. Describe, in few lines, the experiments in document 4a with the corresponding results in document 4b.

4. Deduce the role of macrophages in the production of immunoglobulin (antibodies) and the mechanism of macrophages action.



Culture	Type of sensitized lymphocytes placed in the chamber.		Cells secreting antibodies anti-Z (plasmocytes) in ur spleen cells.
	Upper	Lower	
1	-	T + B	1 000
2	-	B	72
3	T	B	1011
4	T	-	0

Document 3



Medium	1	2	3	4
Quantity of Ig	-	+	+++	+++

(-) null; (+) weak; (++) abundant

Document 4b

Exercise 2 (4 pts) Immunity against bacterial toxins**Session 2001-2**

Tetanus is a disease that results from the invasion of an organism by a bacterium which liberates a toxin, the tetanus toxin (TT), in the internal body medium. Diphtheria is another disease that results from the invasion of a bacterium which liberates a toxin, the diphtheria toxin (DT), in the internal body medium.

Document 1 shows a series of experiments conducted on Guinea pigs whose purpose is to determine the consequences of the injection of the toxoid (attenuated toxin, non-virulent) and the utilization of blood serum extracted from the animals who received the toxoid.

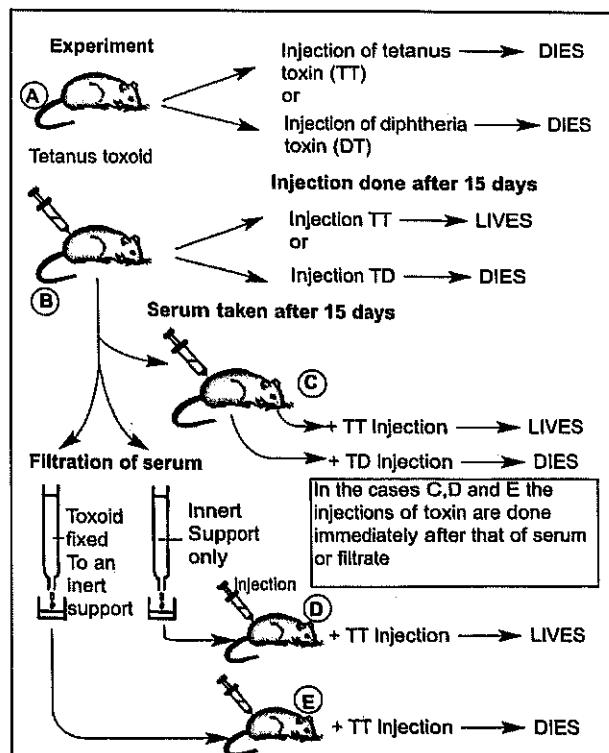
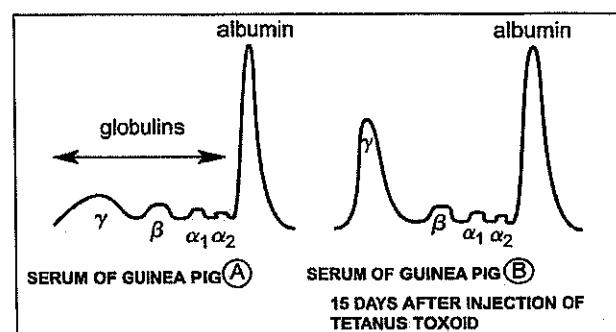
1. Describe in a few lines the experiments A and B in document 1 specifying the results of each.
2. Draw out from experiments A and B in document 1 one characteristic of this immune response.

The experiments exhibit a: humoral mediated immune response.

3. Draw out from the experiments in document 1 all the information that confirm this response.

Document 2 presents the results of the separation of serum proteins by electrophoresis before and after the injection of tetanus toxoid.

4. What can we deduce from the results in document 2?

**Document 1****Document 2**

Mice	Treatment done	Consequences
Lot A	irradiation + grafting of bone marrow	production of B and T lymphocytes
Lot B	ablation of thymus + irradiation + grafting of bone marrow	production of B lymphocytes and immature T lymphocytes
Lot C	ablation of thymus + irradiation + grafting of thymus	no production of B or T lymphocytes

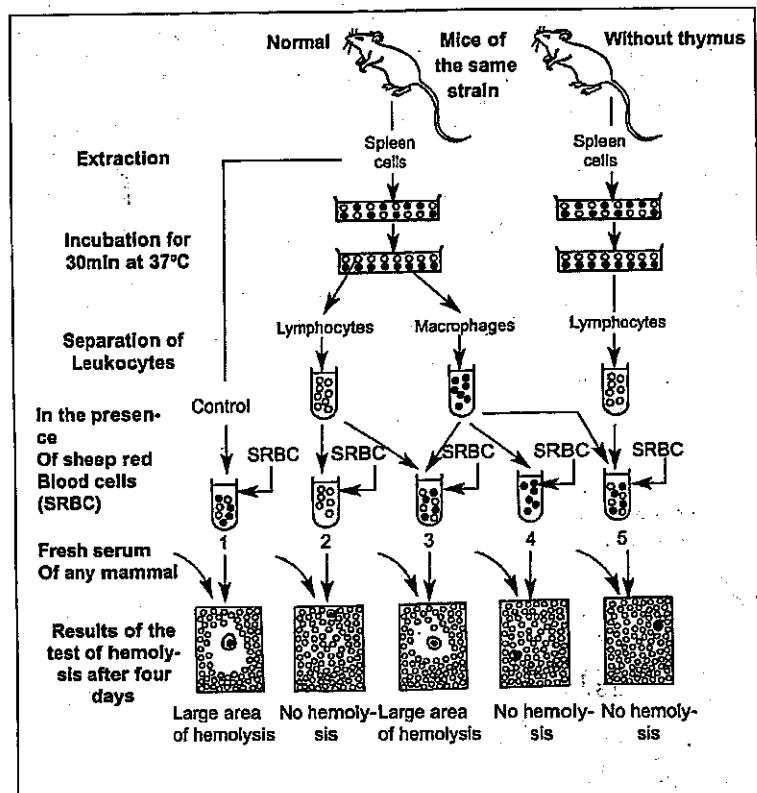
Document 3

Exercise 3 (4 pts) Induction of immunity**Session 2002-1**

In the framework of studying the mechanism of humoral mediated immune response, we perform the experiments shown in the adjacent document.

1. Describe, in a few lines, the performed experiments with the results obtained in each.
2. Interpret the results obtained in the different experimental situations.
3. Formulate a hypothesis concerning the content of the used fresh mammalian serum.

N.B.: Hemolysis would not be obtained if a fresh serum of a certain mammal is not added. Zones in which the red blood cells are destroyed are called areas of hemolysis.

**Document 1**

Exercise 4 (5 pt) An immune deficiency**Session 2002-2**

A young child, who suffers from a rare immunity disease, is submitted to gene therapy. Before treatment, the child presents severe immune deficiency associated with parasitic diseases and diverse infections. Document 1 represents the results of the blood analysis of the sick child and that of another healthy child.

Lymphocytes and antibodies in the blood	Measurements done on the sick child before treatment	Measurements done on the healthy child
T lymphocytes (number/ μ L)	0	From 2000 to 4000
B Lymphocytes (number / μ L)	1250	From 1000 to 2000
Circulating antibodies (mg/dL)	0	>400

Document 1

- 1.1. Analyze document 1.
- 1.2. Draw out the cause of the disease.
2. How can you explain this immune deficiency?

The treatment consists of transferring the gene coding for the synthesis of a membrane protein indispensable for the multiplication and the differentiation of TL originating from their stem cells. For this objective, we obtain from the bone marrow of the sick child stem cells that are precursors of TL. We inject the gene in question into these cells, then we introduce the genetically modified stem cells into the sick child. The obtained result is shown in document 2.

Number of days after gene therapy	30	60	90	120	165	185	270
Number of T Lymphocytes per μ L of blood	250	350	500	1000	1750	1750	3000

Document 2

3. Construct a graph representing the variation of the number of T lymphocytes per μ L of blood in function of time after gene therapy.

Six months after the treatment, the concentration of antibodies in the blood of the treated child becomes 323 mg dL-1. The treated child and the healthy child are then submitted to the following vaccines: anti-tetanus, anti-diphtheria and anti-poliomylitis. The responses of the two children to the vaccines are shown in the table in document 3.

	Amount of anti-tetanus toxin antibodies (IU/mL)	Amount of anti-diphtheria toxin antibodies (IU/mL)	Amount of anti-poliomylitis virus antibodies (a.u.)
The sick child after treatment	0.53	0.86	215
The untreated healthy child	>0.20	> 0.20	> 80

Document 3

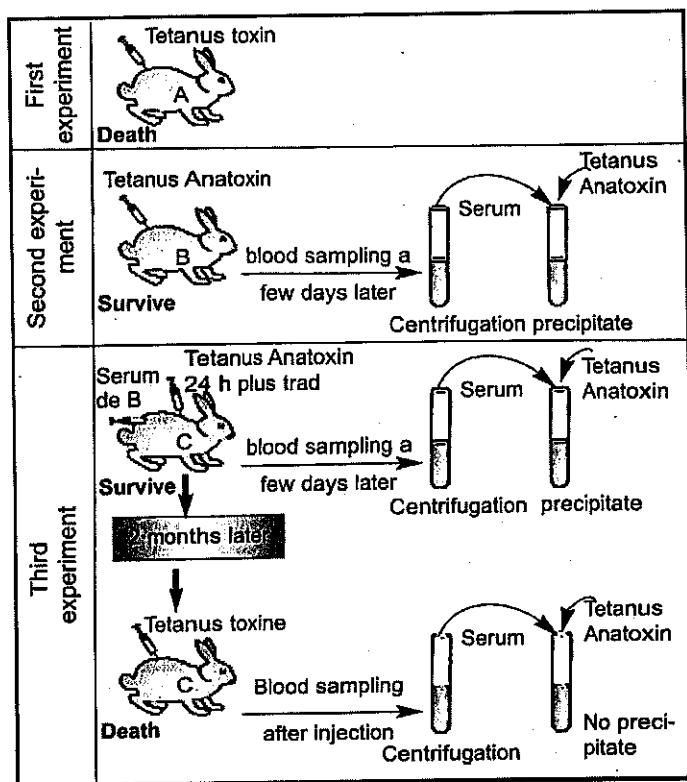
4. Is the used treatment effective? Justify the answer by referring to documents 2 and 3.

Exercise 5 (6 pts) Serotherapy

Serotherapy involves the treatment of an individual against a given antigen after the individual is injected with a blood serum taken from another organism, who is immunized against the same antigen.

The opposite document summarizes certain experiments done on rabbits A, B, and C.

1. Describe, in a few lines, each of the experiments 1, 2, and 3 shown in the opposite document with the results of each.
- 2.1 Explain the formation of the precipitate observed in experiment 2 after the injection of tetanus anatoxin (toxoid).
- 2.2 What interest does the utilization of the tetanus anatoxin present?
3. How do we explain the survival of rabbit C after the injection of the tetanus toxin, 24 hours later?
- 4.1 To what can you attribute the death of rabbit C after we inject it with the tetanus toxin, two months later?
- 4.2 What characteristic of Serotherapy is evidenced here?

**Document 1**

Exercise 6 (4 pts) Immunity against flu virus

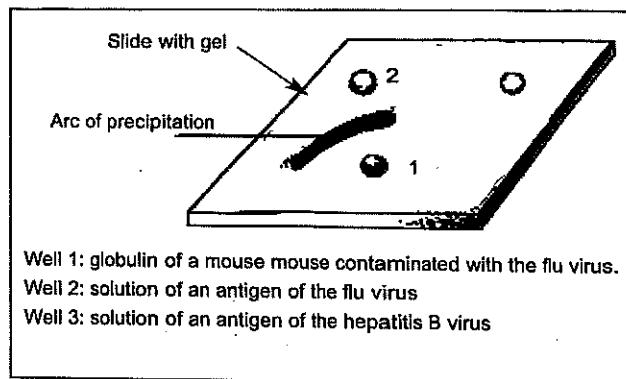
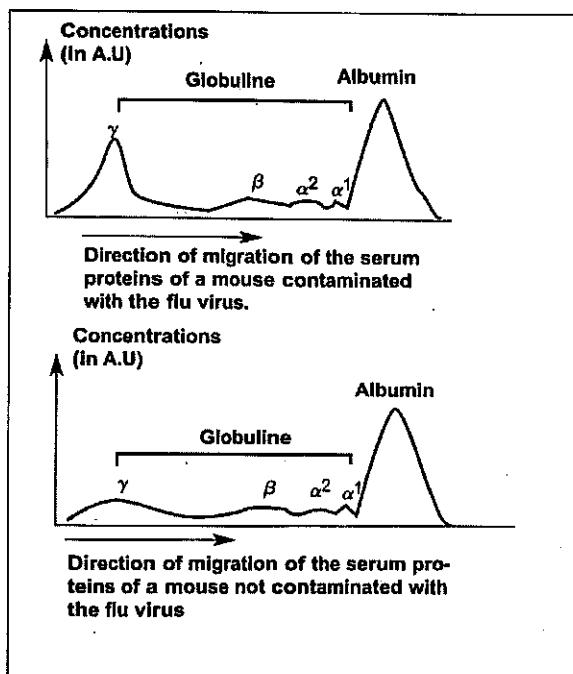
Session 2003-2

Mice are capable of fighting the flu virus. The following documents are related to the immune reactions of a mouse after it has been infected by the flu virus.

Document 1 represents the results of the separation, by electrophoresis, of the serum proteins of a mouse not contaminated with the flu virus and the serum proteins of another mouse contaminated with the flu virus.

Document 2 shows the result of immune diffusion in gel. The precipitation arc reveals the formation of an immune complex between an antigen and the corresponding antibody.

Concentrations

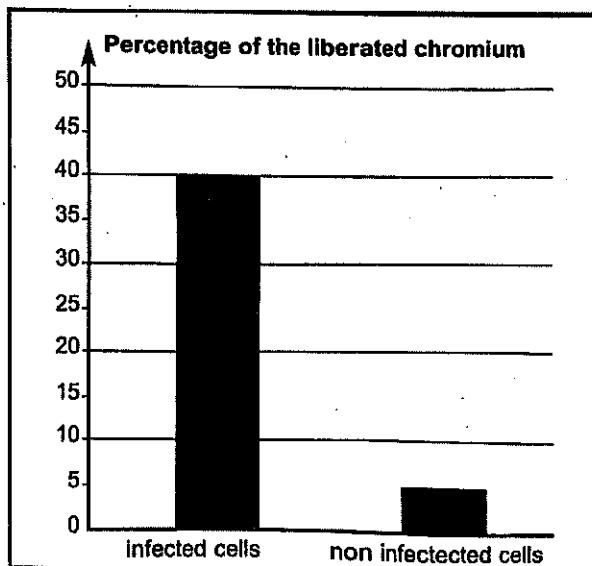
**Document 1****Document 2**

We put some lymphocytes, taken from the spleen of a mouse contaminated with the flu virus since many days, in two different culture media. One medium contains cells infected by this virus and the other medium contains non-infected cells. All the cells are obtained from mice of the same strain. We previously incorporate in these cells (infected and non-infected) a small amount of radioactive chromium 51. The lysis of the target cells leads to the liberation of chromium into the culture medium. The quantity of liberated radioactivity is measured; it is proportional to the number of the destroyed cells.

Document 3 presents the results of this test.

1. Interpret the results presented in each of the documents 1, 2 and 3.
2. Name the immune response revealed by the results of the three documents.

Justify the answer.

**Document 3**

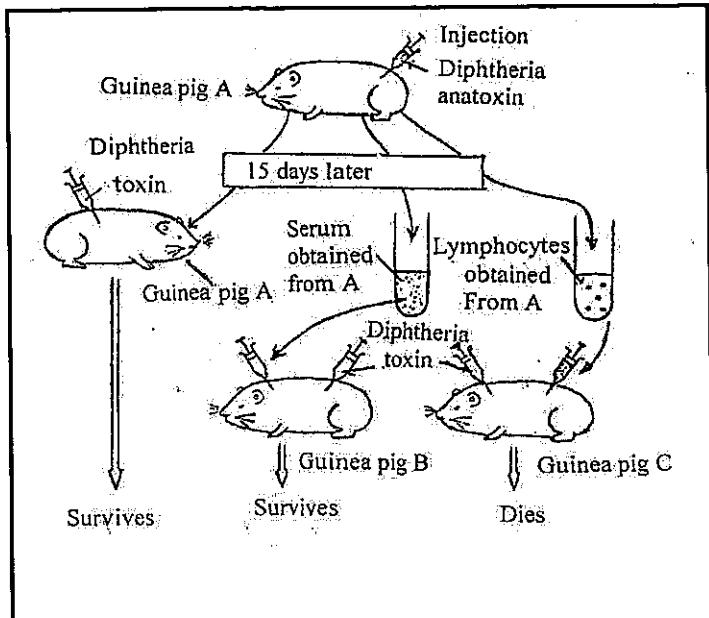
Exercise 7 (3 ½ pts) Specific immune response

Session 2004-1

Diphtheria is a fatal disease due to the action of diphtheria toxin, which spreads throughout the body. This toxin is liberated by a bacillus that remains in the throat.

In order to understand the consequences of the injection of diphtheria toxoid (attenuated toxin, non-virulent), we performed several experiments on guinea pigs. The results are represented in the adjacent document.

5. Describe, in a few lines, each experiment shown in this document.
6. Interpret the results of these experiments.

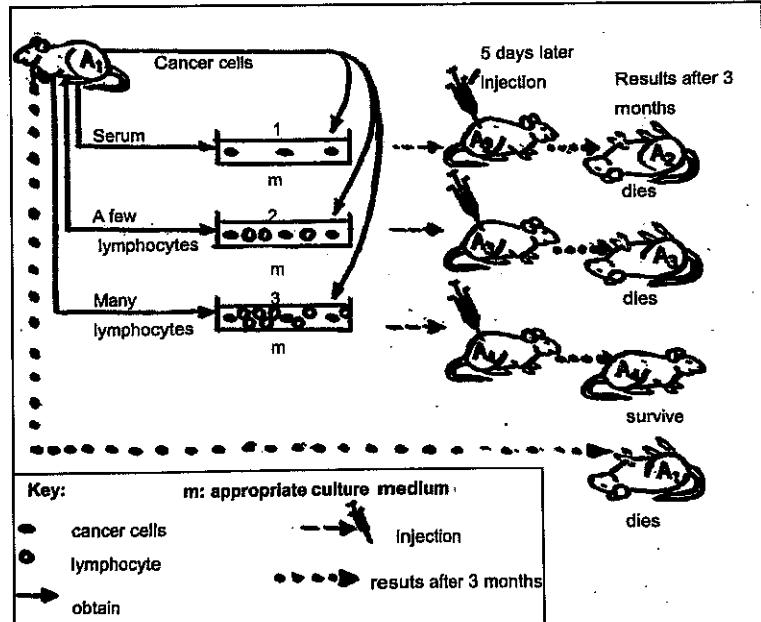
**Exercise 8 (5 pts) Immunity against cancer**

Session 2004-2

Cancer cells are abnormal cells whose rapid and uncontrolled division frequently leads to the death of the individual. Being a part of the organism, these cells present abnormal markers on their membranes.

In the framework of studying the reaction of the organism against these cells, we perform, on mice, the experiment shown in the adjacent document.

1. Describe, in a few lines, the performed experiment.
2. Interpret this experiment.
3. Explain, by referring to the acquired knowledge, the immune mechanism involved in the elimination of the cancer cells by the organism.
4. Formulate a hypothesis, which permits to explain the obtained results after the injection of preparation 2.

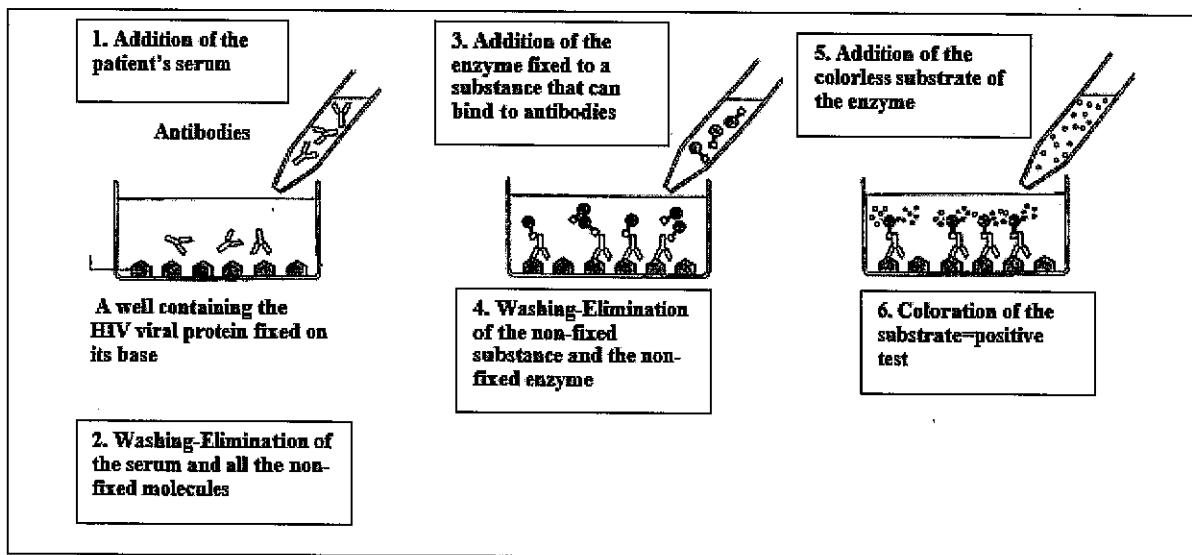
**Document 1**

Exercise 9 (6 pts) Infection by HIV

Session 2005-1

AIDS, or Acquired Immunodeficiency Syndrome is a disease due to a virus called HIV, or Human Immunodeficiency Virus. This disease affects the immune system and develops through many years, more or less rapidly depending on the individual.

Individual A is suspected to be infected by the virus. He consulted a doctor who prescribed blood analysis and a test called ELISA test. Document 1 reveals the different steps and the obtained results of this test.

**Document 1**

1. Write a short text describing document 1.
- 2.1. What does the obtained result indicate?
- 2.2. How can you explain this result?

Document 2 reveals the amount of T4 lymphocytes, measured over time, of a patient **B** who presents severe signs of infection.

Duration in months	3	6	12	18	30	40	50	70
Amount of T4 lymphocytes/mm ³ of blood	550	750	800	500	450	300	200	50

Document 2

3. Draw the curve of the variation of the amount of T4 lymphocytes in function of time.
- 4.1. Analyze the results shown by the table.
- 4.1. Draw out the cause of the observed immune deficiency starting from the 40th month.

Knowing that the blood analysis done for patient **A** shows that the amount of T4 lymphocytes is equal to 800/mm³ of blood, and in reference to document 2,

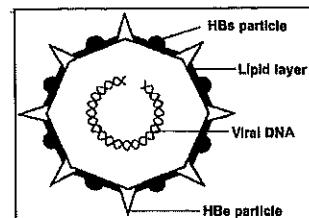
5. Draw out the duration of infection in patient **A**.

Exercise 10 (5 pts) Immunity against hepatitis virus

Session 2005-2

Hepatitis B is a disease caused by a virus that attacks hepatic cells (document 1). It is manifested by the inflammation of the liver and in severe cases by necrosis of the liver, due to the destruction of hepatic cells. The analysis of the serum of a person severely affected by hepatitis B reveals the presence of anti-HBs and anti-HBe antibodies.

1. Indicate the antigens of hepatitis B virus recognized by the immune system. Justify the answer.



Document 1

We remove a sample of hepatic cells from a healthy person (lot A) and another sample from a person who has hepatitis B (lot B). Experiments done on these cells and the obtained results are shown in the table in document 2.

Cells of lot A	Cells of lot B
Deposits of these cells on a plate covered by anti-HBs antibodies	
	Washing the plate
	Deposits of anti-HBs antibodies labeled with fluorescence on a plate
	Washing the plate
Result: Absence of labeling	Result: yellow coloration

Document 2

2. Describe, in a few lines, the above experiments and the obtained results.
3. What do these results indicate?

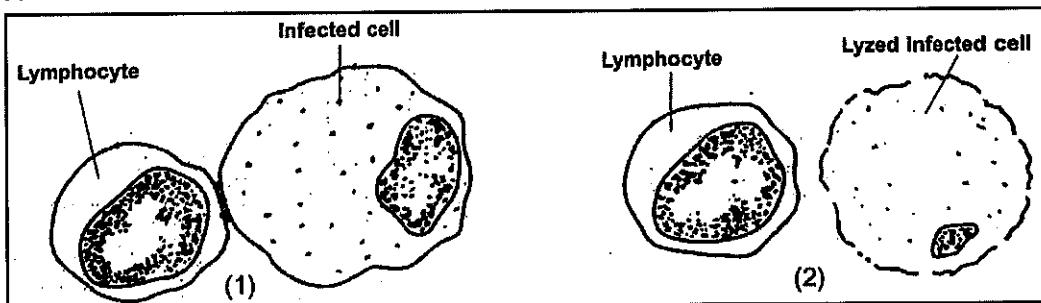
We obtain samples of hepatic cells from a healthy person and from a person infected with hepatitis B and we place them in identical culture mediums. These cells are cultured in the presence of T lymphocytes obtained from a sick person (mediums 1 and 2) and in absence of T lymphocytes (medium 3). The experiments done and the obtained results are presented in document 3.

Medium 1	Medium 2	Medium 3
TL + hepatic cells of a healthy person	TL + hepatic cells of a person infected by the virus	hepatic cells of a person infected by the virus
No lysis	Lysis of infected cells	No lysis

Document 3

4. Interpret the obtained results.

The microscopic observation of the sample of medium 2 at two different intervals of time 1 and 2 shows certain cells schematized in document 4.

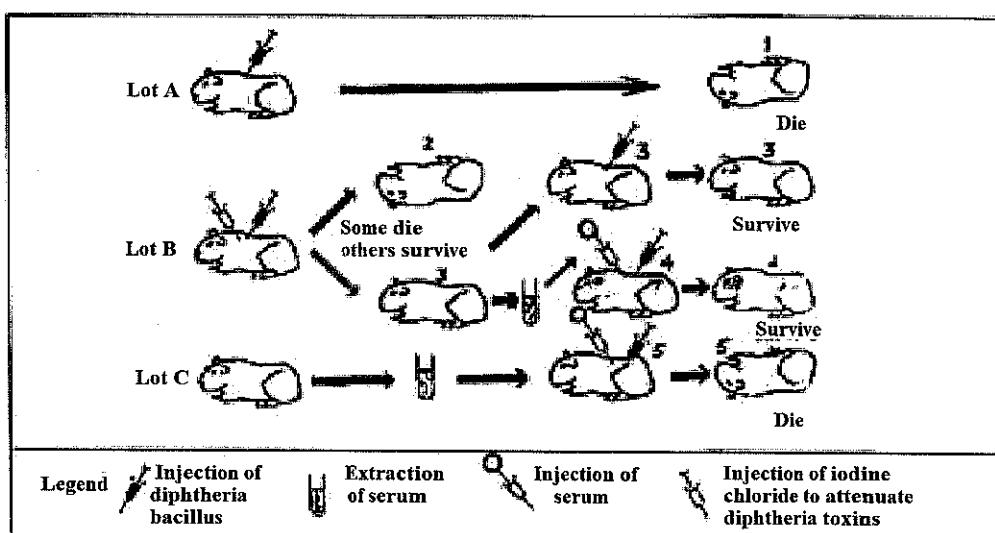


Document 4

5. Explain briefly, by referring to document 4 and to acquired knowledge, the mechanism of the lysis of cells by TL.
6. Draw out from the preceded information the types of immune response developed against hepatitis B virus.

Exercise 11 (8 pts) Medical applications of immunity**Session 2006-1**

In the framework of studying transmission of immunity against diphtheria, a human disease caused by bacillus that secretes a deadly protein; the following experiments are conducted on Guinea pigs. Document 1 shows the experimental procedure used and the obtained results.

**Document 1**

- Describe, in a short text, each experiment performed and its obtained result.
- Interpret the results of these experiments.
- Indicate the two medical applications that you can draw out from these experiments? Justify the answer.

To understand why some infectious diseases can infect an organism only one time during life, even when the same organism is confronted with the pathogenic agent again, we perform the following experiment.

We inject a Guinea pig with an antigen X and we measure the amount of plasma anti-X antibodies. After 50 days, when the amount of anti-X antibodies in the plasma becomes nearly nil, we inject the same Guinea pig again with antigen X and another antigen: antigen Y.

Time (days)	0	8	15	30	50	60	75	85	100
Amount of anti-X antibodies (a.u.)	0	0	1	0.8	0.2	1.5	3	2.8	2.6
Amount of anti-Y antibodies (a.u.)	-	-	-	-	0	0	1	0.5	0

↑^{1st} contact
with antigen X

2nd contact with antigen X and 1st
contact with antigen Y

Document 2

- Draw, on the same graph, the curves showing the variation of anti-X and anti-Y antibodies as a function of time.
- Analyze the variations of the amount of anti-X. document 2.
- Draw out the characteristics of the secondary immune response.
- What do the results of antigen Y injection confirm?

Exercise 12 (4 pts) Conditions and mechanism of cytotoxicity

Session 2006-2

For determining the relation between the T4 lymphocytes and the T8 lymphocytes, also called cytotoxic T lymphocytes (Tc), we perform the following experiments:

- We remove from the spleen of a mouse, immune cells and we culture them in different mediums, document 1. We add to the culture mediums infected cells taken from an infected mouse of the same species. We detect cytotoxicity from the infected cells that are destroyed by the immune cells present in the mediums, document 2.

Document 1

Medium 1	Immune cells in serum
Medium 2	Immune cells in a medium that leads to the elimination of T4 lymphocytes
Medium 3	Immune cells in a medium that leads to the elimination of T8 lymphocytes

Experiment 1	Immune cells removed from medium 1 + infected cells from a mouse of the same species	Presence of cytotoxicity
Experiment 2	Immune cells removed from medium 2 + infected cells of a mouse of the same species	Absence of cytotoxicity
Experiment 3	Immune cells removed from medium 3 + infected cells from a mouse of the same species	Absence of cytotoxicity
Experiment 4	Immune cells removed from mediums 2 and 3 + infected cells from a mouse of the same species	Presence of cytotoxicity

Document 2

1. Interpret the results obtained in these experiments.

- The following microscopic observations reveal the mode of action of Tc lymphocytes in the presence of infected cells.

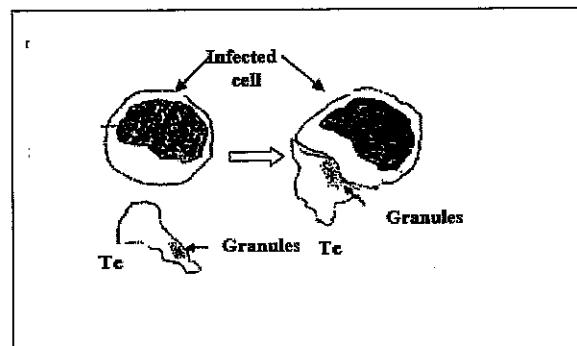
1st observation: In the presence of infected cells, the Tc lymphocytes that are rich in granules containing perforin, come in contact with these cells, document 3.

2nd observation: In the presence of non-infected cells, the Tc lymphocytes do not reveal granules containing perforin in their cytoplasm, and do not come in contact with these cells.

3rd observation: The membrane of the infected cells shows many pores in the region of contact with the Tc lymphocytes.

4th observation: In some mutant mice the Tc lymphocytes present a deficiency in perforin. No pores are observed at the level of the membrane of the infected cells in the region of contact with Tc lymphocytes, and the consequence is the non-destruction of the infected cells.

2. Analyze these microscopic observations
3. Draw out the role of perforin in the destruction of infected cells.
4. Explain, from what has been preceded and based on the acquired knowledge, how the T8 lymphocytes become active cytotoxic T lymphocytes and how do they destroy the target cells.

**Document 3**

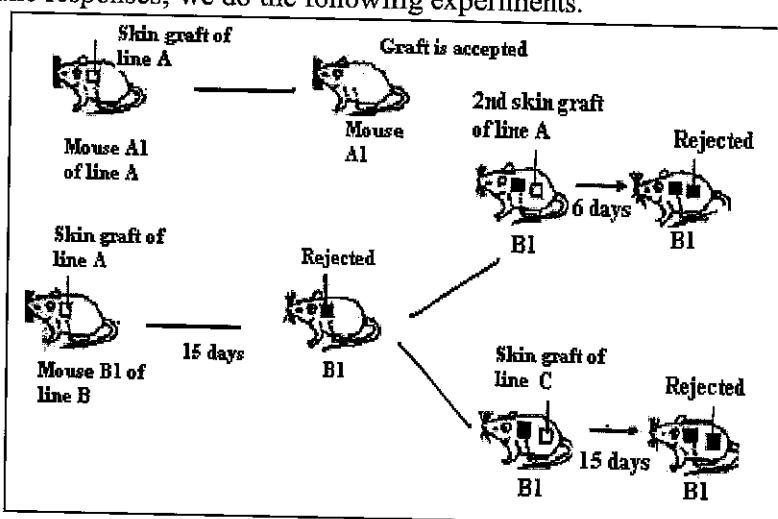
Exercise 13 (5 pts) Specific cell mediated immunity

Session 2007-1

In the framework of studying immune responses, we do the following experiments.

1st Series of experiments: We perform skin graft between mice A, B, and C of different lines, document 1.

1. Interpret these experiments.
2. Indicate two characteristics of the immune system revealed by these experiments.



Document 1

2nd Series of experiments: We graft skin of mouse A into mouse B under different conditions. The experiments done and the obtained results are shown in document 2.

3. Specify, starting from document 2, the organs involved in graft rejection.

Nº of experiment	Experimental conditions	Results
1	Control mouse B	Graft is rejected
2	Mouse B deprived of its thymus	Graft is accepted
3	Irradiated mouse B (destruction of bone marrow)	Graft is accepted

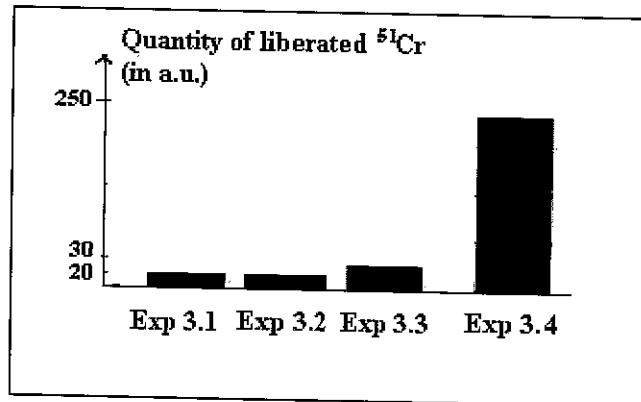
Document 2

3rd Series of experiments: We remove cells infected by virus X from a mouse of line A. We incubate the cells with radioactive chromium ^{51}Cr . This ^{51}Cr is absorbed and binds to proteins in the cells. After incubation, we wash these cells and culture them with different effector cells obtained from the same mouse A. The supernatants are then collected for measuring the quantity of ^{51}Cr released by the lysed target cells. Document 3a shows the experiments that are carried out, and document 3b shows the obtained results.

4. Interpret the obtained results.
5. Explain, by referring to the acquired knowledge, how the effector cells in document 3a intervene in the lysis of infected cells.

Nº of experiment	Effector cells of mouse A
3.1	None
3.2	Macrophages
3.3	LT4 + LT8
3.4	LT4 + LT8 + macrophages

Document 3a



Document 3b

Exercise 14 (5 ½ pts) An immune disease

In order to determine the cause of juvenile diabetes in rats, the following experiments were carried out on mutant rats of the same strain, in which diabetes appears in the first few months of their life.

1st experiment: 100 newborn mutant rats were brought, and divided into two lots, lot A and lot B. Lot A was subjected to the ablation of the thymus, the organ where T lymphocytes undergo maturation, and lot B was used as control. A few months later; the number of the rats that presented diabetes was determined, document 1.

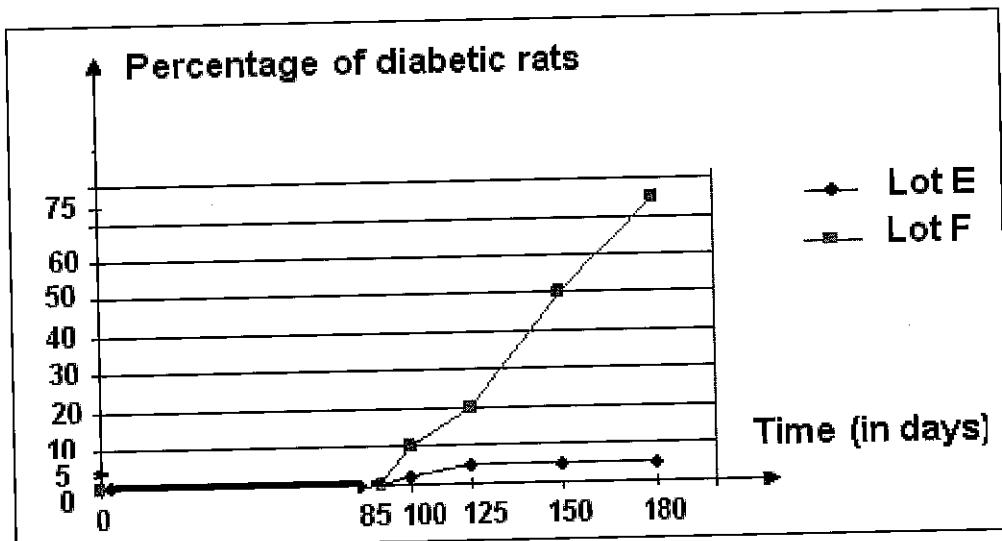
Number of diabetic rats	
Lot A	5/50
Lot B	30/50

Document 1

2nd experiment: Two lots of healthy non-mutant rats, lot C and lot D, were brought. The rats of lot C were injected with TL taken from diabetic mutant rats, and the rats of lot D were injected with TL taken from healthy rats. The rats of lot C, only, developed diabetes.

1. Formulate the hypothesis at the origin of these experiments.
2. Interpret each of the carried-out experiments.
3. What can one deduce regarding the formulated hypothesis?
4. What name can be attributed to this kind of disease? Justify the answer.

3rd experiment: Two lots of mutant rats, lot E and lot F, were brought. Lot E was treated, from birth, with cyclosporine, an immunosuppressant medicine, and lot F was used as control. Document 2 reveals the percentages of diabetic rats in these two lots of rats.



Document 2

5. Represent in a table the various data provided by document 2.
6. Interpret the obtained results.
7. Draw out the mode of action of cyclosporine.

Exercise 15 (5 pts) Characteristics of the immune response

Session 2008-1

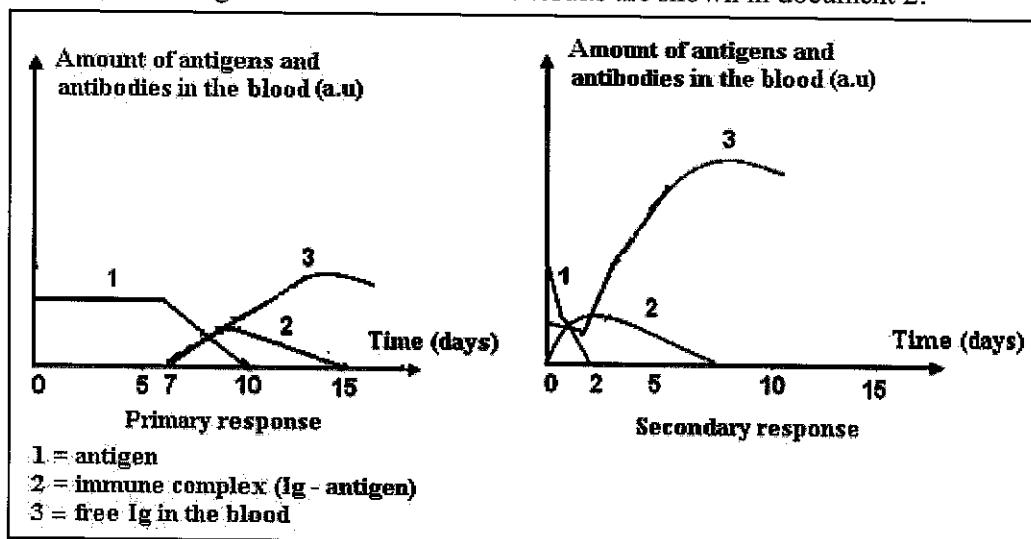
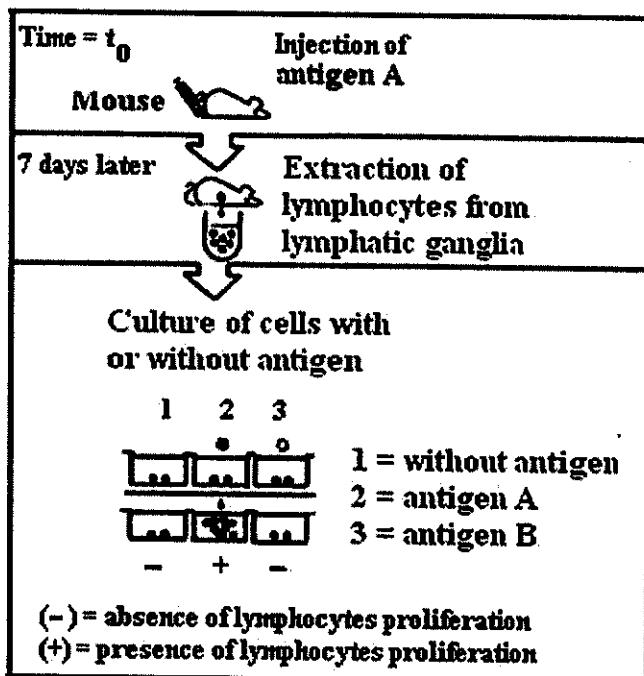
In order to study one of the characteristics of the immune response, the experiment shown in document 1 were carried out.

1. Write a short text describing the experiment carried out as well as the obtained results.
2. Interpret the obtained results.

In a second experiment, the same steps are repeated without a seven days' time delay. The cells of the lymphatic ganglia are directly extracted after the mouse immunization against antigen A. The results do not show any proliferation of lymphocytes.

3. Explain the necessity of the seven days' time delay for the lymphocytes proliferation.

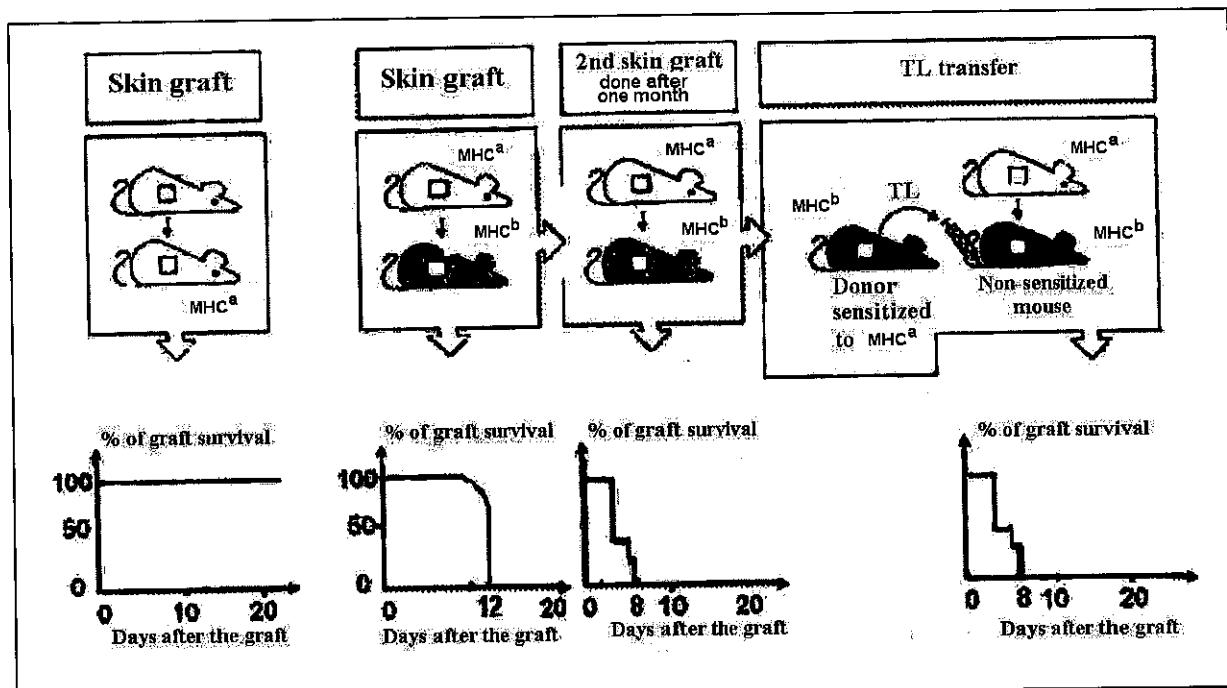
In a third experiment, we estimate the variations in the amounts of antigens and the produced antibodies (Ig) during two separate injections of the same antigen to an individual. The results are shown in document 2.

**Document 2**

4. Compare the variations in the amounts of antigens then in the amounts of antibodies during both contacts.
5. Conclude the characteristics of the immune memory.
6. Explain the appearance then the disappearance of the immune complexes following the antigen's injection.

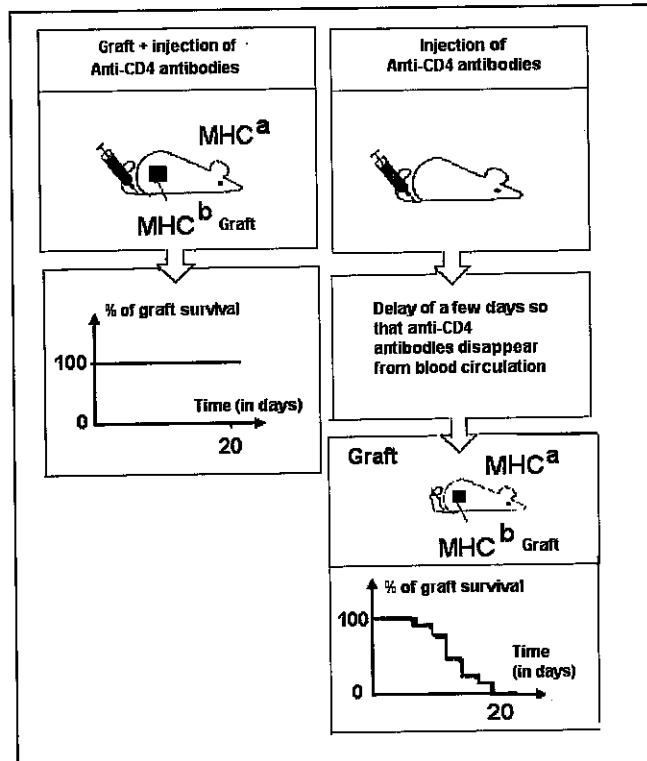
Exercise 16 (5pts) Immunity against grafts**Session 2008-2**

In order to know the mechanisms responsible for rejecting or accepting grafts, experiments on mice of the same line or different line are done. The experiments and their results are shown in document 1

**Document 1****1. Interpret these experiments.**

Two types of T-lymphocytes (TL) are recognized: TL4 with a CD4 receptor and TL8 with CD8 receptor. The experiments revealed in document 2 are carried out in order to determine the role of TL involved in graft rejection.

2. Interpret the obtained results.
3. Justify that these experiments are not sufficient to assure, which of the two types of TL is involved in graft rejection.
4. Suggest an experiment that allows solving this problem.
5. Explain how the anti-CD4 antibodies intervene in accepting grafts.
6. Draw out a practical medical application.



Exercise 17 (5 pts) Infection by HIV

Session 2009-1

In an attempt to understand how the HIV that causes AIDS infects selectively T4 cells, we perform the following experiments on many lots of T4 cells (Lymphocytes characterized by the presence of CD4 proteins on their membranes) and T8 cells (lymphocytes characterized by the presence of CD8 proteins on their membranes).

Document 1 presents the experimental procedure and the obtained results.

1-Interpret the obtained results.

Studies and knowledge of the immune system and the immune reactions of persons infected by HIV allow for the preparation of an anti-HIV vaccine. We test the efficiency of this vaccine on Rhesus monkeys.

	Experimental Procedure	Results
Lot 1	T4 and T8 cells are placed directly in the presence of HIV	Infection of T4 cells, but no infection of T8 cells
Lot 2	T4 cells are incubated for 20 minutes with several types of antibodies* that do not bind to the membrane protein CD4, then placed with HIV	Infection of T4 cells only
Lot 3	T4 cells are incubated for 20 minutes with antibodies* that bind to the membrane protein CD4, then placed with HIV	No infection of T4 cells

*Anti-bodies block the biological activity of the molecules to which they bind

Document 2 reveals the

Document 1

variation of the proportion of T8 cells specific to HIV during infection time in vaccinated and non-vaccinated monkeys.

	Time (in weeks)	0	1	2	4	6	8	10	12
Proportion of T8 specific for the HIV (in a.u)	Lot 1 : Vaccinated monkeys	0.1	7	6.5	6	4	3	2	2
	Lot 2 : Non-vaccinated monkeys	0	0	0.5	2	1.5	1	1.3	1.5

Exposure to HIV**Document 2**

2-Draw, on the same graph, the curves obtained from the tabulated data.

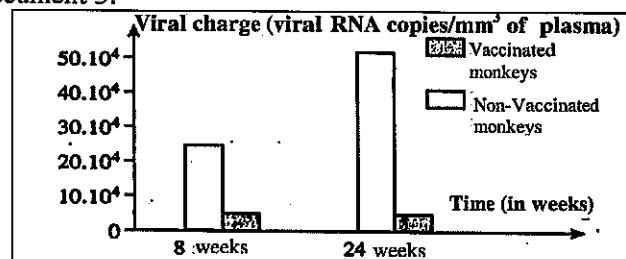
The immune response in the vaccinated monkeys is rapid and amplified.

3-Justify this affirmation by referring to the results of document 2.

We measure the viral charge (the number of viral RNA copies /mm³ of plasma that is an indicator of the concentration of the virus in blood) in the vaccinated and non-vaccinated monkeys after 8 and 24 weeks of exposure to the virus. The results are shown in document 3.

4-1-Compare the obtained results.

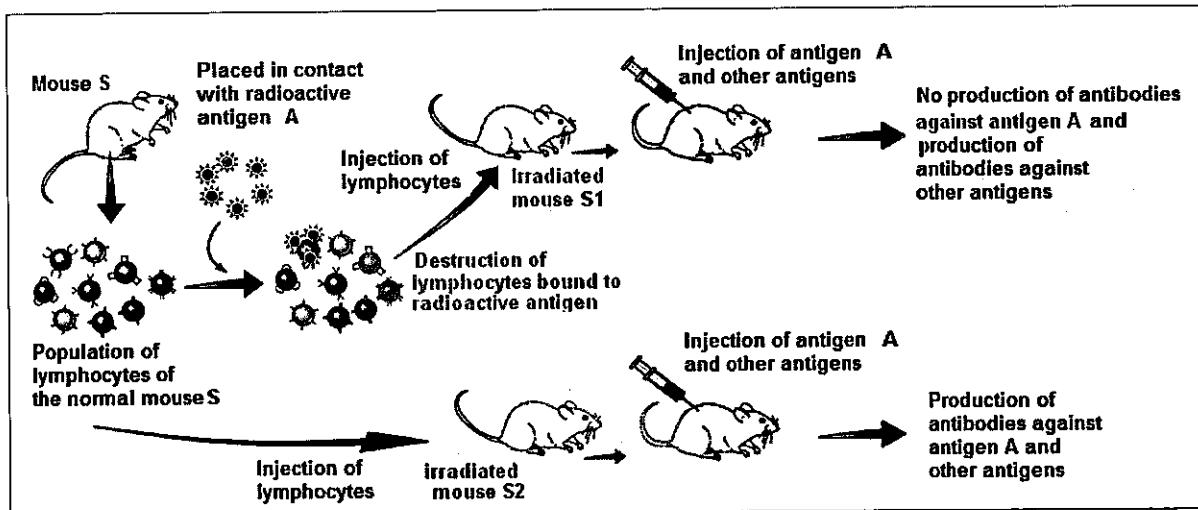
4-2-Draw out a relation concerning the effect of the studied vaccine.

Document 3

Exercise 18 (5 pts) Clonal selection

Session 2009-2

In the framework of studying clonal selection of B lymphocytes which are at the origin of antibodies, we perform several experiments on mice of line S that are not immunized against an antigen A (document 1). According to the theory of clonal selection, each lymphocyte acquires during its development the ability to react with a specific antigen, even without being previously exposed to it.

**Document 1**

N.B. irradiation leads to the destruction of immune cells.

1- Refer to document 1 to show that:

- 1-1 B lymphocytes are ready to respond to an antigen before encountering it;
- 1-2 B lymphocytes possess a surface receptor;
- 1-3 The immune response is specific.

To determine the phenomenon responsible for the secretion of the most effective antibodies, researchers performed the following experiment. They injected mice with a chemical substance recognized by the immune system as a foreign antigen. This antigen is characterized by having several antigenic determinants.

At different times following the injection, researchers sacrificed the mice and dissected their lymphatic ganglia to detect the B lymphocytes which recognize the injected antigen. Document 2 shows the results of this experiment.

Time since the injection of the antigen (in days)	Aspect of the lymphatic ganglia	Number of the different detectable B lymphocytes clones	Efficiency of the immune response
5	Beginning of swelling	10	Average
10	Strong swelling	1 or 2	Very high

Document 2

- 2- Explain the swelling of the lymphatic ganglia mentioned in document 2.
- 3- To what can we attribute the number of B Lymphocytes clones 5 days following the injection of the antigen?
- 4- Formulate a hypothesis that explains the decrease in the number of B lymphocytes clones detected 10 days following the injection of the antigen.

Exercise 19 (5 pts) Specificity of lymphocytes and antibodies

Session 2010-1

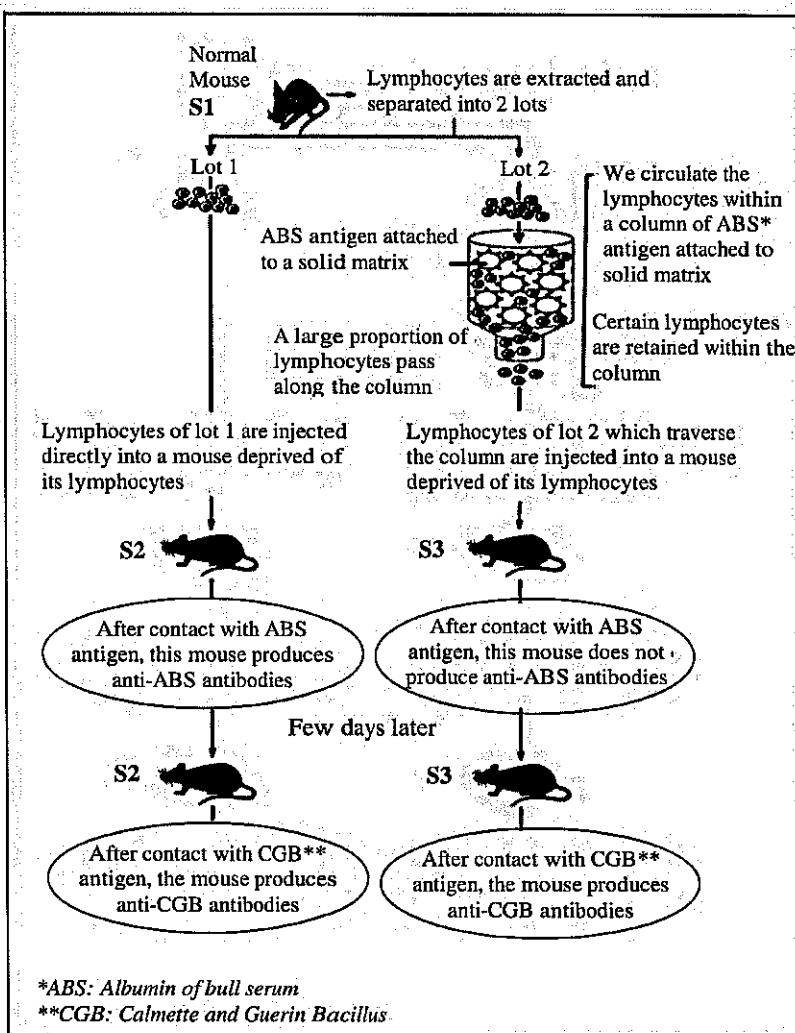
In the midst of the twentieth Century, two hypotheses were proposed to explain the high diversity of antibodies.

First hypothesis: Any lymphocyte encountering any antigen is capable of producing antibodies specific to this antigen.

Second hypothesis: Only some lymphocytes which correspond to an antigen are capable of producing antibodies specific to this antigen.

To verify one of these two hypotheses, an experiment was performed on mice of the same strain. The steps and results of this experiment are represented in document 1.

- 1- Write a text which describes the experiment shown in document 1.
- 2-1-Interpret this experiment.
- 2-2-Specify which hypothesis is validated.
- 3- Name the different types of lymphocytes implicated in the immune response revealed by this experiment.



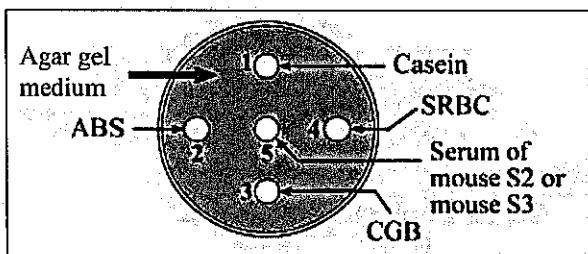
*ABS: Albumin of bull serum.

**CGB: Calmette and Guerin Bacillus.

Document 1

Document 2 shows a serological test called immuno diffusion in gel where antibodies and antigens are deposited in wells in agar gel medium. We deposit an antigenic substance in each of the wells 1, 2, 3 and 4 and deposit either serum taken from mouse S2 or serum taken from mouse S3 in the central well 5.

- 4- Specify where precipitation arc (s) would be formed with each serum after 24 hrs of antigens' deposit. Justify the answer.
- 5- Schematize the mechanism which leads to the formation of the precipitation arcs.



Document 2

Exercise 20 (5 pts) Cytotoxicity of Tc lymphocytes

Session 2010-2

The cells that are infected by a virus express on their plasma membranes some antigens of this pathogen. These antigens can be recognized by specific receptors of the cytotoxic lymphocytes (Tc). In the attempt to prove the cytotoxicity of the Tc lymphocytes, we perform the experiment schematized in document 1, where cells infected by virus A are incubated with ^{51}Cr , a substance that binds to intracellular proteins after being absorbed by the cell.

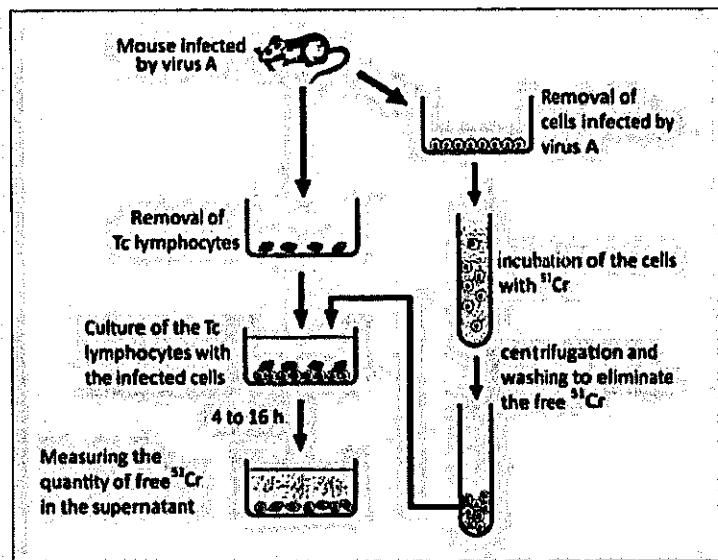
1. Describe the experiment schematized in document 1.

2. Justify, by referring to document 1, how the presence of free ^{51}Cr in the supernatant reveals the cytotoxic role of Tc lymphocytes.

3. Specify the type of the immune response revealed by the experiment of document 1.

Dermal cells of mice of strain X or of strain Y, infected or not by a virus, are cultured *in vitro*.

Tc lymphocytes removed from other mice of strain X, infected or not by a virus, are added to the culture medium. Document 2 presents the experimental conditions and the obtained results.



Document 1

Origin of the cultured dermal cells Origin of the added Tc lymphocytes	Healthy mice X	Mice X infected by virus A	Mice X infected by virus B	Mice Y infected by virus B
Healthy mice X	No destruction of dermal cells	No destruction of dermal cells	No destruction of dermal cells	No destruction of dermal cells
Mice X infected by virus A		Destruction of the infected dermal cells by the Tc lymphocytes	No destruction of dermal cells	
Mice X infected by virus B		No destruction of dermal cells	Destruction of the infected dermal cells by the Tc lymphocytes	No destruction of dermal cells

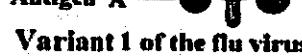
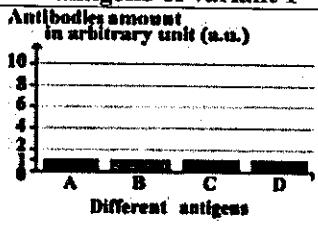
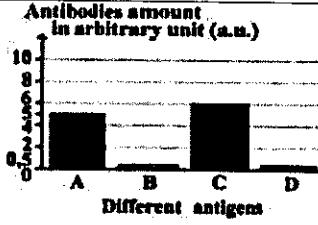
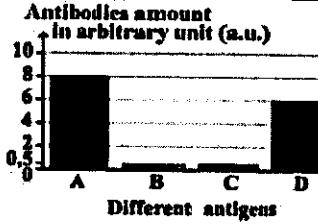
Document 2

4. Interpret the results of document 2.
5. Explain the mechanism that leads to the destruction of target cells by Tc lymphocytes.

Exercise 21 (5 pts) Immunological memory**Session 2011-1**

When an organism encounters the same pathogen more than once during its lifetime, the immune response against this pathogen becomes more and more efficient. The flu virus exists in different variants having different antigens. We study the immune responses triggered by an individual upon contact with the flu virus three times during his life time.

The document below presents the age of this individual at the time of contact with one of the three variants of the flu virus and the evolution in the amount of antibodies specific to the antigens of variant 1.

Age of the individual at the time of infection	Antigens of the variant	Evolution in the amount of antibodies specific to the antigens of variant 1										
 2 years old individual in contact with variant 1 of the flu virus	Antigen B  Antigen D  Antigen C  Antigen A  Variant 1 of the flu virus	 <table border="1"> <caption>Antibodies amount in arbitrary unit (a.u.)</caption> <thead> <tr> <th>Different antigens</th> <th>Antibodies amount (a.u.)</th> </tr> </thead> <tbody> <tr> <td>A</td> <td>1</td> </tr> <tr> <td>B</td> <td>1</td> </tr> <tr> <td>C</td> <td>1</td> </tr> <tr> <td>D</td> <td>1</td> </tr> </tbody> </table>	Different antigens	Antibodies amount (a.u.)	A	1	B	1	C	1	D	1
Different antigens	Antibodies amount (a.u.)											
A	1											
B	1											
C	1											
D	1											
 The same individual at the age of five years in contact with variant 2 of the flu virus	Antigen E  Antigen F  Antigen C  Antigen A  Variant 2 of the flu virus	 <table border="1"> <caption>Antibodies amount in arbitrary unit (a.u.)</caption> <thead> <tr> <th>Different antigens</th> <th>Antibodies amount (a.u.)</th> </tr> </thead> <tbody> <tr> <td>A</td> <td>5</td> </tr> <tr> <td>B</td> <td>1</td> </tr> <tr> <td>C</td> <td>5</td> </tr> <tr> <td>D</td> <td>1</td> </tr> </tbody> </table>	Different antigens	Antibodies amount (a.u.)	A	5	B	1	C	5	D	1
Different antigens	Antibodies amount (a.u.)											
A	5											
B	1											
C	5											
D	1											
 The same individual at the age of 20 years in contact with variant 3 of the flu virus	Antigen E  Antigen D  Antigen G  Antigen A  Variant 3 of the flu virus	 <table border="1"> <caption>Antibodies amount in arbitrary unit (a.u.)</caption> <thead> <tr> <th>Different antigens</th> <th>Antibodies amount (a.u.)</th> </tr> </thead> <tbody> <tr> <td>A</td> <td>8</td> </tr> <tr> <td>B</td> <td>1</td> </tr> <tr> <td>C</td> <td>1</td> </tr> <tr> <td>D</td> <td>8</td> </tr> </tbody> </table>	Different antigens	Antibodies amount (a.u.)	A	8	B	1	C	1	D	8
Different antigens	Antibodies amount (a.u.)											
A	8											
B	1											
C	1											
D	8											

- 1- Name the specific immune response revealed in the above document. Justify the answer.
- 2- Justify the following statements by referring to the document.
 - 2-1- The secondary immune response is more amplified than the primary immune response.
 - 2-2- The secreted antibody is specific to the antigen and not to the variant of the virus.
 - 2-3- The organism keeps memory for an encountered antigen for more than ten years.
- 3- Name two cells implicated in the immune response triggered against variant 1 of the flu virus and specify the role of each cell.
- 4- Explain how the secreted antibodies contribute to the destruction of the flu virus.
- 5- Specify if the revealed immune response is capable alone to eliminate cells infected by the virus.

Exercise 22 (5 pts) Immune response against a virus**Session 2011-2**

The EBV virus infects 90% of the world population, but in a benign manner. This virus persists in the body. Its target cells are B lymphocytes.

Document 1 shows the activity of the EBV in "naive B Lymphocytes" (B lymphocytes that have never encountered the specific antigen) and in memory B lymphocytes specific for this antigen.

- Determine by referring to document 1, how the EBV virus persists and is produced in the body.

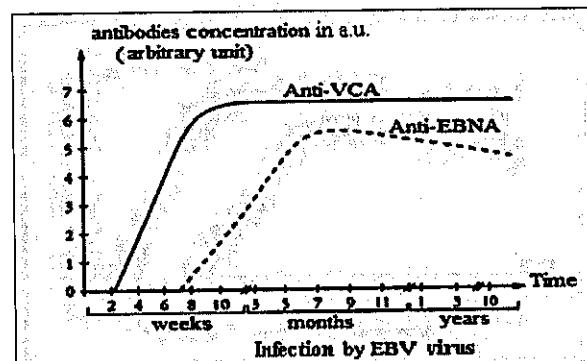
To better understand one of the immune responses triggered against the EBV virus, we follow up the evolution of anti-VCA and anti-EBNA antibodies directed respectively against two peptides VCA and EBNA that are found on the surface of this virus. The results are shown in document 2.

- Name the immune response revealed by these measurements. Justify the answer.
- Analyze the results of document 2.
- What can we draw out?

To Petri dishes containing appropriate culture medium, we add Lymphocytes (BL and TL) taken from different individuals infected or not by different viruses, EBV or other viruses. All the lymphocytes used in each experiment have the same HLA. Document 3 presents the conditions and the results of these experiments.

- Describe, in a short text, the experiments and the obtained results presented in document 3.
- Explain the obtained results of these experiments.

Activity of EBV	Naive B Lymphocyte	Memory B Lymphocyte
State of EBV in the lymphocyte	Active	Dormant
Presentation of viral peptides on the surface of the lymphocyte	Yes	No
Production of new viruses released into blood and able to infect other BL	Yes	No except if it is reactivated

Document 1**Document 2**

Experiment	Experimental conditions	Results
1	TL of an individual infected by EBV ↓ BL infected by EBV	100% lysed BL
2	TL of an individual infected by EBV ↓ BL not infected by EBV	No lysed BL
3	TL of an individual infected by EBV ↓ memory BL infected by EBV	No lysed BL
4	TL of an individual infected by EBV ↓ BL infected by another virus	No lysed BL
5	TL of an individual not infected by EBV ↓ BL infected by EBV	No lysed BL

Document 3

Legend: → : Add

Exercise 23 (5 pts) Tetrahydrocannabinol and immune response

Session 2012-1

A recent experimental study was performed on mice to demonstrate the action of tetrahydrocannabinol (THC) on the immune system. THC is an active ingredient of cannabis (drug) that is suspected to modify the immune response against cancer cells. In order to study the tumor development and the immune response in the presence of THC, the following experiments were performed.

Experiment 1: We take two lots of non-immunized healthy mice, to which we implant cancer cells having the same strain as that of the mice: Lot 1 did not receive any injection of THC; Lot 2 was subjected to four injections of THC per week before and after the implantation of cancer cells. The results of the two lots are presented in documents 1 and 2.

Document 1 represents the variation of the tumor volume as a function of time after implantation. Document 2 shows the proliferation of T lymphocytes as a function of the percentage of the cancer cells implanted relative to the number of T lymphocytes before proliferation.

- Interpret the results of each of the documents 1 and 2.

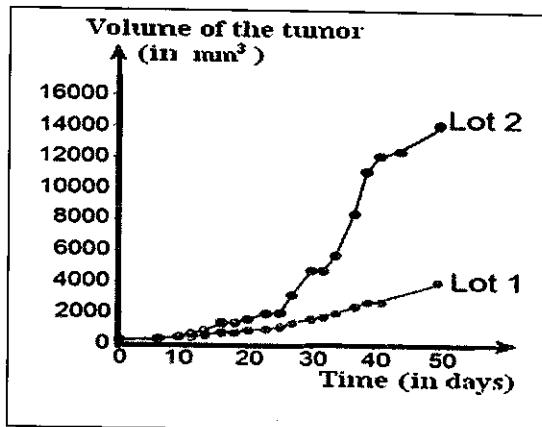
Experiment 2: The level of interleukins secreted in the mice of experiment 1 was measured at the level of the tumor and the spleen. These values are presented in document 3.

- Determine, with reference to document 3 and to acquired knowledge, the target cells of THC.
- Draw out referring to all what preceded, the action of THC on the immune response against the tumor.

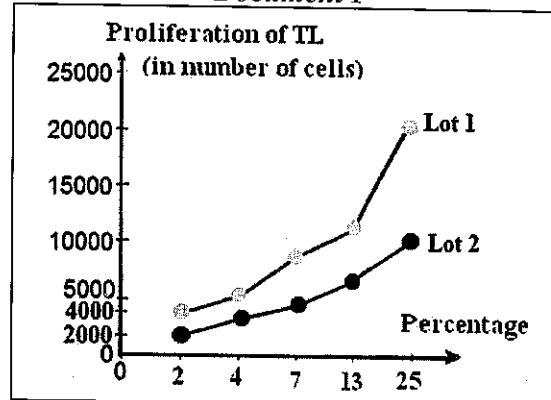
In the framework of studying the effect of THC on the secondary immune response, we perform experiment 3.

Experiment 3: New mice belonging to the same lots are immunized against this tumor before being subjected to implantation of cancer cells. From each lot, eight mice were subjected to the implantation of a variable number of cancer cells and the percentage of mice rejecting the tumor was calculated (Document 4).

- Construct a histogram that translates the results in document 4.
- Analyze the results of experiment 3.
- What can you draw out?



Document 1



Document 2

	Interleukins secreted at the level of the tumor (pg.mL⁻¹ for 500 mg of tumor)	Interleukins secreted at the level of the spleen (pg.mL⁻¹ for 10⁶ cells)
Lot 1	190	37
Lot 2	73	21

Document 3

Number of live implanted tumor cells	1 × 10⁵	2 × 10⁵	3 × 10⁵
	Percentage of mice rejecting the tumor	Lot 1	100%
Lot 1	100%	100%	100%
Lot 2	100%	60%	50%

Document 4

Exercise 24 (5 pts) Cellular cooperation and production of antibodies**Session 2012-2**

In the framework of determining the conditions of the production of antibodies during the immune response, we perform a series of experiments on mice of the same strain.

Experiment 1: Mice are subjected to the ablation of the thymus followed by irradiation that destroys all cells of the immune system. These mice are then divided into 4 lots and treated as shown in document 1.

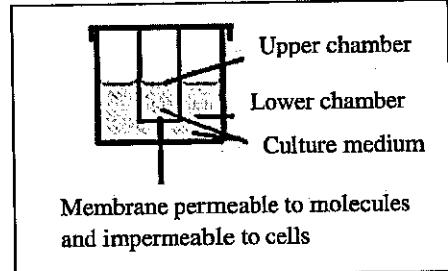
Ablation of the thymus then irradiation of the mice				
	Lot 1	Lot 2	Lot 3	Lot 4
Injection of lymphocytes removed from mice of the same strain	T	B and T	B and T	B
Injection of an antigen: SRBC (sheep red blood cells)	Yes	Yes	No	Yes
One week later, removal of serum from the mice and addition of SRBC to the serum				
Results : agglutination of SRBC	absence	presence	absence	absence

Document 1

- Interpret the experimental results of experiment 1.
- Specify the aim of destroying the cells of the immune system before starting the experiment.

Experiment 2: A mouse receives an injection of sheep red blood cells (SRBC). Three days later, we extract lymphocytes from its spleen. These lymphocytes are distributed into 4 identical lots then cultured in Marbrook chamber (document 2) according to the procedure described in document 3.

Few days later, the culture medium is filtered and the collected liquid is added to SRBC. The results are shown in document 3.

**Document 2**

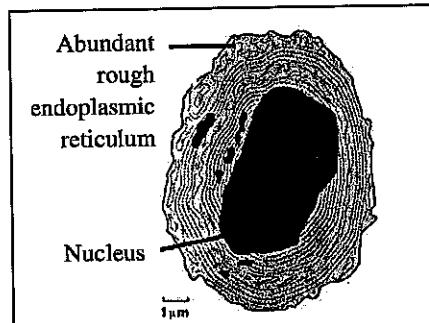
Culture medium	1	2	3	4
Lymphocytes placed in the upper chamber	none	T	none	none
Lymphocytes placed in the lower chamber	T and B	B	B	T
Results : agglutination of SRBC	Strong	Strong	Null	Null

Document 3

- Analyze the results of media 1 and 2. What can you draw out?

Document 4 illustrates an electronography of an antibody secreting cell that is found in large quantities, in media 1 and 2 of document 3 and absent in media 3 and 4.

- Name this cell. Justify the answer.
- Explain the variation in the quantity of this type of cells in the four media of experiment 2.

Document 4

Exercise 25 (5 pts) Cell lysis

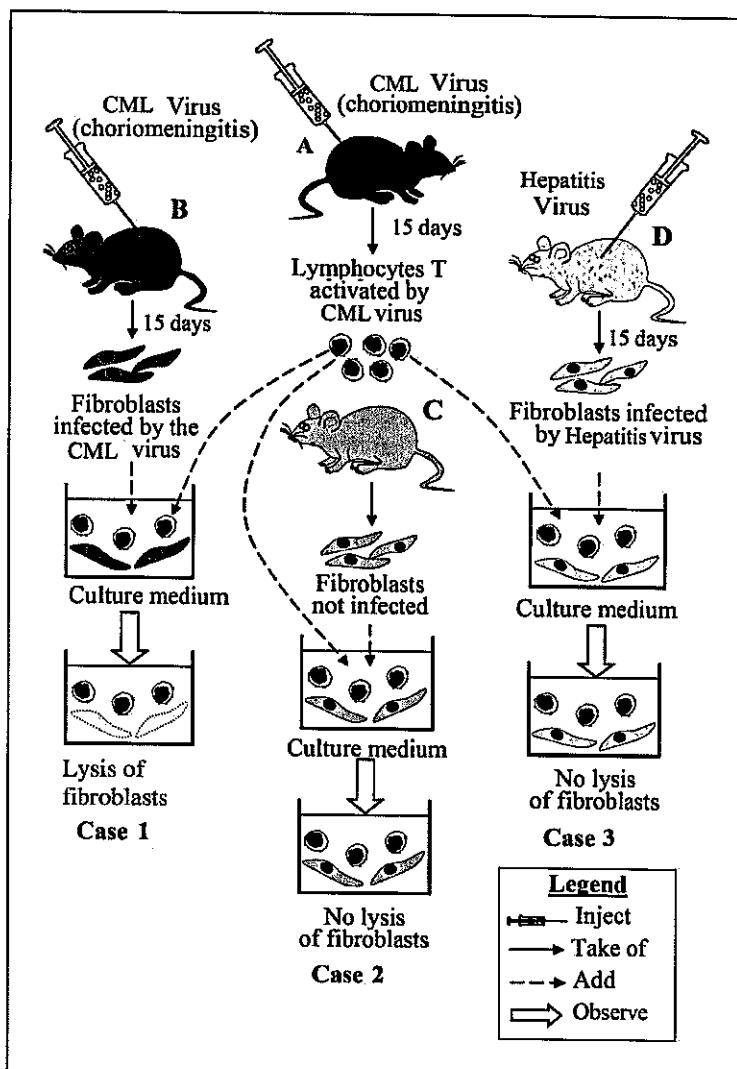
Session 2013-1

Choriomeningitis virus (CML) is a virus transmitted by rodents. The disease is manifested by symptoms similar to those of flu with fever. This disease is transmitted to humans by contaminated food or dust from infected mice.

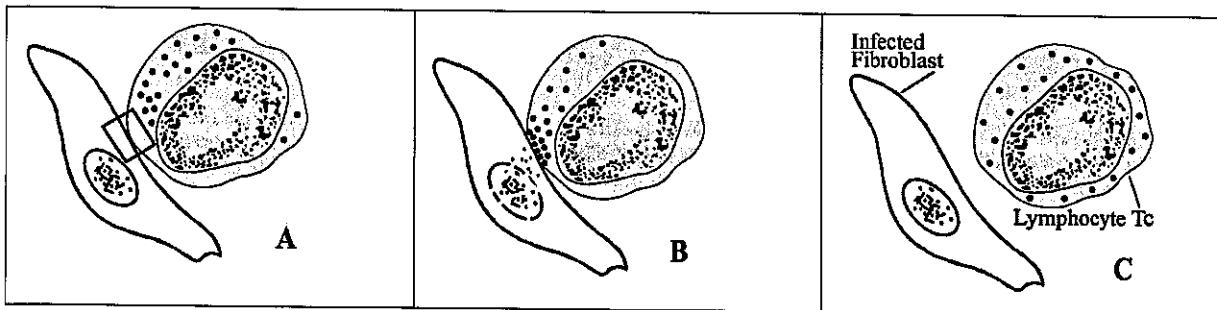
To better understand the immune mechanisms responsible for the lysis of infected cells, a set of experiments are performed on mice of the same line (document1).

- Pick out from the text the means of contamination of humans by CML virus.
- Describe the experiments schematized in document 1.
- Interpret the results of the experiments of document 1.

Document 2 shows the schematic representations of the cellular interactions observed in the culture medium in case1.



Document 1



Document 2

- Arrange, in chronological order, the schematic representations of document 2. Justify the answer.
- Explain the mechanism of cell lysis observed in document 2.

Exercise 26 (5 pts) Vaccine against AIDS

In the framework of researches concerning AIDS, scientists followed up 1600 non treated persons that are infected by HIV (Human Immunodeficiency Virus). They measured, at the beginning of the infection, the viral concentration in the blood and recorded the percentage of persons reaching the phase of AIDS. The results are presented in document 1.

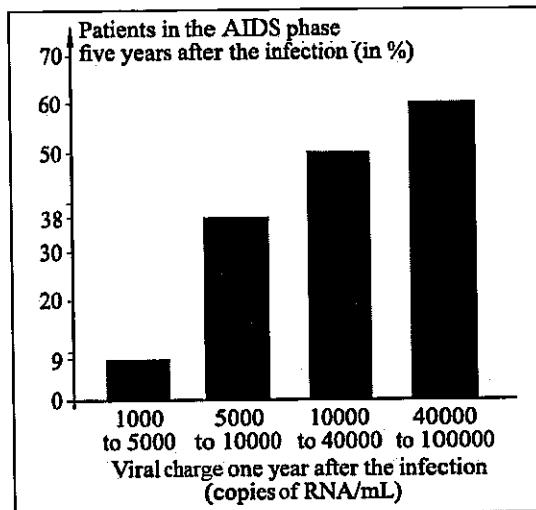
- Justify, by referring to document 1, the following statement: "in the absence of treatment, there is a relation between the onset of the phase of AIDS and the early evolution of the viral charge".

In the case of HIV, vaccines that activate only the production of anti-HIV antibodies don't protect against all the known strains of the virus. Currently, the scientific community agrees on the fact that: to be effective, a vaccine should also stimulate the production of cytotoxic T lymphocytes directed against HIV. This allowed the elaboration of vaccines against HIV.

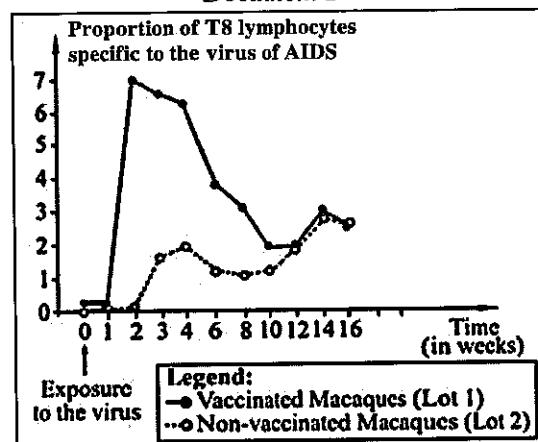
- Indicate how vaccination protects against a given antigen.

One of these vaccines was tested on two lots of macaques monkeys that are not infected by the virus of AIDS. The monkeys of the first lot (lot 1) receives a series of five vaccine injections. The monkeys of the second lot (lot 2) are not vaccinated. Then, all the monkeys are exposed to the virus. The proportion of T8 lymphocytes specific to the AIDS virus is then evaluated in the blood of the monkeys (document 2). The viral charge is measured in the two lots of monkeys at the 8th and at the 24th week following the exposure to the virus (document 3).

- Show, by referring to document 2, that the immune response of the vaccinated monkeys is more rapid and more amplified than that of the non-vaccinated monkeys during the first 3 months of the infection.
- Determine if the immune response triggered in lot 1 is durable.
- Interpret the results of document 3.
- Show, by referring to what precedes, that the tested vaccine has a limited efficiency and doesn't allow the eradication of the disease.



Document 1



Document 2

Time after exposure to the virus	Viral charge (number of viral RNA copies / ml of plasma)	
	Lot 1	Lot 2
8 th week	$5 \cdot 10^4$	$25 \cdot 10^4$
24 th week	$5 \cdot 10^4$	$50 \cdot 10^4$

Document 3

Exercise 27 (5 pts) Infection by HIV

Session 2014

HIV is a retrovirus whose genetic material consists of RNA. When the virus infects a target cell, it uses the transcription and translation machineries of the host cell in order to synthesize its different components. The infected cell produces new viruses and then dies.

- Indicate the type of the specific immune response that is triggered against cells infected by a virus. Justify the answer.

To better understand the mechanism of infection of a target cell by HIV, the following studies are performed:

Leucocytes are cultured then exposed to HIV. The survival of these cells is measured during the days that follow the exposure. The results are presented in document 1.

- Represent in a table the obtained results shown in document 1.
- 3-1 Analyze the obtained results (doc 1).
- 3-2 Conclude the target cells of HIV.

Document 2 shows the stage of recognition of a target cell by the HIV.

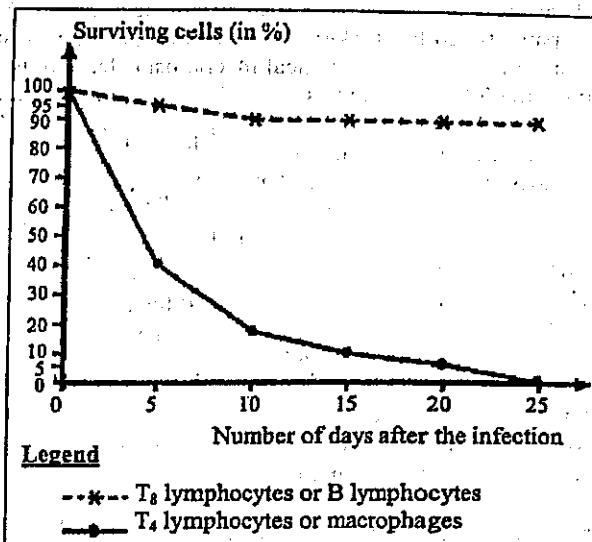
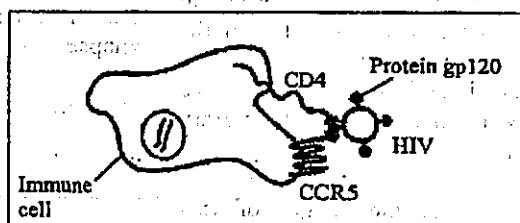
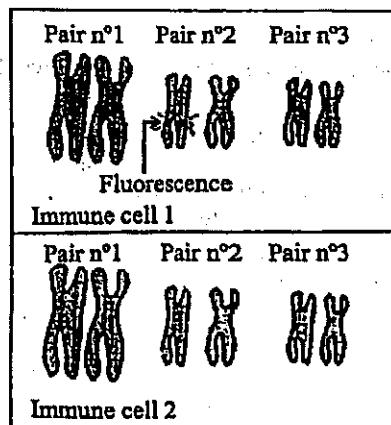
- Specify, by referring to document 2, the characteristic that allows a cell to be a target for HIV.

In order to identify the infected cell, two immune cells 1 and 2 are extracted from a patient and isolated. DNA probe having complementary sequence to the HIV viral genome is added. Once fixed, the probe is visualized by fluorescence using an appropriate technique.

The obtained results are presented in document 3.

For simplification, only three pairs of chromosomes are represented.

- Identify which immune cell (1 or 2) is infected by HIV.
- Explain the mode of infection of an immune cell by HIV starting from its recognition till the production of new viruses.

**Document 1****Document 2****Document 3**

Exercise 28 (5 pts) Fight against Ebola

Session 2015-1

Ebola is a very contagious and fatal virus that causes hemorrhagic fever. It is transmitted through blood, saliva, feces as well as through sexual contacts.

Infected individuals who survived, show first a high amount of specific anti-Ebola antibodies, followed by the disappearance of the virus with an important increase in specific cytotoxic T cells (TcL).

- 1-** Identify the immune response(s) triggered against Ebola.

In order to develop fighting or therapeutic modalities against this disease, researchers performed experiments that are described below.

- In December 2011, researchers developed a vaccine. They isolated a surface protein of the virus and injected it to a first lot of mice. To a second lot, they injected the same protein in the form of immune complexes called EIC (Ebola Immune Complexes). To a third lot they injected the EIC and a substance, the PIC. The injections are repeated four times for each lot. Two weeks after each injection, serum is collected from the mice and the antibodies amounts were measured. The obtained results are presented in document 1.

- 2-** Determine the most efficient vaccine against Ebola.

The molecule PIC is an agonist to proteins that are indispensable for phagocytosis.

- 3-** Indicate the roles and the moments where macrophages intervene in the specific immune response triggered against Ebola.

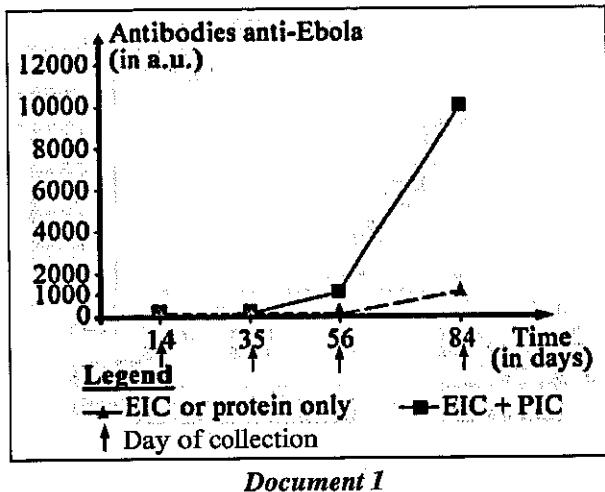
Two lots of mice have been vaccinated using the mixture EIC+PIC, the first lot received three boosters for the vaccine and the second received four boosters. After that both lots were contaminated by Ebola virus. The results concerning the survival of the mice are presented in document 2.

- 4-** Deduce one condition for the vaccination against Ebola to be successful.

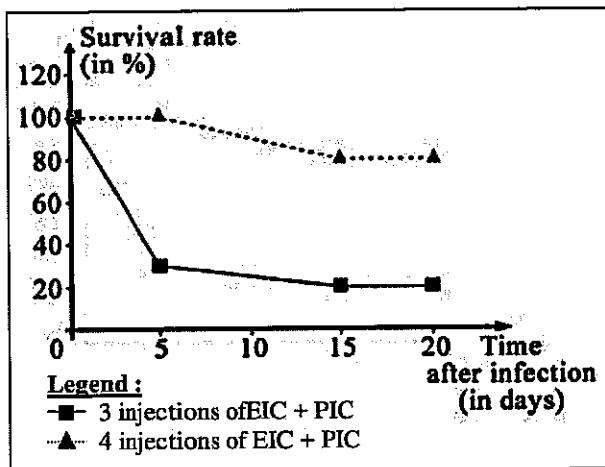
- In June 2012, Canadian researchers performed the following experiment: two lots of monkeys, infected by the Ebola virus, received a mixture of three antibodies specific to particular epitopes of the virus. The obtained results are presented in document 3.

- 5-** Explain the obtained results.

- 6-** Distinguish serotherapy from vaccination concerning: the nature of the injected substance, the latency period and the duration of the protection established against Ebola.



Document 1



Document 2

Lots of monkeys	Performed treatment	Number of monkeys	Number of surviving monkeys
A	Infection by the virus then injection of antibodies 24 hours after infection	4	4
B	Infection by the virus then injection of antibodies 48 hours after infection	4	2

Document 3

Exercise 29 (5 pts) Cervical cancer and HPV virus

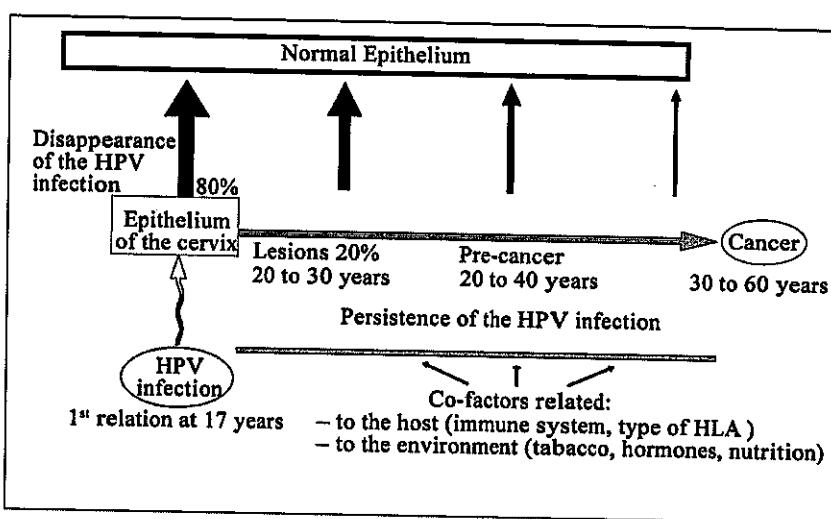
Session 2015-2

Cervical cancer is considered to be the second cancer that affects women in the developing countries, and the eighth in the developed ones.

To better understand the cause of this cancer and in order to prevent it efficiently, researchers performed different studies.

- A study involving thousands of women suffering from cervical cancer shows that 75% of these women have encountered the human papillomavirus (HPV) during their sexual life.

Document 1 shows the evolution of the state of the cervical epithelium after HPV infection.

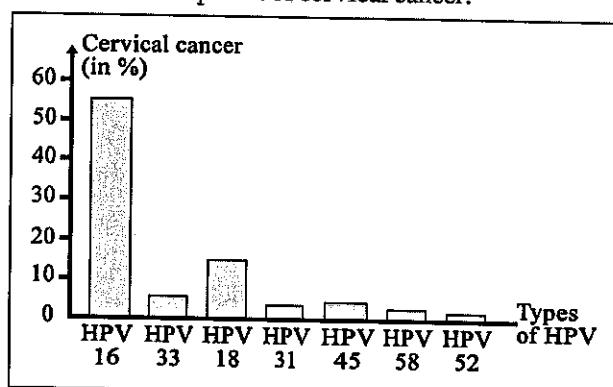


1. Justify the following statement: « Cervical cancer is a slowly induced viral cancer ».
2. Pick out, from document 1, two other risk factors for the development of cervical cancer.

3. Indicate the type of specific immune response triggered to fight against a viral infection. Justify the answer.

- In a second study, 150 types of HPV have been identified, some of which are qualified as being "high-risk", induce a genetic mutation which causes cervical cancer. Document 2 shows the percentages of women having cervical cancer as a function of the types of HPV that have infected them.

4. Deduce from document 2 the two types of HPV of high risk .



- Researchers have elaborated prophylactic vaccines aiming to ensure a preventive protection of individuals against the infection. These vaccines stimulate the production of antibodies directed against certain types of HPV viruses. The characteristics of two of these vaccines are regrouped in document 3.

5. Determine the most efficient vaccine.
6. Explain how the antibodies produced during this vaccination allow protection against cervical cancer.
7. Suggest two preventive means against cervical cancer.

Types of targeted HPV	Vaccine	
	Gardasil	Cervarix
Quadrivalent Vaccine HPV6, HPV11, HPV16 and HPV18	Bivalent Vaccine HPV16 and HPV18	
Suggested amount	Almost 20 µg	20 µg
Vaccination Schedule	0, 2 and 6 months	0, 1 and 6 months
Amount of antibodies produced compared to that of the natural infection	8 times higher	100 times higher

Document 3

Exercise 30 (5 pts) AIDS and treatments

The human immunodeficiency virus (HIV) is responsible for the weakness of immune defenses in the organism, which leads to the death of affected persons. Document 1 shows the evolution of the concentration of T4 cells, measured in patients contaminated by HIV.

- 1- Analyze the results of document 1.
- 2- Draw out, from document 1, the cause of the appearance of opportunistic diseases.

In order to find a treatment that limits the consequences of opportunistic diseases, a series of studies is performed, some of which are represented below:

Study 1: Lymphocytes are removed from a monkey and B, T4 and T8 cells are separated.

- B cells are placed in chambers of culture 1 (1a, 1b and 1c) where molecules of antigen X are present at their bottoms. Only 0.01% of B cells remains fixed to the bottom of each chamber and is not eliminated by rinsing.
- T8 cells are placed in chambers of culture 2 (2a and 2b) where monkey cancerous fibroblasts are present at their bottoms. Only 0.01% of T8 cells remains fixed to the bottom of each chamber and is not eliminated by rinsing.
- Then, lymphocytes activated by the same antigens (X or cancerous fibroblasts) are added to certain chambers.

Document 2 shows the experimental conditions as well as the results.

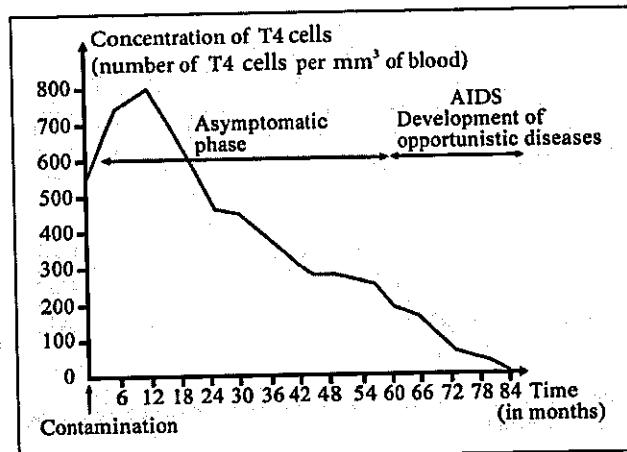
- 3- Interpret the results obtained in document 2.
- 4- Explain, by referring to all what precedes, the appearance of opportunistic diseases observed in document 1.

Study 2: Two groups of researchers have produced two treatments.

The first treatment is based on the principle of vaccination against some opportunistic diseases such as pneumonia. This treatment was tested on two categories of patients having a different number of T4 cells. The results are represented in document 3.

In the second treatment, three medicines are administered during 5 years to individuals whose number of T4 cells, at the beginning of treatment, is between 200 and 350 T4 cells/mm³ of blood. The results are shown in document 4.

- 5- Explain the importance of vaccination.
- 6- Determine if the first treatment is efficient against the development of opportunistic diseases.
- 7- Show that the second treatment may delay the AIDS phase.



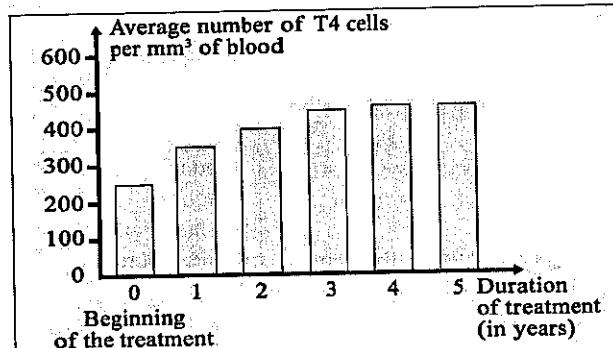
Document 1

Chamber	Existing lymphocytes	Added lymphocytes	Result
1a	B cells retained by antigen X	-	No antibodies
1b		Activated T4 cells	Presence of antibodies
1c		Activated T8 cells	No antibodies
2a	T8 cells retained by monkey cancerous fibroblasts	-	No lysis of fibroblasts
2b		Activated T4 cells	Lysis of fibroblasts

Document 2

Category	Average number of T4 cells/ mm ³ of blood	production of antibodies
1	> 500	Strong
2	< 200	Weak

Document 3



Document 4

Exercise 31 (5 pts) Conditions of LT action

Session 2016-2

The Choriomeningitic leukemia virus (CML) is slightly pathogenic and infects nervous cells. In the framework of studying the immune response against the infection by this virus, two experiments were performed.

Experiment 1: different viruses are injected into mice of different strains, Y and Z. The experimental conditions as well as the obtained results are shown in document 1.

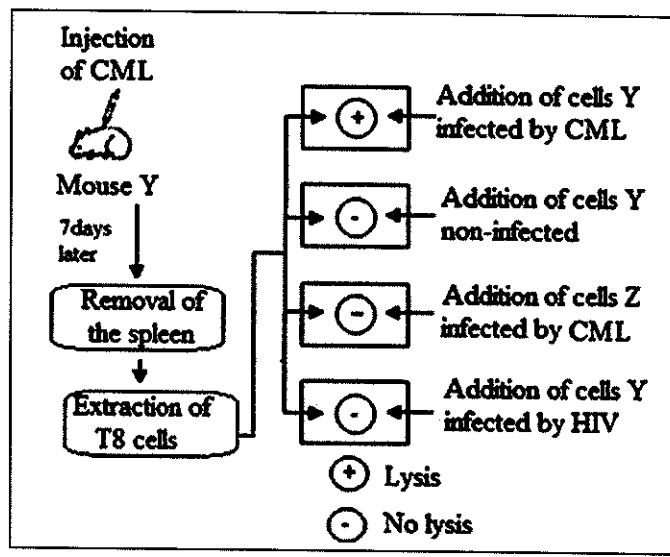
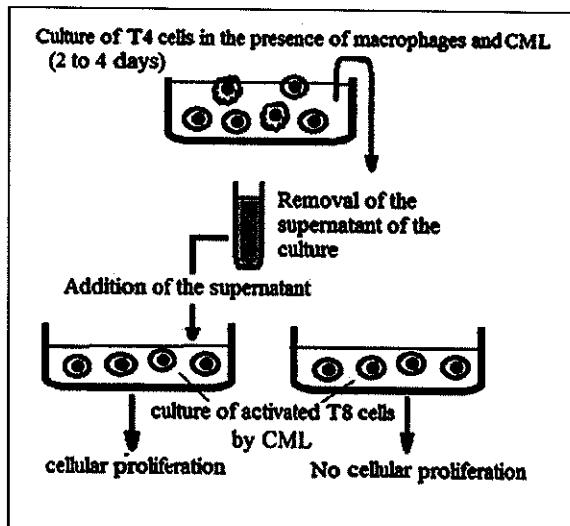
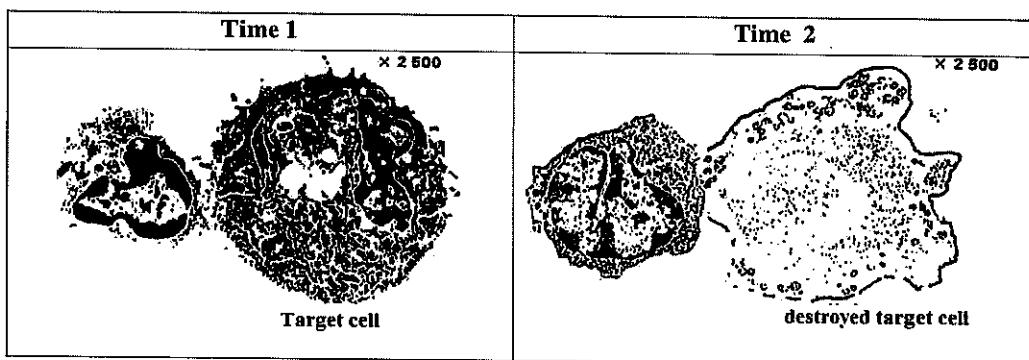
- 1- Name the specific immune response triggered against a virus and that triggered against a bacterium.
- 2- Interpret the results of experiment 1.

Experiment 2: T4 cells are cultured in the presence of macrophages and CML. The experimental conditions as well as the results are shown in document 2.

- 3- Determine the role and the mode of action of T4 cells as revealed in experiment 2.
- 4- Explain the role of macrophages in the culture of T4 cells in experiment 2.

Document 3 shows two electronographs, made at two successive times, of a target cell infected by CML in the presence of an activated T8 cell taken from experiment 2.

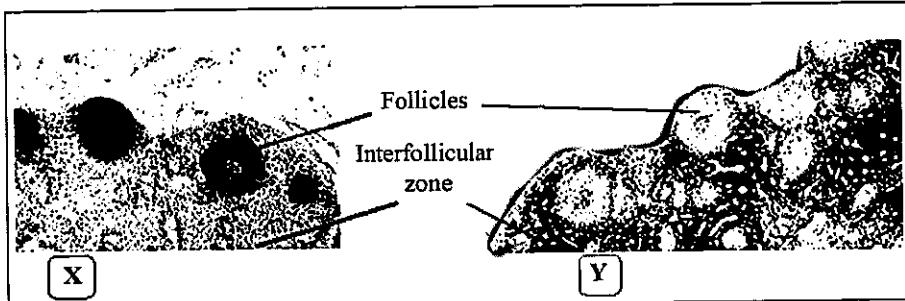
- 5- Draw a scheme showing the molecules involved in the recognition taking place between the activated T8 cell and the target cell.
- 6- Explain the mechanism shown in document 3.

**Document 1****Document 2****Document 3**

Exercise 32 (5 pts) Hypertrophy of lymph nodes**Session 2017-1**

A temporary hypertrophy (swelling) of the lymph nodes is observed in an individual infected by an antigen like the tetanus toxin. In order to better understand the mechanisms involved in this hypertrophy, the following experiments are performed.

Experiment 1: The constituents of the lymph nodes of this individual are studied by using radioactive markers. Microradiographs are then performed. The radioactive labeled zones appear in black on the microradiographs.

**Document 1**

Document 1 shows the results of labeled radioactive B lymphocytes (X) and of labeled radioactive T lymphocytes (Y).

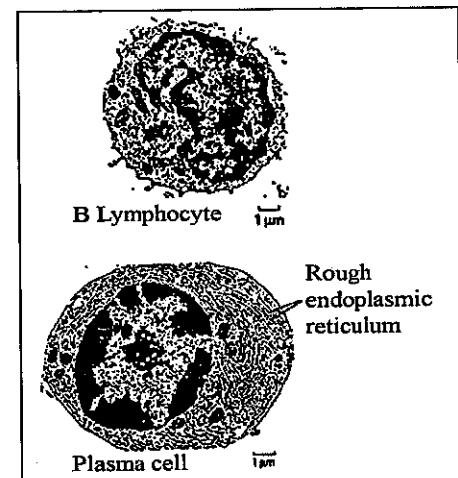
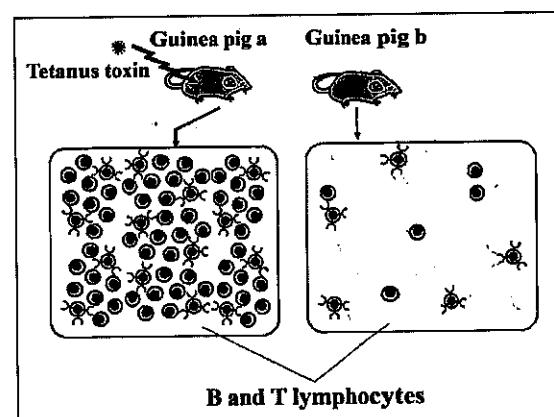
- Deduce the localization of each lymphocyte population at the level of lymph nodes.

Document 2 shows microphotographs of the cells identified in the lymph nodes of the individual who is infected with tetanus toxin.

- Specify the type of the immune response triggered against this antigen and revealed in document 2.
- 3-1- Name the molecules secreted by this plasma cell.
3-2- Explain how the plasma cell is a cell adapted to the secretion of these molecules.

Experiment 2: cells are extracted from the lymph nodes of a guinea-pig (a) which is injected with tetanus toxin and from the lymph nodes of a healthy guinea pig (b). They are then purified to obtain only B and T lymphocytes. The results are schematized in document 3.

- Interpret the results presented in doc 3.
- Justify, referring to what precedes, the temporary hypertrophy of the lymph nodes observed in this individual.
- Explain the role of TL involved in the immune response revealed in document 2.

**Document 2****Document 3**

Exercise 33 (5 pts) Roles of macrophage

Session 2017-2

The monocytes circulate in the blood and can migrate to the tissues where they become macrophages.

- Indicate the origin of monocytes.

In order to study the mode of action of macrophages and their cooperation with certain cells of the immune system, the following experiments are performed.

Experiment 1 :

Cells are extracted from the ganglia of a guinea pig which is immunized against antigen X. T4 lymphocytes and macrophages are isolated and placed in different culture media. The experimental conditions and the results are shown in document 1.

- Determine the conditions indispensable for the proliferation of T4 lymphocytes.

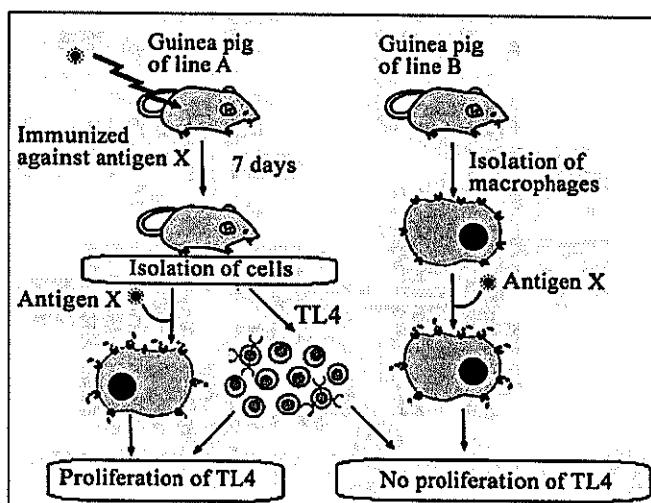
Culture medium	Conditions of the culture	Results
1	T4 lymphocytes and antigen X	No proliferation of T4 lymphocytes
2	T4 lymphocytes and macrophages	No proliferation of T4 lymphocytes
3	T4 lymphocytes, macrophages and antigen X	Proliferation of T4 lymphocytes
4	T4 lymphocytes	No proliferation of T4 lymphocytes

Document 1

Experiment 2 :

An experiment is performed on two different strains of guinea pigs, A and B. The experimental conditions as well as the results are shown in document 2.

- Indicate the condition indispensable for the proliferation of T4 lymphocytes shown in this experiment. Justify the answer.



Document 2

Experiment 3:

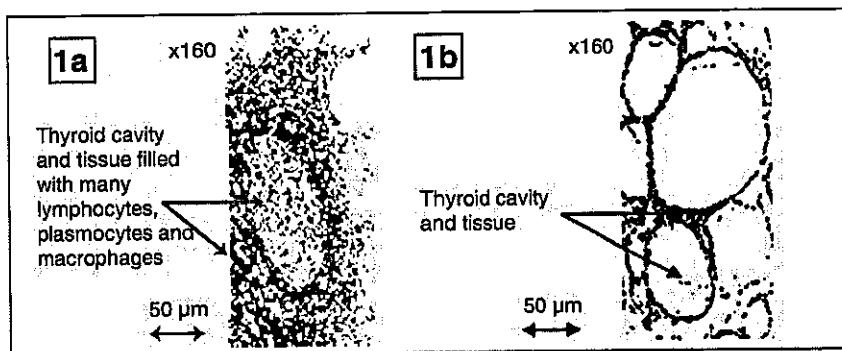
Macrophages are incubated with the same antigen X labeled with radioactive ^{131}I odine isotope. At phase I, radioactivity is detected inside the macrophage, and in phase II a rapid degradation of antigen X is noticed. After some time 80% of radioactivity is detected in the culture medium in the form of ^{131}I odine isotope linked to peptides, while the remaining 20% are found attached to the cell surface.

- Draw out the role of macrophages shown at phase I of experiment 3.
- Explain the results obtained at phase II in experiment 3.
- Explain the mode of action of the macrophages that permits the proliferation of T4 lymphocytes.
- Specify the consequence of the absence of the macrophages on the specific immune responses.

Exercise 34 (5 pts) Diagnostic of galactosemia

Sarah has a swelling of the neck at the level of thyroid gland and suffers from many troubles of metabolic origin. Blood analysis of Sarah shows that the concentration level of the thyroid hormones is noticeably lower than the normal values. The synthesis of these thyroid hormones necessitates the presence of a protein named thyroglobulin.

A biopsy is performed on the thyroid gland of Sarah. Document 1 represents the results of the microscopic observations of the sections of thyroid gland of Sarah (1a) and those of the normal thyroid gland (1b).

**Document 1**

1. Formulate a hypothesis that can explain the results of biopsy of the thyroid gland of Sarah.

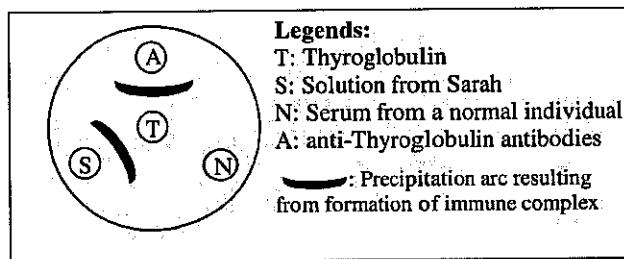
The immune and thyroid cells extracted from the thyroid gland of Sarah, are cultured in 3 different media. The conditions as well as the results are shown in document 2.

2. Interpret the results shown in document 2.
3. Identify the nature of the specific immune response revealed in document 2.
4. Explain the following statement: "Macrophages induce specific immune response".

Culture	Cultivated Cells	Results
1	Thyroid cells + B Lymphocytes	Absence of antibodies
2	Thyroid cells + B Lymphocytes + Macrophages	Absence of antibodies
3	Thyroid cells + B Lymphocytes + Macrophages + T ₄ Lymphocytes	Presence of a large amount of antibodies

Document 2

Afterwards, immunodiffusion gel test is applied. A solution containing the protein thyroglobulin (T) is deposited in the central well, and three other different solutions are separately deposited in three peripheral wells: A solution of antibodies from Sarah (S), anti-thyroglobulin antibodies (A), and serum from a normal individual (N). The results are shown in document 3.

**Document 3**

5. Show that Sarah suffers from an auto-immune disease directed against the self.

Exercise 35 (5 pts) Therapy against autoimmune disease**Session 2018-2**

Type 1 diabetes (T1D) is due to an autoimmune disease. The current treatment that is based on insulin injection attenuates the symptoms of type I diabetes disease without curing it. For this reason, a research is carried out to verify the effectiveness of a new therapeutic approach to stop the progression of the autoimmune disease which is at the origin of this type of diabetes.

Measurements of the mass of certain components of the pancreas are performed during autopsies in healthy individuals and in individuals suffering from type 1 diabetes. Document 1 shows the obtained results.

1.1. Compare the obtained results.

1.2. Draw out the cause of type 1 diabetes.

The NOD mice (Non Obese Diabetic) develop a disease similar to T1D starting from the age of 10 weeks.

Document 2 represents islets of Langerhans of NOD mice at two different stages of diabetes: an early stage of diabetes (2 a) and a more advanced stage (2b). In this document, T8 lymphocytes appear in the form of black spots.

Note that these mice are not subjected to any viral infection.

2. Identify the type of the immune response involved in this autoimmune disease.

3. Explain the mode of action of T8 Lymphocytes on their target cells.

A new treatment for T1D is tested on two lots of NOD mice at the age of 4 weeks, before the onset of the disease:

- Lot A receives an injection of a saline solution that has no effect (control lot).
- Lot B is subjected to this new treatment.

Document 3 shows the occurrence of diabetes in these two lots of NOD mice.

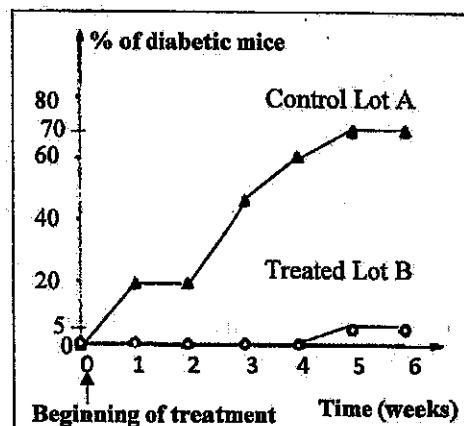
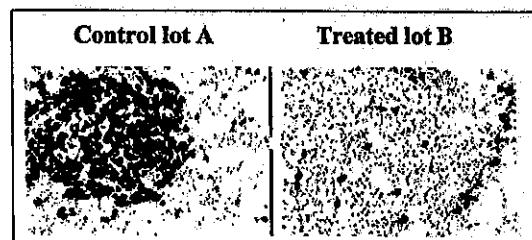
4. Draw a table representing the results of document 3.

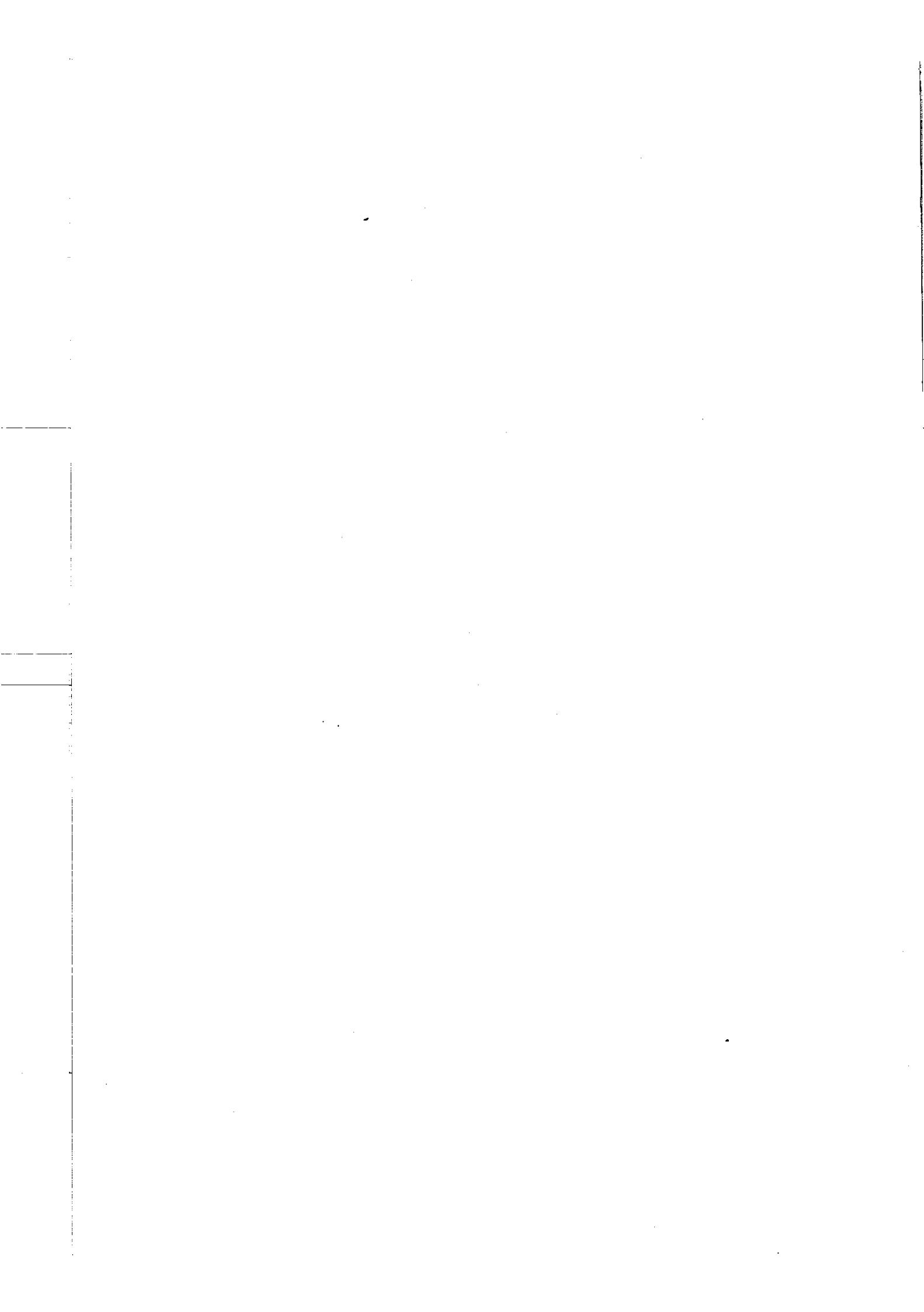
5. Verify if this new treatment is effective against type 1 diabetes.

Document 4 presents the results of labeling cytotoxic T8 lymphocyte in the pancreas of beginning of treatment. T8 lymphocytes appear in form of black spots inside the islet of Langerhans.

6. Draw out how this new treatment slows down the progression of T1D.

	Healthy individual	Individual suffering from type 1 diabetes
Mass of the islets of Langerhans (mg)	1400	415
Mass of alpha cells (mg)	220	200
Mass of beta cells (mg)	850	0

Document 1**Document 2****Document 3****Document 4**



Immunity

Official exercises answer key

Exercise 1 (7.5 pts) Relation between immune cells**Session 2001-1**

1. Lot A is a control. The strong agglutination indicated the presence of antibodies in large quantity. Thus, the control mouse, elaborates in presence in KPN a sufficient amount of antibodies. (½ pt) Lot B shows a weak agglutination which signifies a weak concentration of antibodies. These mice have only BL (absence of thymus and grafting of bone marrow), this means that BL alone reacts weakly against the antigen KPN and hence the low secretion of antibodies. (½ pt) Lot C does not show agglutination thus no secretion of antibodies. This means that TL alone (coming from the grafted thymus) has no effect on the secretion of antibodies. (½ pt)
Lot D presents strong agglutination thus there is secretion of large amount of antibodies. The presence of both (grafting of thymus and bone marrow) BL and T L together favors the secretion of antibodies. (½ pt)
2. When B Lymphocytes are alone in contact with the antigen, some plasma cells are produced (72). On the contrary when T lymphocytes are alone in contact with the antigen, no plasma cells are formed. While the B lymphocytes which are in direct or indirect contact (through the membrane) with the T lymphocytes are at the origin of a great number of plasma cells (1000) therefore, necessities a cooperation between B and T lymphocytes. (½ pt)
This cooperation does not require direct contact between BL and TL; in spite of the fact that they are separated by a permeable membrane, the differentiation of B lymphocytes into plasma cells occur effectively. We can deduce that B and T lymphocytes communicate by intermediary of molecules in solution. (½ pt)
3. Medium 1 contains serum and macrophages. We add to this medium KPN. After few days, the dosage indicates the total absence of immunoglobulin in the serum. (½ pt)
Medium 2 contains serum to which are added B and T lymphocytes and KPN. The dosage indicates a weak quantity of Ig in the serum. (½ pt)
Medium 3 contains serum and macrophages. We add to this medium B and T lymphocytes and KPN, the dosage indicates a large quantity of Ig in the serum. (½ pt)
Medium 4 contains serum and macrophages. We add to this medium KPN. After one hour, we can't out the washing. The macrophages remain adherent, we then add, BL and TL. The dosage indicates the presence of large quantity of Ig in the serum. (½ pt)
4. The presence of macrophages alone does not trigger the production of antibodies. This production of antibodies takes place if BL and TL are together but this production considerably increased in the presence of macrophages which indicate the cooperation between macrophage, BL and TL. (1 pt)
The production of Ig was not changed after the washing, we can deduce that This cooperation of macrophage does not take place by humoral path way but by contact. (1½ pt)

Exercise 2 (4 pts) Immunity against bacterial toxins**Session 2001-2**

1. The injection of tetanus toxin (TT) into guinea pig A provokes its death. The injection of diphtheria toxin (DT) provokes the death of the guinea pig.
We inject the tetanus toxoid into the guinea pig B then we inject it with (TT), after 15 days the guinea pig remains alive. we inject it with diphtheria toxin (DT), it dies. (1 pt 1/2)
2. When guinea pig B gets in contact with tetanus toxoid it presents a specific immunity to antigen TT and not to antigen DT. (¾ pt)
3. The givens that confirm that the immune response is of humeral mediation are:
The immunity in guinea pig B is transferred to guinea pig C by the serum.
When the serum is filtrated with the toxoid fixed to an inert support, there is no more immunity present in the filtrate injected in the guinea pig. (1 pt)
4. By comparing the results of electrophoresis, we find a peak of γ - globulins only in the guinea pig B, that is to say an increase in the quantity of antibodies. This guinea pig-has thus produced antibodies, after injecting it with the tetanus toxoid. (3/4 pt)
5. The presence of bone marrow (Lot A) allows the production of B and T lymphocytes, on the contrary, in its absence (Lot C) there is no production of BL and of TL. The thymus alone (Lot B) does not allow the production of BL and TL. The ablation of the thymus (Lot B) prevents the production of TL only. We deduce from what has preceded that the bone marrow is the site of BL and TL production, but TL needs the presence of the thymus to achieve maturation. (1 pt)

Exercise 3 (4 ½ pts) Induction of immunity**Session 2002-1**

1. Spleen cells were extracted from two mice of the same strain, one of them was normal while the other was without a thymus. These cells were incubated for 30 minutes at 37°C. Lymphocytes and macrophages were separated from the cells of the normal mouse and lymphocytes only from the mouse that is without a thymus.

A control tube was already prepared and contained the spleen cells (a mixture of lymphocytes and macrophages) of the normal mouse.

We added SRBC and fresh mammalian serum in the tubes containing:

1. lymphocytes and macrophages of the normal mouse (control).

2. lymphocytes of the normal mouse.

3. a mixture of lymphocytes and macrophages of the normal mouse.

4. macrophages of the normal mouse.

5. lymphocytes of the mouse without thymus and macrophages of the normal mouse.

The contents of these 5 tubes were subjected to the hemolysis test.

Four days later, only 1 and 3 presented areas of hemolysis. (2 pts)

2. The areas of hemolysis of SRBC apparent in tubes 1 and 3 due to the presence of macro phages and lymphocytes together, both derived from the normal mouse, in the presence of the fresh serum. The presence of lymphocytes alone (tube 2), macrophages alone (tube 4) or lymphocytes and macrophages derived from different mice, the first is normal and the other, with thymus, (tube 5) does not induce hemolysis of SRBC even in the presence of fresh serum.
This indicates that hemolysis necessitates the cooperation between macrophages and lymphocytes in condition that the latter are derived from a normal mouse. (2 pt)
3. Hypothesis: The presence of enzymes in the fresh serum favors hemolysis.
or: The presence of certain chemical compounds that favors hemolysis (1/2 pt)

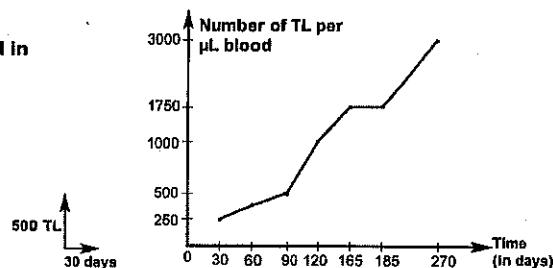
Exercise 4 (5 pt) An immune deficiency**Session 2002-2**

- 1.1. The table shows the total absence of TL in the sick child where as in the healthy child between 2000 to 4000/ μ L. Similarly, the sick child has no antibodies whereas the healthy child has 400 mg/dL. On the contrary, the number of the BL in the sick child is 1250/ μ L (as in the healthy child where the number is between 1000 to 2000/ μ L). (¾ pt)

- 1.2. We can say that the disease is due to the absence of TL and antibodies. (1/4 pt)

2. The presence of BL in a normal amount and the absence of antibodies show a humoral immune deficiency which is explained by the absence of TL that are necessary for the activation and the differentiation of the BL to become antibody secreting plasma cells. Moreover, the total absence of TL indicates that a cell mediated immune response, by the Tc cells, does not take place. (1 ½ pt)

3. (1 ½ pt) ■ Variation of the number of TL per μ L of blood in function of time



4. The used treatment is effective since document 2 reveals that 200 days after the treatment the number of TL becomes normal (185 - 270). Also, six months after treatment, the number of the antibodies of the treated child becomes 323 mg/dL which is very close to the normal (>400). After submission to the three different vaccines the amount of antibodies. reached very high values showing that the that the TL in the sick child had multiplied and consequently the T4 are capable of strongly stimulating the production of antibodies 2 or 4 times more than in the healthy child depending on the vaccination. (3/4 pt)

Exercise 5 (6 pts) Serotherapy**Session 2003-1**

1. First experiment: We inject rabbit A with the tetanus toxin; rabbit A dies. (1/2 pt)
 Second experiment: We inject rabbit B with the tetanus anatoxin; rabbit B remains alive. A few days later we take a blood sample from this rabbit from which we obtain the serum by centrifugation. We add to the serum the tetanus anatoxin; a precipitate is formed. (1 pt)
 Third experiment: We inject rabbit C with the serum of rabbit B. Then, 24 hours later, we inject this rabbit with the tetanus toxin. The rabbit remains alive. A few days later, a blood sample is taken from this rabbit from which we obtain the serum, by centrifugation. We add to the serum the tetanus toxoid. A precipitate is formed. 2 months later, we inject rabbit C with the tetanus toxin; rabbit C dies. We take a blood sample from rabbit C after its injection with the tetanus toxin. We obtain the serum from the blood sample, by centrifugation, to which we add the tetanus anatoxin; no precipitate is formed. (1 pt 1/2)
- 2.1. The precipitate is obtained upon mixing the serum of rabbit B with the tetanus anatoxin due to the formation of immune complex. The serum contains anti-tetanus antibodies, which causes the agglutination of the tetanus anatoxin. (1/2 pt)
- 2.2. The tetanus anatoxin does not provoke the death of the rabbit as the toxin, but it conserves its antigenic power (determinant), which permits the body to form the corresponding antibodies. (1/2 pt)
3. Immunity is transferred from rabbit B to rabbit C, by the intermediation of the serum. This explains the resistance of rabbit C to the tetanus toxin that was injected 24 hours later. (1 pt)
- 4.1. Absence of antibodies (½ pt)
- 4.2. Protection for a short duration. (½ pt)

Exercise 6 (6 pts) Immunity against flu virus**Session 2003-2**

1. The concentration of γ -globulins in the mouse contaminated with the flu virus is considerably elevated compared to the non-contaminated mouse. On the contrary, the concentration of β , α_1 and α_2 globulins, and that of albumin are identical in the two mice. This reveals that the production of γ -globulins is linked to the sickness (reaction to infection). (½ pt)
 The γ -globulins of the contaminated mouse, deposited in the central well 1 of the slide of immunodiffusion in gel, formed a precipitation arc with the solution of the flu virus antigen 1 deposited in well 2. A reaction had taken place between the anti-flu virus γ -globulins and the antigen of the virus; which indicates that γ -globulins are antibodies. On the contrary, the absence of a precipitation arc between wells 1 and 3, shows that the anti-flu virus γ -globulins had not bound to the antigen of hepatitis B virus; which indicates that these γ -globulins have a specific action. (¾ pt)
 Document 3 reveals that the percentage of liberated chromium is 8 times greater in the case of infected cells (40%) than in the case of non-infected cells (5%). Since the percentage of the liberated chromium is proportional to the number of destroyed cells; therefore, the infected cells only are destroyed significantly by the lymphocytes taken from the spleen of the contaminated mice. (¾ pt)
2. Documents 1 and 2 reveal a humoral specific immune response (½ pt)
 in document 1 there is increase in the production of γ -globulins in the serum following the viral infection (¼ pt) whereas in document 2 there is formation of an immune complex between antibodies and a specific antigen (¼ pt)
 Document 3 reveals a cell mediated immune response (¼ pt) because specific Tc lymphocytes cause the lysis of the cells infected by the flu virus. (3/4 pt)

Exercise 7 (3 ½ pts) Specific immune response**Session 2004-1**

1. We inject diphtheria toxoid into guinea pig A. 15 days later:
We inject into guinea pig A diphtheria toxin, it survives.
We obtain serum from guinea pig A and we inject it into guinea pig B then we inject diphtheria toxin, it survives.
We obtain lymphocytes from guinea pig A and we inject it into guinea pig C then we inject the diphtheria toxin, it dies.

2. The injection of diphtheria toxin into guinea pig A after 15 days from being injected with diphtheria toxoid, does not provoke its death. This implies that diphtheria toxoid provided immunity to this guinea pig against this toxin.

The death of guinea pig C, who received lymphocytes from guinea pig A together with diphtheria toxin, shows that the lymphocytes of guinea pig A did not ensure protection against this toxin.
On the contrary, the survival of guinea pig B, after the injection of the serum of guinea pig A and with diphtheria toxin shows that this serum contains the immune elements, which ensure protection against this toxin.

Exercise 8 (5 pts) Immunity against cancer**Session 2004-2**

1. We obtain cancer cells and serum from mouse A₁ and we put them in an appropriate culture medium. Five days later, we inject this mixture into mouse A₂ which dies after three months.
We obtain cancer cells and a few lymphocytes from mouse A₁ and we put them in an appropriate culture medium. Five days later, we inject this mixture into mouse A₃ which dies after three months.
We obtain cancer cells and many lymphocytes from mouse A₁ and we put them in an appropriate culture medium. Five days later, we inject this mixture into mouse A₄ which survives.
Mouse A₁ dies after three months. (2pts)
2. The presence of cancer cells in mouse A₁ provokes its death after three months. This indicates that cancer is fatal. Similarly, mice A₂ and A₃ die after A₂ received cancer cells and serum from A₁ and A₃ received cancer cells and a few lymphocytes from A₁. This indicates that the serum as well as the limited number of lymphocytes do not protect the mice from cancer. On the contrary, mice A₄, who received cancer cells cultured with many lymphocytes remains alive. Thus, lymphocytes in large quantities destroy cancer cells. (1pt)
3. Macrophages phagocytose foreign cells, digest them and express their non-self peptides on MHC-II molecules on their surfaces, these complexes are recognized by T4 cells specific to these antigens which secrete interleukins 2 that activate specific T8 cells having recognized the non-self peptides expressed on MHC-I on the surface of target cells which are recognized by the TL as foreign bodies. This recognition activates the T₈, which, after proliferation, differentiate into cytotoxic TL. These cells will destroy (lyse) the cancer cells after recognizing them and secreting perforin molecules that perforate the target cell membrane and by secreting granzymes that induce the degradation of the target cell leading to its death. (1½ pt)
4. Hypothesis: The lymphocytes which are specific to the tumoral antigen are not present in this culture medium.

Or

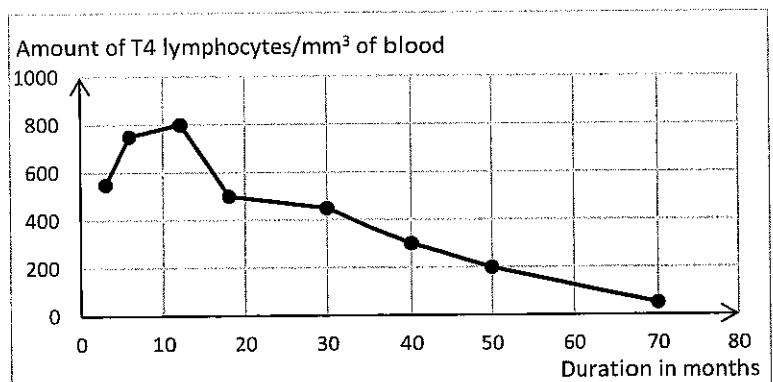
Specific TL are not enough to destroy, in 5 days, all the cancer cells. (½ pt)

Exercise 9 (6½ pts) Infection by HIV**Session 2005-1**

1. We add the serum of the patient to a well containing the viral protein of HIV fixed on its base. Then we wash the well to eliminate the serum and all the non-fixed molecules. We then add an enzyme fixed to a substance capable to bind to antibodies. We wash again the well to eliminate the non-fixed substance and the enzyme. We finally add to the well a colorless substrate of the enzyme, a coloration appears indicating that the test is positive. **(1 pt)**
- 2.1. Patient A is seropositive. **(½ pt)**
- 2.2. The positive test is caused by the serum of patient A that contains anti-HIV antibodies since individual A is infected and its immune system reacts to synthesize the specific antibodies. **(½ pt)**

3. (1 ½ pt.)

Variation of the number of T4 lymphocytes as a function of time



- 4.1. During the first 12 months, the amount of T4 increased from 550 / mm³ of blood to a maximum of 800/mm³ of blood, on the contrary, starting from the 12th month, the amount of T4 decreased to become 50/mm³ of blood after 70 months. **(1 pt)**
- 4.2. The total immune deficiency observed, takes place starting from the 40th month, is due to the low number of T4 (destruction). **(½ pt)**
5. The duration of the infection is almost 12 months. **(1 pt)**

Exercise 10 (5 pts) Immunity against hepatitis virus**Session 2005-2**

1. The recognized antigens are the antigens HBs and HBe (1/2 pt) because the immune system has reacted and formed anti-HBs and anti-HBe antibodies. (1 ½ pt)
2. We put each lot of cells A and B on a plate covered with anti-HBs antibodies, then we wash the plate. We put on this plate anti-HBs antibodies labeled by fluorescence then we wash again. The result shows the absence of labeling with the cells of lot A and a yellow coloration with the cells of lot B. (1 pt)
3. The yellow coloration exists only in the cells of lot B, the infected cells. This means that only these cells present the HBs particles that have bound to the anti-HBs antibodies labeled by fluorescence, due to the yellow coloration. (1 ½ pt)
4. The TL cultured in the presence of healthy cells (medium 1), do not provoke the lysis of these cells. On the other hand, when these cells are cultured in the presence of cells infected by the virus (medium 2), the lysis of the cells takes place. This implies that the TL provoke the lysis of infected cells only. The culture of the infected cells in the absence of TL, does not lead to the lysis of these cells. This implies that the presence of TL Is necessary for the lysis. (1 pt)
5. The TL approaches the infected cell and comes in contact with it. The T8 recognizes the HLA-I non-self peptide complex on the surface of the infected cell by its TCR membrane receptor, then it liberates proteins called perforin that perforate the membrane forming ducts; it also releases the granzymes that traverse these ducts and induce the destruction of the DNA provoking the lysis of the cell. (1 pt)
6. We conclude that the immune response against the hepatitis B virus requires two types of responses: humoral and cellular (½ pt)

Exercise 11 (8 pts) Medical applications of immunity**Session 2006-1**

1. We inject Guinea pigs of lot A with diphtheria bacillus, they die. We inject Guinea pigs of lot B with attenuated diphtheria toxin (iodine chloride + diphtheria bacillus), some Guinea pigs die (2) while Others survive. We inject the Guinea pigs who survived with diphtheria bacillus again, they survive. We extract serum from the surviving Guinea pigs and we inject it into other Guinea pigs together with injection of diphtheria bacillus, they survive.

We extract serum from Guinea pigs of lot C and we inject it with diphtheria into other Guinea pigs, they die. (1 ½ pts)

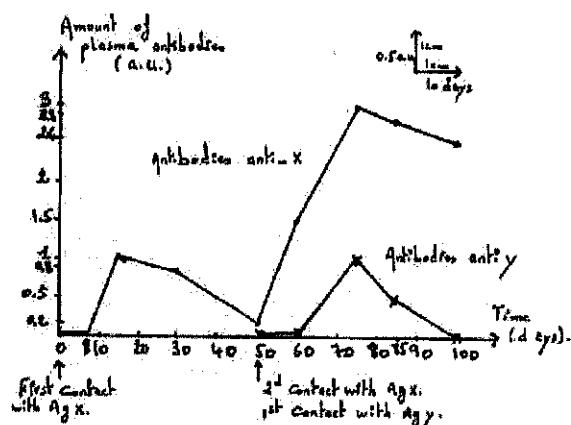
2. After the injection of diphtheria bacillus to lot A, we obtain death while injection of iodine chloride D.B (attenuated diphtheria toxin) to Guinea pigs of lot B does not kill all the Guinea pigs, and those who survive do not die even when they are injected with the DB. Therefore, attenuated toxin is not deadly, and it causes immunity against diphtheria bacillus.

After the injection of the serum of immunized Guinea pigs (3) into non-immunized guinea pigs (4), with DB, we obtain survival of them while after the injection of non-immunized Guinea pigs (5) with the serum of non-immunized Guinea pigs, we obtain their death; this means that attenuated diphtheria toxin provides, against diphtheria bacillus, immunity transferred by the serum. (2 pts)

3. Vaccination and serotherapy. Because in the case of vaccination, we give an attenuated toxin, which allows the body to launch an immune response upon contact with the concerned antigen (Guinea pigs 3). In the case of serotherapy, we give the serum which contains antibodies, that are against the concerned antigens (Guinea pigs 4). (1 pt)

4. (1 ½ pt)

Variation of the amount of anti-X and anti-Y antibodies as a function of time.



- 5.1. The amount of plasma anti-X antibodies is nil at the beginning and starts to increase 8 days after the first contact with antigen X. It reached a maximum of 1 a.u. on day 15 then it decreases progressively to reach 0.2 a.u. on day 50. The second contact with the antigen X on day 50, causes a rapid increase in the amount of anti-X antibodies to reach a maximum of 3 a.u. on day 75, then it decreases slowly to become 2.6 a.u. on day 100. (1 pt)
- 5.2. Therefore, the secondary immune response is relatively, faster, more amplified and more persistent. (½ pt)
6. The results of antigen Y injection confirm that the immune response is specific, and at the first contact it is always slow, less amplified and non-persistent. (½ pt)

Exercise 12 (4 pts) Conditions and mechanism of cytotoxicity**Session 2006-2**

1. In experiment 1 where cytotoxicity is observed, all the immune cells are present. On the other hand, cytotoxicity does not appear in absence of T4 (experiment 2) inspire of the presence of T8 and neither in absence of T8 (experiment 3) although T4 is present, which is confirmed by experiment 4 where cytotoxicity is observed where the immune cells taken from mediums 2 and 3 are placed together with the infected cells. This indicates that T4 only or T8 only are incapable to provoke cytotoxicity, thus, the presence of both is obligatory (1½ pt). Or, the appearance of cytotoxicity necessitates the cooperation between T4 and T8.
2. The microscopic observations reveal that in the presence of infected cells a contact takes place between Tc rich in perforin and these cells (1st observation) while in the presence of non-infected cells, the Tc do not show perforin and are not in contact with these cells (2nd observation). On the other hand, there is the appearance of pores in the region of contact between Tc and the infected cells (3rd observation) and these pores do not appear in the case of a deficiency in perforin (4th observation), Tc are thus, incapable to provoke the destruction of infected cells. (1 pt)
3. From what has preceded, we can say that perforin is necessary when there is a contact between immune cells and infected cells, which is responsible for the formation of pores at the level of the membrane of the infected cells, followed by their destruction. (½ pt)
4. After recognizing the antigen, the activated T4 multiply and differentiate into cells that secrete interleukins. Interleukin 2 acts on certain T8 lymphocytes provoking their multiplication and their differentiation into effector cells: Tc lymphocytes. Tc binds to infected cells and secretes perforin that provokes the appearance of pores on the membrane of the infected cells. These pores permit the passage of granzymes that attack the DNA of the infected cells leading to their destruction. (1 pt)

Exercise 13 (5 pts) Specific cell mediated immunity**Session 2007-1**

1. The skin graft of mouse A received by mouse A₁ of the same line is accepted while the skin graft of a mouse A received by mouse B₁ of line B different from A, is rejected after 15 days, this means that the graft succeeds only between mice of the same line. This same mouse B rejects a second graft of A, 6 days after grafting, on the other hand it takes more time of 15 days to reject the graft received the first time from a mouse of line C this means that the rejection of the graft is faster upon second recognition. (1pt)
2. Recognition of the non-self by the immune system, the presence of an immune memory, and specificity of the immune response. (½ pt)
3. Skin graft taken from a mouse of line A to mouse B (control) leads to the rejection of the graft. On the other hand, the graft is accepted when it is done on mouse B that is deprived of its thymus (experiment 2) or on mouse B that is subjected to the irradiation of the bone marrow (exp 3). Therefore, the thymus and the bone marrow are involved in graft rejection. (1pt)
4. The quantity of ⁵¹Cr released by the lysed cells in a medium deprived of effector cells of mouse of line A and in a medium containing macrophage is 20 a.u. This quantity increases to become slightly higher equal to 30 a.u. in a medium containing LT4 and LT8 and increases very sharply to 250 a.u. in a medium containing LT4, LT8 and macrophages. This means that, lysis of infected cells requires the co-operation between these three types of immune cells. (1pt)
5. The macrophages digest the free viruses, recognized as non-self, and transform them into peptides and present them on HLA molecules of class II. These macrophages are thus, antigen presenting cells (APC). These latter migrate towards the lymphatic ganglia where they activate the specific LT4 that recognize the HLA-II non-self-peptide complexes by their TCR, then they proliferate and differentiate into interleukin secreting cells that secrete IL-2. IL-2 activates the specific LT8 (LTc), which adheres by the TCR to the membrane of the target cell expressing HLA-I non-self peptide complexes and releases perforin that perforate the membrane and granzymes that induce the degradation of the DNA of the target cell leading to its lysis. (1½ Pt)

Exercise 14 (5 % pts) An immune disease

Session 2007-2

- Hypothesis: T lymphocytes of the mutant rats are at the origin of juvenile diabetes. (0.5pt)
- The number of the rats that have juvenile diabetes is 5/50 in lot A, which had undergone ablation of the thymus. On the contrary, the number of the rats that have juvenile diabetes is 6 times larger (30/50) in the control lot B. This indicates that the thymus, the place of maturation of T lymphocytes, is implicated in the appearance of diabetes.

The 2nd experiment revealed that the healthy rats of lot C, injected with TL taken from mutant rats, developed diabetes, whereas the healthy rats of lot D, injected with TL taken from healthy rats, did not develop diabetes. This implies that the appearance of the disease is linked to the presence of the TL of the mutant rats.

- Thus, the formulated hypothesis is valid, the TL of the mutant rats are responsible for the disease. (1.5pt)

4. Auto-immune disease. (0.25pt)

The T lymphocytes are directed against the self; they recognize it as modified self and attack it. (0.25pt)

5. (1pt)

Time (in days)	0	85	100	125	150	180
Diabetic rats (in %)	0	0	2	5	5	5
Lot E	0	0	2	5	5	5
Lot F	0	0	10	20	50	75

Variation of the percentages of diabetic rats as a function of time in lots E and F

- The percentage of diabetic rats was null in the two lots of rats until day 85. At day 100 the percentage increased to become 2% in the rats treated with cyclosporine, lot E, and 10% 5 times more in the untreated rats, lot F. The percentage continued increasing to become 5% in lot E, and 20% 4 times more in lot F at time 125 days. This percentage remained stable at 5% in the treated rats from day 125 to day 180, whereas it continued to increase in the untreated rats after day 125 to become 75% 15 times more. This implies that the treatment with cyclosporine had attenuated the appearance of diabetes in the mutant rats of lot E.
- Thus, cyclosporine is a medicine that attenuates the action of TL responsible for the appearance of diabetes in the mutant rats. (1.5pts)

Exercise 15 (5 pts) Characteristics of the immune response

1- Antigen A is injected into a mouse. 7 days later, cells of the lymphatic ganglia are extracted and put in 3 culture mediums: without antigens in medium (1), with antigen A in medium (2), and with antigen B in medium (3). We observe the absence of lymphocytes proliferation in the 1st and 3rd culture mediums and the proliferation of lymphocytes occurs in the second medium. **(1pt)**

2- A high proliferation of lymphocytes extracted from the mouse immunized against antigen A was observed when they are put in culture with this antigen. On the contrary, no proliferation was observed when they are alone or in contact with antigen B. This implies that the proliferation of the lymphocytes, selected after the first contact with antigen A, cannot occur unless the lymphocytes are put again in contact with the same antigen. Or this means that the immune response is specific. **(1pt)**

3- "7 days' time delay" is necessary to induce the immune response. Macrophages phagocytose the antigens express their peptides on the MHC-II and become APC that migrate towards the lymphatic ganglia. APC bind to the lymphocytes via their specific receptors TCR and activate them. These selected lymphocytes rapidly multiply and proliferate upon a second contact with the same antigen. **(1pt)**

4- During the first contact with the injected antigen (primary response), the antigen's amount in the blood decreases starting from day 7, to disappear within 10 days. However, during the secondary response, the antigen's amount in the blood decreases and disappears after 2 days, more quickly than at the time of the primary response.

At the time of 1st contact with the injected antigen, the amounts of circulating antibodies in the blood are null and does not appear until the 7th day. They increase to reach a maximum at day 13. On the contrary, during the 2nd contact, these antibodies are present starting from day 0, they start to increase at day 2, and reach a maximum at day 7, greater and faster than in the 1st contact. Beyond this day, the number of antibodies in both cases decreases however remains higher in the 2nd contact.

5- Thus the immune memory favors a faster, stronger, and more lasting response. **(1.5pt)**

6- The appearance of immune complexes is due to the neutralization of the antigen by the antibodies secreted by plasmocytes. The disappearance of these complexes is due to the opsonization and phagocytosis carried out by macrophages. **(0.5pt)**

Exercise 16 (5pts) Immunity against grafts**Session 2008-2**

1. The skin graft from mouse MHC^a to another mouse of the same line shows a 100% survival of the graft that persists beyond 20 days. On the other hand, the graft carried out between two mice of different lines MHC^a and MHC^b survives 100% until day 10 after which the % of survival while it decreases to become null, and the graft is rejected at the end of day 12. This implies that graft survival can take place when it is done between members of the same line. Graft rejection is done between individuals of different lines.

This % is even weaker when mouse MHC^b receives, after one month, a second skin graft from MHC^a and the graft is rejected on the 8th day < 12th day this implies that the rejection of the graft is faster after a 2nd contact with the same antigen.

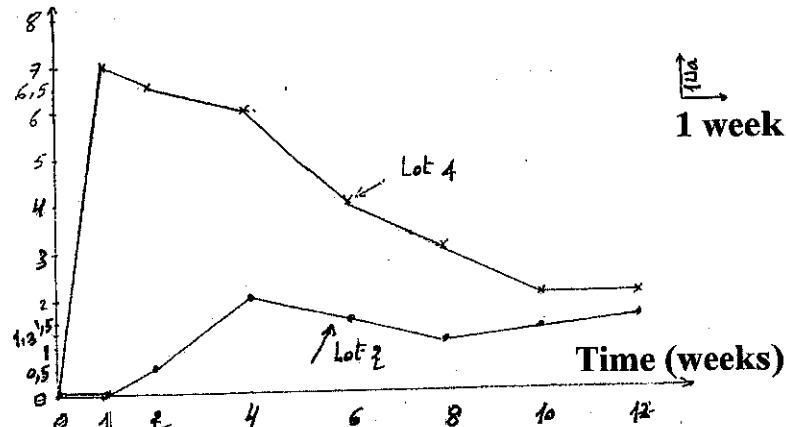
The injection of TL, obtained from mouse MHC^b immunized against MHC^a, into a mouse MHC^b which receives a 1st skin graft from MHC^a, led to the rejection of the graft in 8 days as in the case of the 2nd graft of the immunized mouse, this means that TL are the cells that reject grafts. (1.5pts)

2. Graft between different lines, done along with the injection of anti-CD4 antibodies, succeeds and the percentage of its survival is maximal. On the other hand, if the injection of anti-CD4 antibodies is carried out a few days before the graft, a time delay for these antibodies to disappear from blood circulation, the graft is rejected. This means that the anti-CD4 antibodies, when they are present, prevent graft rejection. Therefore, TL4 cells have a role in graft rejection. (1pt)
3. This experiment is not sufficient to determine which type of cells is involved in graft rejection. part 2 reveals that the TL4 cells have a role in graft rejection but it cannot be determined whether TL8 have the same role. (0.5 pt)
4. For that, it is necessary to repeat the experiments of document 2, and add two more mice. The 1st mouse is injected with anti-CD8 antibodies and the 2nd mouse with anti-CD4 and anti-CD8 antibodies, before performing the graft between the two different lines. We follow the variation of the graft: survival or rejection in order to determine which cell is behind graft rejection, TL4 or TL8 or both. (1pt)
5. Antibodies specific for CD4 are simultaneously injected with the grafting. They fix to TL4 receptors to block them. The blocked TL4 are not activated and do not proliferate nor do they differentiate into cells that secrete interleukins 2. Thus, the TL8, that are necessary for killing the cells of the graft, are not activated and the graft is successful. (0.75 pt)
6. Anti-CD4 antibodies can be used as immunosuppressor drugs during grafting. (0.25pt)

Exercise 17 (5 pts) Infection by HIV

1. The results of lot 1 show that T4 and not T8 cells get infected by HIV. This means that T4 are the target cells of HIV. Moreover, results show that infection of T4 cells occurs in lot 1 and lot 2 where CD4 proteins are free, while no infection of T4 cells occurs in lot 3 where the CD4 proteins are fixed to antibodies. This means that HIV attacks only T4 cells possessing free CD4 on their membrane. (1 pt)

2. Graph showing the variation of the proportion of T8 specific to HIV in function of time in vaccinated and non-vaccinated monkeys. (2 pts)

Proportion of T8 cells specific for HIV (a.u.)

3. Document 2 reveals that in the vaccinated monkeys, the proportion of T8 specific to HIV start increasing from the time of viral exposure to reach 7 a.u. after one week while, in the non-vaccinated monkeys, the proportion of T8 starts increasing after a longer period of time (two weeks) following infection. This explains why the immune response is rapid in the vaccinated monkeys. Also, document 2 shows that the proportion of T8 in the vaccinated monkeys reach a value 7 a.u which is 3.5 times higher than the value attained in the non-vaccinated monkeys which is 2 a.u so, the immune response in the vaccinated monkeys is amplified. (1 pt)

- 4.1. In the non-vaccinated monkeys, the viral charge is around $25 \cdot 10^4$ copies/mm³ of plasma after 8 weeks following viral infection and increases to reach $50 \cdot 10^4$ copies in mm³ of plasma after 24 weeks. These values are always higher than the viral charge in the vaccinated monkeys which remains constant at $5 \cdot 10^4$ copies/mm³ of plasma at weeks 8 and 24. (¾ pt)

- 4.2. We can conclude that this vaccine attenuates the multiplication of HIV. (¼ pt)

Exercise 18 (5 pts) Clonal selection

Session 2009-2

- 1-1 B lymphocytes are ready to respond to an antigen before encountering it, because we observe anti-A antibodies production in mouse S2 which received all lymphocytes. On the contrary, there is no production of anti-A antibodies in mouse S1 which received all lymphocytes except the lymphocytes that can recognize antigen A (already destroyed by radioactivity after the fixation on the radioactive antigen A). This indicates that the lymphocytes that recognized antigen A were present before any contact with this antigen. (1 pt)
- 1-2 The experiment shows that the radioactive antigen is fixed on plasma membrane of the B lymphocytes that recognized this antigen. This implies the presence of a membrane receptor capable of the fixation of this antigen. (1 pt)
- 1-3 The immune response is specific to this antigen because we observe the production of antibodies against all antigens except anti-A antibodies in mouse S1 that received all lymphocytes except the lymphocytes that can recognize antigen A. (1 pt).
- 2- The swelling started after 5th day and the strong swelling observed on the 10th day correspond to the activation of lymphocytes(T4 and BL) and to their rapid and important proliferation on day 10 leading to the formation B lymphocytes clones that recognize the antigen. (1 pt)
- 3- The 10 clones of BL 5 days following the injection is attributed to the presence of 10 different antigenic determinants at the level of the antigen.(½ pt)
- 4- Hypothesis: There is an important clonal selection of B lymphocytes where only lymphocytes that can recognize the most effective antigenic determinant are kept. (1/2 pt)

Or

There is an important clonal selection of B lymphocytes where only lymphocytes that can recognize the most frequent antigenic determinant are kept.

Exercise 19 (5 pts) Specificity of lymphocytes and antibodies**Session 2010-1**

- 1- We extract lymphocytes from a normal mouse S1 and separate them into two lots. Lymphocytes of lot 1 are directly injected into mouse S2 deprived of its lymphocytes. After contact with ABS antigen (albumin of bull serum), this mouse produces anti- ABS antibodies. We circulated the lymphocytes of lot 2 in a column of solid matrix attached to ABS antigen. Some lymphocytes are retained within this column and a great proportion of these lymphocytes pass along the column. The lymphocytes of lot 2 which traverse the column are injected in mouse S3 deprived of its lymphocytes. After the contact with ABS antigen, mouse S3 doesn't produce anti- ABS antibodies. Several days later, following contact with CGB antigen (Calmette and Guerin Bacillus), mice S2 and S3 produce anti-CGB antibodies. (1 ½ pt).

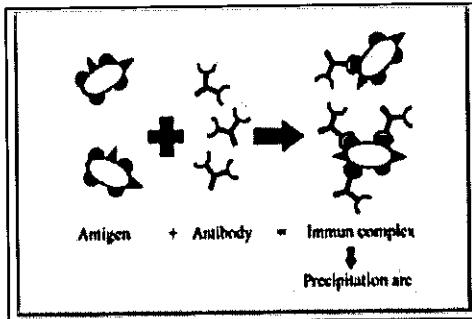
- 2-1**-Mouse S2, deprived of its lymphocytes, and which receives lymphocytes extracted from a normal mouse S1, produces anti-ABS antibodies upon its contact with ABS antigen. On the contrary, no anti-ABS antibodies are produced by mouse S3 also deprived of its lymphocytes, and which receive lymphocytes that traverse a column of ABS antigens attached to solid matrix, upon its contact with the same antigen. This means that mouse S2 only receives lymphocytes specific to ABS antigen. (½ pt) However, these two mice S2 and S3 were able to produce anti- CGB antibodies upon their contact with CGB antigen. This means that both mice possess BL specific to CGB antigen. (½ pt).
- 2-2**- This experiment validates the second hypothesis, since the production of specific antibodies, against a certain antigen, necessitates the presence of a category of BL specific to that antigen. **Therefore**, lymphocytes are specific to an antigen. (½ pt).

- 3- B lymphocytes (¼ pt) and T4 Lymphocytes (¼ pt)

- 4- With the serum of mouse S2, a precipitation arc is formed between well 2 and well 5; and between well 3 and well 5 (¼ pt), because mouse S2 produced anti -ABS antibodies and anti-CGB antibodies specific to ABS and CGB antigens respectively. (¼ pt).

With serum of mouse S3, the precipitation arc is formed between well 3 and well 5 (¼ pt), because mouse S3, in the absence of lymphocytes specific to ABS antigen, produces only anti-CGB antibodies which have an antigen binding site specific to CGB antigen. (¼ pt).

- 5- Drawing (½ pt)



Exercise 20 (5 pts) Cytotoxicity of Tc lymphocytes**Session 2010-2**

1. We remove from mouse X infected by virus A cells infected by virus A and Tc lymphocytes. We incubate the infected cells with ^{51}Cr , then we perform centrifugation and wash to eliminate the free ^{51}Cr . After that we culture the removed Tc lymphocytes with the infected cells. After 4 to 16 h we measure the quantity of free ^{51}Cr in the supernatant. (1 ½ pt)
2. The disappearance of ^{51}Cr after washing then its reappearance in the supernatant after culturing the infected cells with Tc lymphocytes indicate that these latter have destroyed the infected cells liberating the ^{51}Cr bound to intracellular proteins. (1pt)
3. Specific cell mediated immune response (1/4 pt). Since the Tc cells have destroyed the infected cells. (1/4 pt)
4. Dermal cells of healthy mice are not destroyed by the Tc regardless from which mice they are removed.

Dermal cells of mice X infected by virus A or by virus B are only destroyed by Tc lymphocytes of mice X infected by the same virus: respectively virus A or virus B. Thus the cells infected by a virus are destroyed only by Tc of mice infected by the same virus. However dermal cells of mice of strain Y infected by virus B are not destroyed by Tc of mice of different strain X even though they are infected by the same virus B; thus the infected cells are destroyed only by Tc of mice of the same strain. (1 1/2 pt)

5. The Tc lymphocytes recognize and bind, by its TCR, to target cells expressing the modified self: self MHC carrying a non self peptide of the antigen that is at the origin of their activation. They will then release, by exocytosis, perforine molecules forming hollow polyperforine channels through the cell membrane, and then they release granzymes molecules that penetrate the target cell through these channels leading to its DNA degradation and to the target cell destruction. (1/2 pt)

Exercise 21 (5 pts) Immunological memory

Session 2011-1

1	Specific humoral immune response; since following the entry of the virus of variant 1, having the antigens A, B, C and D, the amount of antibodies specific to each of these antigens rises to 1a.u..	1
2	<p>2-1- During the first contact with the variant 1 of flu virus at the age of 2 years, the amount of antibodies specific for each of the antigens A, B, C and D was 1a.u. which corresponds to a primary immune response . However, during the second contact at the age of 5 years with the variant 2 of flu virus having the antigens C and A in common with variant 1, the amounts of antibodies specific to A and to C increase respectively to 5 a.u. and to 6 a.u ($> 1\text{a.u.}$) , while the amounts of antibodies specific to B and to D remains low (0.5 a.u.). This means that the immune response triggered upon the second contact with the same antigen or secondary immune response is more amplified than the primary immune response.</p> <p>2-2- During the 3rd contact at 20 years of age, with the variant 3 of flu virus having the antigens A and D in common with the variety 1, only the amount of antibody specific to these antigens increases respectively to 8 a.u ($> 1\text{a.u.}$) and 6 a.u ($> 1\text{a.u.}$) thus the secreted antibody is specific to the antigen and not to the variant of the virus.</p> <p>2-3- The organism keeps memory for an encountered antigen for more than ten years since the amount of antibodies has increased to 6 a.u($> 1\text{a.u.}$) 18 years after the first contact with the antigen D. Or Since the amount of anti B antibodies remains constant at 0.5 a.u from the age of 5 years to 20 years.</p>	2.25
3	<ul style="list-style-type: none"> - Macrophages: after phagocytosis of the antigen, they become APCs that activate specific T4L - T4L: once activated they secrete the interleukin 4 that activate the LB - LB: they identify the free antigens through their membrane antibodies to be activated - Plasma cells: secrete specific antibodies against the antigen. 	1
4	The specific antibodies neutralize their corresponding antigens of the flu virus by binding to them through their specific antigenic binding sites forming immune complexes. Thus the antibodies become able to bind through their constant part on macrophages that phagocytose the whole immune complexes thus destroying the virus (opsonization).	0.75
5	No. Since the infected cells cannot be identified by the antibodies which block only extracellular antigens.	0.5

Exercise 22 (5 pts) Immune response against a virus

Session 2011-2

1	The virus persists in the body because it remains in the dormant state in the memory BL.(0.25pt) The virus is produced by naive B lymphocytes that are once infected and by the memory BL once reactivated.(0.25pt)	0.5
2	Specific humoral immune response (0.25pt) because the actors in this response are antibodies anti-VCA and anti- EBVA . (0.25pt)	0.5
3	3-1- Anti-VCA antibodies appear in blood two weeks after infection and reach their maximum concentration 6.5 a. u. within eight weeks after the infection then stabilizes for the following 10 years. However Anti-EBVA antibodies appear later at the 7th week (7 w >2 w) and reach their maximum concentration 5.5a.u. (5.5 < 6.5 a. u.) after more than7 months (7months>8 w) then their concentration decreased slightly to reach 4.5 a. u. (4.5 < 6.5 a.u.) after 10 years. (1pt) 3-2- We can conclude that the body develops two different humoral immune responses against two different peptides (different antigens) of EBV virus, and that the response triggered against the VCA is faster, more amplified and more sustainable than the one triggered against the EBNA. (0. 5pt)	1.5
4	Experiment 1: Lymphocytes TL of an individual infected with the virus EBV are added into the medium containing BL infected with EBV, we obtain100% of lysis LB. Experiment 2: Lymphocytes TL of an individual infected with the virus EBV are added into the medium containing BL non infected with EBV, no lysis of BL is obtained. Experiment 3: Lymphocytes TL of an individual infected with the virus EBV are added into the medium containing memory BL infected with EBV, no lysis of BL is obtained. Experiment 4: Lymphocytes TL of an individual infected with the virus EBV are added into the medium containing BL infected with another virus, no lysis of BL is obtained . Experiment 5: Lymphocytes TL of an individual non infected with the virus EBV are added in the medium containing BL infected with EBV, no lysis of BL is obtained .	1.25 (5 x0.25)
5	The Lymphocytes T cytotoxic having receptors that recognize infected cells presenting at with their surface self HLA having non self peptide which has activated the same T8 lymphocytes which is identified in experiment 1 (there is 100%of lysed BL). In experiment 2, non-infected BL do not present non-self peptides this is why we do not observe any lysis. In experiment 3, B memory cells infected by the same virus as TL do not present non-self peptides. They are not identified by Tc and they are not lysed. In experience 4, BL infected by another virus present another non-self peptides. They are not identified by Tc and they are not lysed. In experiment 5, TL from an individual non-infected with EBV are not activated and differentiated into Tc and do not cause the lysis of BL infected by the virus.	1.25 (5 x0.25)

Exercise 23 (5 pts) Tetrahydrocannabinol and immune response

Session 2012-1

1	<p>Document 1: The volume of the tumor (VT) is constant between day 0 and day 10 in both lots. On the other hand, this volume increases to 4000mm³ between day 10 and day 50 in lot 1. Similarly, in lot 2 which received THC injection VT increases slightly to 2000 mm³ from day 10 till day 25 followed by a sharp increase to a much higher value than lot 1 (14000 mm³) between day 25 and day 50. This shows that THC favors the development of the tumor after 10 days (1/2 pt)</p> <p>Document 2: The number of T cells in the mice of lot 1 increases from 4000 cells to 20000 cells when the percentage of implanted tumor cells relative to the number of lymphocytes before proliferation increases from 2 % to 25%. Similarly, in lot 2 which received THC, the number of TL increases but slightly from 2000(< 4000) to 10000(< 20000).</p> <p>This shows that the proliferation of TL varies in the same direction as the quantity of cancer cells and THC reduces this rate of proliferation against tumor cells. (1/2 pt)</p>	1												
2	<p>The level of IL secreted at the level of the tumor in lot 2 is 73 pg / mL for 500 mg of tumor which is less than 190 pg / mL for Lot 1. Similarly, in lot 2 the level of IL secreted at the level of the spleen is 21 pg / mL for 10⁶ cells which is less than that secreted at the level of the spleen of Lot 1 (37 pg / mL for 10⁶ cells). Thus, THC decreases the secretion of interleukins and since interleukins are secreted by T4 cells then the target cells of THC are the T4 cells.</p>	1												
3	<p>THC acts on T4 lymphocytes secreting IL indispensable for the activation of the specific immune response (humoral mediated and cell mediated). Thus, a decrease in the quantity of IL provokes a decrease in the proliferation of T lymphocytes leading to a decrease in the immune response. Hence the tumor develops.</p>	3/4												
4	<p>Histogram: percentage of mice rejecting the tumor as a function of number of live implanted tumor cells.</p> <table border="1"> <caption>Data from Histogram: Percentage of mice rejecting the tumor</caption> <thead> <tr> <th>Number of live implanted tumor cells</th> <th>Lot 1 (%)</th> <th>Lot 2 (%)</th> </tr> </thead> <tbody> <tr> <td>1x10⁵</td> <td>100</td> <td>100</td> </tr> <tr> <td>2x10⁵</td> <td>100</td> <td>55</td> </tr> <tr> <td>3x10⁵</td> <td>100</td> <td>50</td> </tr> </tbody> </table>	Number of live implanted tumor cells	Lot 1 (%)	Lot 2 (%)	1x10 ⁵	100	100	2x10 ⁵	100	55	3x10 ⁵	100	50	1 1/2
Number of live implanted tumor cells	Lot 1 (%)	Lot 2 (%)												
1x10 ⁵	100	100												
2x10 ⁵	100	55												
3x10 ⁵	100	50												
5	<p>5-1-Document 4 shows that the percentage of mice rejecting the tumor in lot 1 is constant 100 percent regardless of the number of implanted tumor cells while in lot 2 receiving injections of THC, it decreases from 100 % to 50% when the number of implanted tumor cells increases from 1x10⁵ to 3x10⁵. (1/2)</p> <p>5-2- Hence THC weakens the secondary immune response. (1/4pt)</p>	3/4												

Exercise 24 (5 pts) Cellular cooperation and secretion of antibodies**Session 2012-2**

1	<p>There is agglutination of SRBC in lot 2 where there was an injection of lymphocytes B and T and SRBC at the same time whereas there is no agglutination in lot 1 where there were only injection of lymphocytes T with SRBC and in lot 4 where there are only injection of B lymphocytes with SRBC; This shows that the agglutination requires the cooperation of TL and BL or the presence of the TL and BL at the same time.</p> <p>There is agglutination of SRBC in lot 2 where there was an injection of lymphocytes B and T and SRBC at the same time however there is no agglutination in lot 3 where there were injection of lymphocytes B and T without SRBC. This shows that the contact with the antigen a week in advance is necessary to get agglutination.</p>	1 1/2
2	This will ensure that the immune response triggered by the mice is due only to the injected cells.	1/2
3	<p>There is strong agglutination of SRBC in the media 1 and 2 where B and T lymphocytes are found together whether they are in the same medium (medium 1) or separated by a membrane that is impermeable to cells but permeable to molecules (medium 2). (1/2 pt) Therefore, the agglutination of SRBC that is due to the production of anti-SRBC antibodies requires the cooperation of B and T lymphocytes via molecules and not by direct contact. (1/2 pt)</p>	1
4	<p>Plasmocyte (1/2 pt) because this cell has a voluminous cytoplasm that is rich in rough endoplasmic reticulum, cytoplasmic organelle that is indispensable for the synthesis of proteins such as antibodies. (1/2 pt)</p>	1
5	<p>Plasmocytes are derived from the differentiation of lymphocytes B which are absent in medium 4 where there is only TL, hence plasmocytes are absent in this medium. The differentiation of LB into plasmocytes is stimulated by IL 4 that is secreted by TL that are absent in medium 3, hence plasmocytes are absent in this medium. However B and T cells are present in media 1 and 2. IL 4 stimulates directly the B cells in the lower chamber (medium 1) or crosses the permeable membrane and stimulates B cells (medium 2). Hence the abundance of plasmocytes in these two media.</p>	1

Exercise 25 (5 pts) Cell lysis

Session 2013-1

1	The means of human contamination by the CML virus are: food and dust contaminated by infected mice.	0,5
2	Mouse A is injected with CML virus (choriomeningitis). 15 days later T lymphocytes activated by CML of this mouse are taken of and added to three culture media. Mouse B is injected with CML virus (choriomeningitis). 15 days later, the fibroblasts infected by CML of this mouse are taken of and are added with the activated T lymphocytes of mouse A to a culture medium. Lysis of these fibroblasts is observed. Non infected fibroblasts of mouse C are taken of and added with the activated T lymphocytes of mouse A to a culture medium. No lysis of these fibroblasts is observed. Mouse D is injected with hepatitis virus. 15 days later, the fibroblasts infected by hepatitis virus of this mouse are added with activated T lymphocytes of mouse A to a culture medium. No lysis of these fibroblasts is observed.	1,5
3	There is lysis of the fibroblasts of mouse B that are infected by the CML virus in the medium containing T lymphocytes activated by the same virus, while there's no lysis of non-infected fibroblasts of the mouse C neither of the fibroblasts of the mouse D that are infected by another virus (hepatitis virus) which are placed in a culture medium containing the same T lymphocytes. This shows that activated T lymphocytes destroy only the cells that are infected by the same virus that led to their activation OR activated T lymphocytes destroy only the cells that are infected and that they are specific to the CML antigen.	1
4	1- The order is: C A B (0,25) The first scheme C shows near the infected fibroblast one T lymphocyte with vesicles that are spread in its cytoplasm. In the second scheme A, the T lymphocyte is in contact with the membrane of the infected fibroblast, what corresponds to the double recognition. In the third scheme B, we notice that the granules are in contact with the infected fibroblast and destroy its nucleus. (0,75 pt)	1
5	Tc recognizes the infected body cell and binds by its TCR to the self HLA-I non self peptide complex expressed on the membrane of the infected cell. Then it liberates perforin to form polyperforin channels through the membrane of the infected cell. After that the TcL releases granzymes that penetrates into the infected cell through the polyperforin channels leading to the degradation of its DNA, thus causing lysis of the infected cell.	1

Exercise 26 (5 pts) Vaccine against AIDS

Session 2013-2

1	The percentage of untreated patients in the AIDS phase five years after the infection increases from 9% to 60% when the viral charge one year after the infection increases from 1000 up to 10000 copies of mRNA/mL. This shows that the onset of the AIDS phase and the early evolution of the viral charge vary in parallel to each other.	1
2	Vaccines immunize the organism against a specific antigen by inducing a durable immunological memory. Or Vaccines protect the organism by sensitizing the immune system against the pathogens in order to recognize and destroy them in a more rapid and more efficient manner upon a second contact with the same pathogen.	0,5
3	In vaccinated monkeys, the proportion of T8 lymphocytes increases from 0 to 6.5 greater than that of non-vaccinated monkeys which is 2. This shows that the response triggered in vaccinated monkeys is more amplified. (0,5 pt) After the exposure to the virus, the increase of the proportion of T8 lymphocytes in vaccinated monkey begins after a latency time of one week, less than that in the vaccinated monkeys which is 2 weeks. This shows that the response in vaccinated monkeys is more rapid than that of the non-vaccinated ones. (0,5 pt)	1
4	Between the fourth and the 12 th week, the proportion of T8 lymphocytes, in both lots 1 and 2 decreases while remaining higher in vaccinated monkeys and reach the same value of 2 at week 12. After the 12 th week, the variations of these proportions remain identical. This shows that the immune response triggered by the vaccine is not durable, it does not last except for 12 weeks.	0,5
5	Document 3 shows that the viral charge at the 8th week in vaccinated macaques is $5 \cdot 10^4$ viral RNA copies/ml inferior to that of the non-vaccinated ones $25 \cdot 10^4$ viral RNA copies/ml . At the 24 th week it increases (doubles) to $50 \cdot 10^4$ viral RNA copies/ml in the non-vaccinated macaques while it remains constant at $5 \cdot 10^4$ viral RNA copies/ml in vaccinated macaques value that is 10 times smaller than $50 \cdot 10^4$ viral RNA copies/ml. This shows that vaccine maintains the viral charge weak and constant at the beginning of the infection.	1
6	The chance of reaching the phase of AIDS diminishes in the case where the viral charge is weak at the beginning of the infection (doc.1). The vaccine maintains the viral charge low at the beginning of the infection (doc.3). This diminishes the evolutions of the disease toward the phase of AIDS thus extending the asymptomatic phase. Therefore there is a greater chance to prolong the life of seropositive individuals. From this point the vaccine is efficient. The vaccine amplifies the specific cell mediated immune response the first three months after infection (doc.2) however this amplification is not durable thus the efficiency is limited. In addition the vaccine doesn't ensure a total recovery and the disease is not eradicated.	1

Exercise 27 (5 pts) Infection by HIV

Session 2014

1)	It is a specific cell mediated immune response since the viruses attack the cells leading to their death and the body reacts against these infected cells by Tc lymphocytes.						
	Days after infection	0	5	10	15	20	25
	T ₈ lymphocyte or B lymphocytes%	100	95	90	90	90	90
2)	T ₄ lymphocytes or macrophage%	100	40	18	10	5	0
	Title: the variation in the percentage of the surviving leucocytes cells as a function of days after infection with HIV.						
3)	3.1-The number of surviving cells T8 lymphocytes or B lymphocytes decreases very slightly during the first 10 days from 100% to 95% after the infection (exposing to HIV), while that of T4 or macrophages decreases very rapidly from 100% to 18%, whereas, between 10 and 25 days after infection, the number of T8 or B lymphocytes remains constant at 90% while that of T4 cells or macrophages continues its decrease to become null at day 25.						
	3.2- The target cells of HIV are T ₄ lymphocytes or macrophage.						
4)	The presence of receptors called CD ₄ and co receptor CCR ₅ at the level of cell surface, since from doc.2 the protein gp120 of the HIV has the capacity to recognize and bind to an immune cell having these two receptors.						
5)	The DNA probe binds to HIV viral genome is visualized by fluorescence which is observed in the chromosome pair number 2 in the immune cell 1 and not in the immune cell 2. This means that the immune cell 1 is infected by HIV.						
6)	The protein gp120 of HIV recognizes and binds with receptors CD ₄ and CCR ₅ presented at the surface of the immune cell T ₄ or macrophage, leading to the entrance of the viral genetic material RNA into the host cell in which it changes into the viral DNA under the effect of reverse transcriptase to bind with DNA of the host cell. Then this viral DNA undergoes transcription in the nucleus of the host cell, finally the RNA of virus undergoes translation in the ribosome in the cytoplasm leading to the production of different viral components needed to produce new viruses.						

Exercise 28 (5 pts) Fight against Ebola

Session 2015-1

1	A specific humoral specific immune response is characterized by specific antibodies that are released by plasma cells that are the effectors of it. Since in case of Ebola the surviving individuals have a high amount of anti-Ebola antibodies then a specific humoral immune response is triggered against ebola. A cell mediated specific immune response is characterized by specific TcL against the virus that are the effectors of it. Since the surviving individuals show an important increase in the specific TcL then a specific cell mediated immune response is triggered against ebola.	½ ½
2	The amount of anti-Ebola antibodies is nil and remains constant on the 14th and 35th day, after the first and the second injection of the three types of vaccine. After the 3 rd injection of vaccine, this amount increases to 1000 a.u on the 56 th day in individuals having received EIC + PIC, while its remains constant and nil in individuals having received the vaccine EIC or only the protein. This amount of antibodies increases in the three lots to reach 10000 a.u on the 84 th day in individuals having received the vaccine EIC+PIC which is 10 times higher than the 1000a.u obtained when only the vaccine EIC or only the protein is administrated. This shows that the vaccine EIC+PIC is the most effective.	3/4
3	At the beginning of the specific immune response, macrophages act as antigen presenting cells which induce the specific immune response. At the end of the specific humoral immune response, they perform phagocytosis of the immune complex in order to eliminate antigens.	1
4	Between day 0 and day 20, the percentage of survival decreases from 100% to 80% in the lot receiving 4 injections. This decrease is 4 times more significant than that obtained in the lot receiving only 3 injections which reaches 20 %. Thus, the condition for the vaccination against Ebola to be successful is to give 4 boosters.	1/2
5	The antibodies injected after 24 hours neutralize the antigen and slow down efficiently the propagation of virus which allows the body defenses to react and protect all the monkeys (4/4) which remain alive. However, when the injection is delayed to 48 hours, the viruses multiply more rapidly than the lymphocytes involved in the specific immune response and infect a great number of cells before being neutralized by the specific injected antibodies. This reduces the efficiency of the body defense and sometimes renders it insufficient. This explains the death of two out of the four infected monkeys.	1
6	In serotherapy, the injected substances are the specific antibodies while in vaccination, the injected substances are viral or antigenic proteins. In serotherapy, the latency time is null while in vaccination, the latency time is 2 weeks In serotherapy, the duration of protection time is short while in vaccination, the protection is more durable.	¼ ¼ ¼

Exercise 29 (5 pts) Cervical cancer and the HPV virus**Session 2015-2**

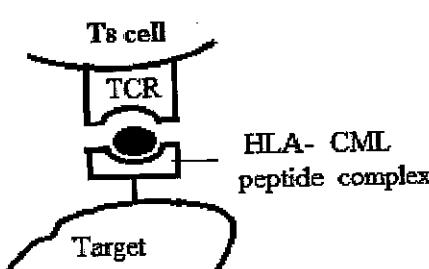
1	Studies involving thousands of women suffering from cervical cancer shows that 75% of them have encountered the human papillomavirus (HPV) at some point of their sexual life. Thus this cancer is induced by a virus. The development of this cancer is slow it requires the persistence of the HPV infection for more than 13 years before inducing a genetic mutation at the origin of the cancer (document 1). Thus, this cancer is induced by a virus and its development needs time.	3/4
2	The environment: tobacco, nutrition... Status of the host: immune system, type of HLA	1/2
3	The specific immune response is cell mediated. Since the virus integrates its DNA into the genome of infected cells and modifies their immunological self. This modified self is only recognized by the T8 which are the effectors of the cell mediated response.	3/4
4	Document 2 shows that the highest percentage of cancers is 54,5% due to HPV 16 and 16% due to HPV 18. These percentages are higher than those of cancers induced by all the other types of HPV (more than 100 type of HPV). Thus the two types HPV 16 and HPV 18 are of high risk.	3/4
5	Both vaccines, Cervarix and Gardasil, require the same amount and the same number of repetitions (3 times) and immunize the body against the two types of HPV of high risk (HPV 16 and 18). On the contrary, the level of produced antibodies induced by the vaccine Cervarix (100 times higher) is more important than that induced by the vaccine Gardasil that is 8 times higher than that produced in the case of natural infection. Thus the more efficient vaccine is Gardasil.	1
6	The antibodies produced due to vaccination neutralize the viruses before they bind to the membrane receptors of the target cells of the cervix and inhibit the viruses from infecting them. The viruses are thus eliminated (the formed immune complexes will be phagocytized by macrophages) and the lesions leading to cancer do not appear. Thus antibodies inhibit the HPV infection and protect the epithelium from genetic mutations that are at the origin of cervical cancer.	3/4
7	Get vaccinated before the first sexual intercourse. Do not smoke, have balanced healthy nutrition.	1/2

Exercise 30 (5 pts) AIDS and treatments

Session 2016-1

1	During the asymptomatic phase, the concentration of T4 cells/mm ³ of blood increases from 550 up to 800 just within 12 months after the contamination. On the contrary, this amount decreases from 800 to 200 at the 60 th month, the beginning of the appearance of opportunistic diseases until it becomes nil at the 84 th month.	1/2
2	The cause of appearance of opportunistic diseases is the low amount of T4 cells, less than 200/mm ³ of blood.	1/2
3	The presence of antibodies is observed in culture medium 1b containing B lymphocytes activated by antigen X and LT4 activated by the same antigen. On the contrary, neither antibody are produced in culture medium 1a containing only B cells activated by antigen X or culture medium 1c containing B cells activated by the antigen and activated T8 cells. This implies that the cooperation only between T4 cells and B cells is indispensable for the secretion of antibodies. Lysis of monkey cancerous fibroblasts is observed in medium 2b containing T8 cells and T4 cells activated by the same antigen. On the contrary, no lysis is observed in culture medium containing only activated T8 cells. This implies that cooperation between T4 and T8 cells is indispensable for cellular lysis.	1
4	Document 2 shows the importance of T4 cells in the activation of specific humoral immune responses whose effectors are B lymphocytes and in cellular immune responses whose effectors are T8 cells. Document 1 shows that the opportunistic diseases appear when the concentration of T4 cells decreases to an amount inferior to 200/mm ³ . Thus, this low amount of interleukin secreted is insufficient to activate proliferation of activated B and T8 cells. This blocks specific immune responses and reduces general immunity of the organism, which renders the environment favorable to the development of opportunistic diseases.	3/4
5	Vaccine ensures the first contact with this antigen and triggers immunological memory. Consequently, the body, after a second contact, develops a secondary response which is more amplified, more rapid and more durable against this antigen.	3/4
6	In the first treatment, the vaccine isn't effective unless the amount of T4 cells/mm ³ of blood is superior to 500 T4 cells/mm ³ (document 3). But opportunistic diseases develop only when the amount is less than 200 T4 cells/mm ³ (2.5 times less than 500). The first treatment is only efficient against pneumonia, one of the multiple opportunistic diseases. Thus this treatment isn't efficient against the development of the opportunistic diseases.	3/4
7	The second treatment ensures the increase in the concentration of T4 cells in blood from 250 to 480 T4 cells/mm ³ between the beginning of treatment and the 5 th year (document 4). This doesn't lead to a concentration less than 200 T4 cells/mm ³ characterizing the AIDS phase which prolongs the asymptomatic phase and delays the AIDS phase.	3/4

Exercise 31 (5 pts) Conditions of LT actions

1	The response triggered against a virus is a specific cell mediated immune response. The response triggered against a bacterium is a specific humoral mediated immune response.	1/2
2	Cells Y infected by CML undergo lysis by T8 cells which are taken from mice of the same strain Y injected by CML. Whereas, cells of the same strain Y that are not infected don't undergo lysis. This shows that T8 cells destroy only infected cells. On the other hand, T8 cells which are taken from mice of strain Y injected by CML lyse the cells of same stain that are infected by CML, but they don't lyse cells of a different strain Z infected by the same virus CML. This implies that T8 cells lyse only infected cells that belong to the same strain. Cells Y infected by CML undergo lysis by T8 cells which are taken from mice of the same strain Y injected by CML. On the contrary, cells of same strain Y which are infected by another virus, HIV, are not lysed. This implies that T8 cells destroy only the cells infected by the same virus that activated them	11/2
3	There's only proliferation of T8 cells when we add the supernatant taken from a culture of T4 cells which are activated by CML in the presence of macrophages. Thus activated T4 cells stimulate the multiplication of T8 cells that recognized the same antigen, by secreting a substance, chemical messengers.	3/4
4	The macrophage phagocytizes and digests the CML virus, the obtained peptides get associated to HLA class II molecules and expressed at the cell surface. The macrophage becomes an antigen presenting cell APC. The APC fixes to T4 cells having specific receptors to the HLA- CML peptide complex thus activating the T4 cells leading to the formation of TH cells that secrete IL-2.	3/4
5	Scheme of the recognition site between T8 cells and the target cell 	3/4
6	The T8 cell performs the double recognition by fixing to the HLA- CML peptide complex of the target cell (time 1). It secretes perforin molecules that form a channel through the plasma membrane of the target cell; then it releases granzymes that penetrate the target cell through the polyperforin channel leading to the degradation of its DNA and consequently to its lysis (time 2).	3/4

Exercise 32 (5 pts) Hypertrophy of lymph nodes

Session 2017-1

1	Document 1 shows that only the follicles appear in black, after using the radioactive marker of B lymphocytes. On the contrary, the black color appears in the interfollicular zone in case of radioactive labelling of T lymphocytes. This shows that B lymphocytes are localized in the follicles while T-lymphocytes are localized at the level of the interfollicular zone.	3/4
2	The immune response triggered against the tetanus toxin is a specific humoral immune response since document 2 shows plasma cells that are antibodies secreting cells resulting from the differentiation of B cells upon their activation by the tetanus toxin. These cells are the effectors of the specific humoral immune response.	1/2
3-1	Anti-tetanus toxin antibodies.	1/4
3-2	The size of plasma cells is bigger than that of lymphocytes 12 μm is higher than 9,3 μm , these cells have a well-developed cytoplasm rich in rough endoplasmic reticulum involved in protein synthesis, and since antibodies are proteins, therefore the plasma cell is a cell adapted to the secretion of antibodies.	1/2
4	Document 3 shows that the number of B and T lymphocytes in the lymph nodes of the guinea pig "a" infected by tetanus toxin is higher than the number of B and T lymphocytes in the lymph nodes of the guinea pig "b" that has never encountered this antigen. Thus the contact with the antigen favors the multiplication (proliferation) of B and T lymphocytes.	1/2
5	After the contact with the tetanus toxin, the B and T cells are activated and proliferate, which increases their number in the lymph nodes leading to the increase in the volume of the lymph nodes.	3/4
6	The revealed immune response triggered against tetanus toxin is humoral which effector cells are B lymphocytes. Once, B lymphocytes are activated by the antigen (tetanus toxin) they can only proliferate in the presence of interleukin 4 released by T4 lymphocytes that are activated by the same antigen and differentiated into interleukin secreting cells. Thus the T4L are responsible for the proliferation of activated the BL.	3/4

Exercise 33 (5 pts) Roles of macrophage**Session 2017-2**

1-	Bone marrow	1/4
2-	Proliferation of T4 lymphocytes takes place only in culture of medium 3 in the presence of T4, macrophages and antigen X. Hence the proliferation of T4 lymphocytes necessitate the association or cooperation between T4 and macrophages in the presence of antigen X.	1
3-	The macrophages and the T4 cells must descend from the same strain. Since there is no proliferation of T4 lymphocytes when T4 cells of strain A are incubated with macrophages from another strain B. However proliferation takes place when T4 cells and macrophages previously in contact with an antigen X that descend from the same strain A.	1
4-	Phagocytosis	1/2
5-	At phase II of the experiment, 20% of radioactivity is detected on the surface of the cell, because a part (80%) of the degraded radioactive protein is eliminated out of the cell; The remaining 20% is degraded into peptides that are associated with MHC II on the surface of macrophages.	1/2
6-	The macrophages that are transformed into APC present the non-self-peptide associated with MHC II on its surface. So TCR of T4 lymphocytes bind to this complex and the T4 becomes activated.	3/4
7-	The induction of specific immune response ceases because the activation of T4 lymphocytes necessitate its binding to APC. So in the absence of activated T4 lymphocytes, no more secretion of interleukin 2 takes place which is responsible for launching the specific cell mediated immune response. Moreover, no interleukin 4 secretion takes place which is responsible for launching of the specific humoral immune response.	1

Exercise 34 (5 pts) A case of thyroiditis

Session 2018-1

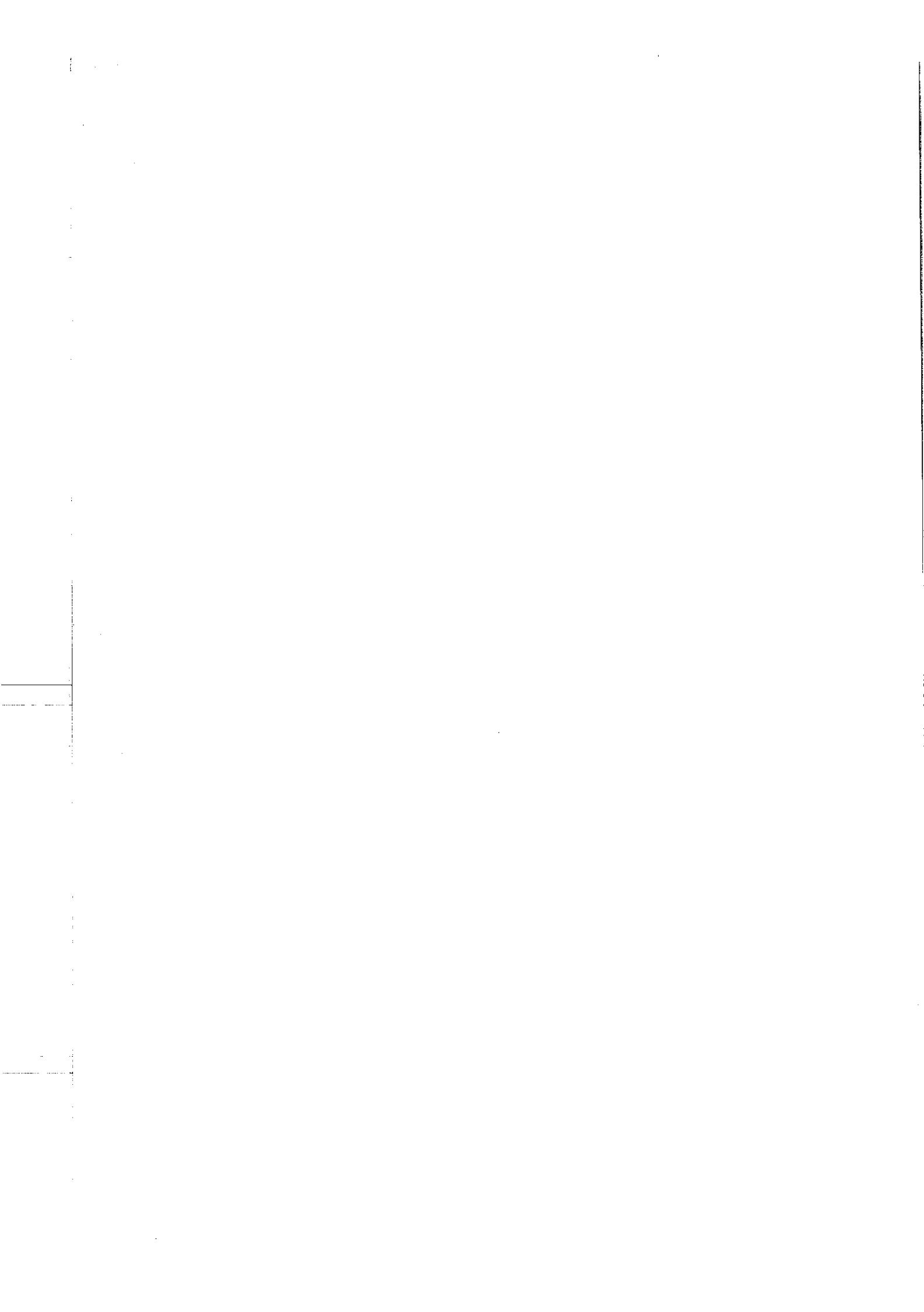
1	Hypothesis : Sarah may have an infection in the thyroid gland. Sarah may have an auto-immune disease. Sarah may have a cancer at the level of the thyroid gland	0.5
2	A large amount of antibodies is secreted in culture 3 in the presence of four types of cells : thyroid gland, B lymphocytes , macrophages and T4 lymphocytes. On the contrary, no antibodies are secreted in the absence of T4 lymphocytes (culture 2) and absence of macrophages (culture 1). This means that secretion of antibodies by B lymphocytes nécessite the presence of T4 lymphocytes and macrophages in the presence of an antigen, in this case the infected thyroid cells of sara.	1.5
3	Document 2 reveals the secretion of antibodies, therefore, the nature of specific immune response is humoral.	1
4	When a macrophage phagocytoses and digests a cell or protein, resulting peptides are attached to HLA class II molecules and presented on the cell surface. The macrophage migrates to the closest lymph node, where it becomes an antigen presenting cell or APC. The T helper cells that are specific for the peptides presented by this APC remain attached to it. Then they are activated and they proliferate	1
5	The anti-thyroglobulin antibodies in well A moves along the gel where it recognizes the thyroglobulin protein, fix to it and form an immune complex which appears as a precipitation arc. A similar precipitation arc (immune complex) is formed between well S and T which means that serum of sara contains antibodies specific to the protein thyroglobulin where they move along the gel and forms an immune complex . No such arc is revealed between well A and well N which lacks the anti-thyroglobulin antibodies since well N contains serum of a normal individual. Thus. Sara cells secrete antithyroglobulin antibodies which attack the thyroglobulin protein in her thyroid gland leading to problems in metabolism and swollen neck. This shows that Sara has auto immune disease.	1

Exercise 35 (5 pts) Therapy against an autoimmune disease

Session 2018-2

1.1	The mass of the islets of Langerhans in a healthy individual is 1400mg which is greater than 415mg (3.37 times less) in individual suffering from T1D. While the mass of Alpha cells in a healthy individual is 220mg slightly greater than that of alpha cells in the affected individual (200 g). However, While the mass of beta cells in a healthy individual is 850mg greater than 0mg in the affected individual	0,5																											
1.2	Type 1 diabetes is due to a lack of beta cells.	0,25																											
2	Document (2a) shows T8 lymphocytes in Langerhans islets of NOD mice. At a more advanced stage (document 2b), the concentration of T8 lymphocytes present in the islet increases and that of the beta cells decreases. As T8 cells have a cytotoxic action against cells, these results show that beta cells are attacked by T8 cells causing their disappearance in the individual DT1 (document 1). Since T8 cells are the effector cells involved in cell mediated immune response thus the immune response involved is a specific cell mediated.	1																											
3	<p>During a cell mediated specific immune response, T8 cells:</p> <ul style="list-style-type: none"> - TL8 recognizes the antigenic peptides presented by MHC found on the membrane of target cells., through its TCR.. - They are then activated by double recognition - Once activated, and under the action of IL-2, T8 cells proliferate and form a clone. - Activated T8 differentiate into killer cells or cytotoxic TL which : <ul style="list-style-type: none"> • Secrete perforin which forms hollow channels through the plasma membrane of target cells. • Secrete granzymes that penetrate the polyperforine channels, leading to the degradation of its DNA. <p>This leads to apoptosis of target cells.</p>	1																											
4	<table border="1"> <thead> <tr> <th colspan="2"></th> <th>Time (weeks)</th> <th>0</th> <th>1</th> <th>2</th> <th>3</th> <th>4</th> <th>5</th> <th>6</th> </tr> </thead> <tbody> <tr> <td rowspan="2">% of diabetic mice</td> <td>Control Lot A</td> <td>0</td> <td>20</td> <td>20</td> <td>40</td> <td>60</td> <td>70</td> <td>70</td> </tr> <tr> <td>Treated Lot B</td> <td>0</td> <td>0</td> <td>0</td> <td>0</td> <td>0</td> <td>5</td> <td>5</td> </tr> </tbody> </table> <p style="text-align: center;">↑ Beginning of diabetes</p> <p>Title: Table showing the variation of percentage of diabetes type 1 in NOD mice with or without treatment, as a function of time.</p>			Time (weeks)	0	1	2	3	4	5	6	% of diabetic mice	Control Lot A	0	20	20	40	60	70	70	Treated Lot B	0	0	0	0	0	5	5	1,25
		Time (weeks)	0	1	2	3	4	5	6																				
% of diabetic mice	Control Lot A	0	20	20	40	60	70	70																					
	Treated Lot B	0	0	0	0	0	5	5																					
5	<p>The results of the document 3 show that the percentage of diabetic mice increases between 0 and 6 weeks from 0 to 70% values that are greater than 5 % obtained between 4 and 6 weeks under the new treatment.</p> <p>The new treatment has thus reduced the risk of developing type 1 diabetes, which confirms their effectiveness against this disease.</p>	0,75																											
6	This treatment seems to protect the beta cells of Langerhans islet from the cytotoxic action of T8 lymphocytes which slows down the occurrence of type 1 diabetes in individuals at risk.	0,25																											

Nervous system



Ch. 5 Function of neuron

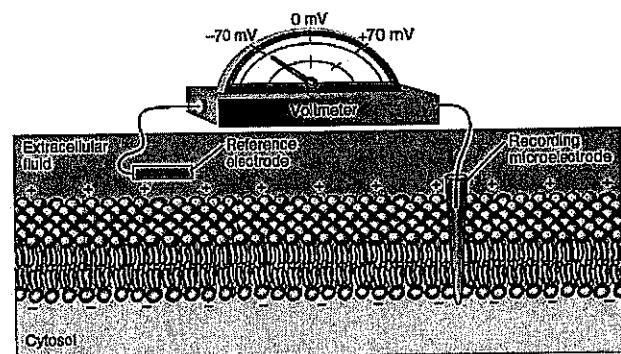
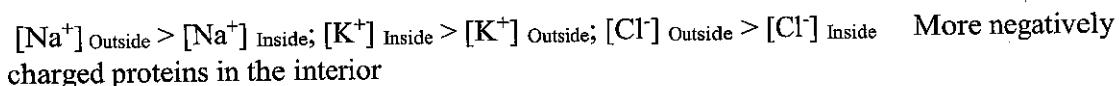


Function of neuron

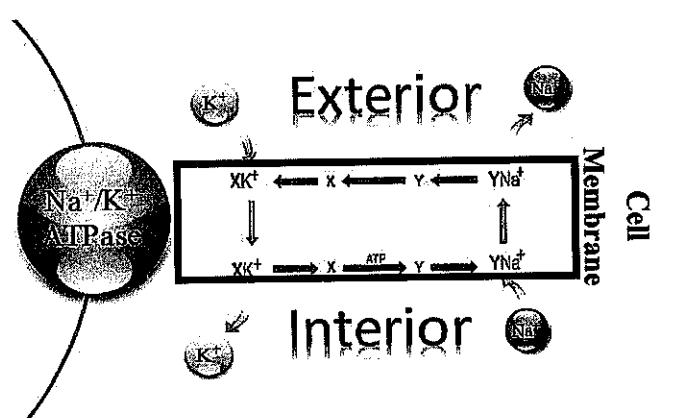
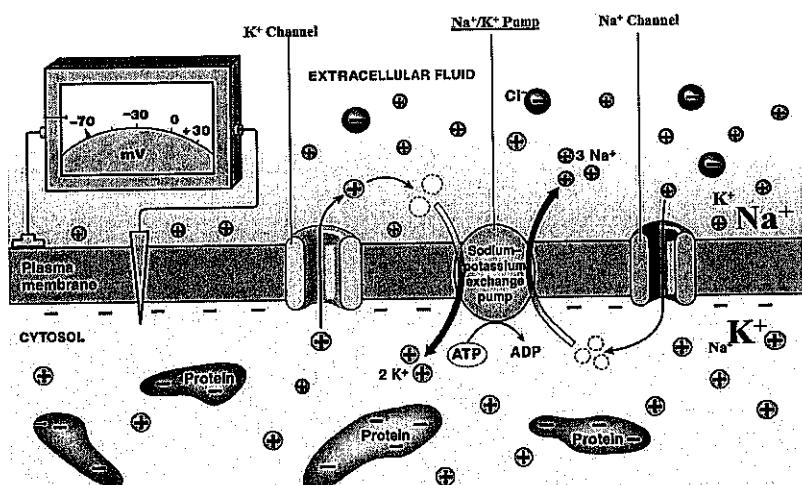
Course abstract

Presence, origin and maintaining of the resting membrane potential difference across neurons

- Resting membrane potential is the potential difference existing between the inside and the outside of non-stimulated neurons.
- According to the chemical analysis of body cells, particularly, the nerve cells (Neurons) show differences in the electric potential between the inside and the outside of these cells. This is what is called the Membrane Potential. It is resulted by the unequal distribution of ions (Na^+ , K^+ , Cl^- , PO_4^{2-} ) between the interior and the exterior of the cell membrane of these neurons. (Generating more electronegative charge in the inside than the outside). Where:

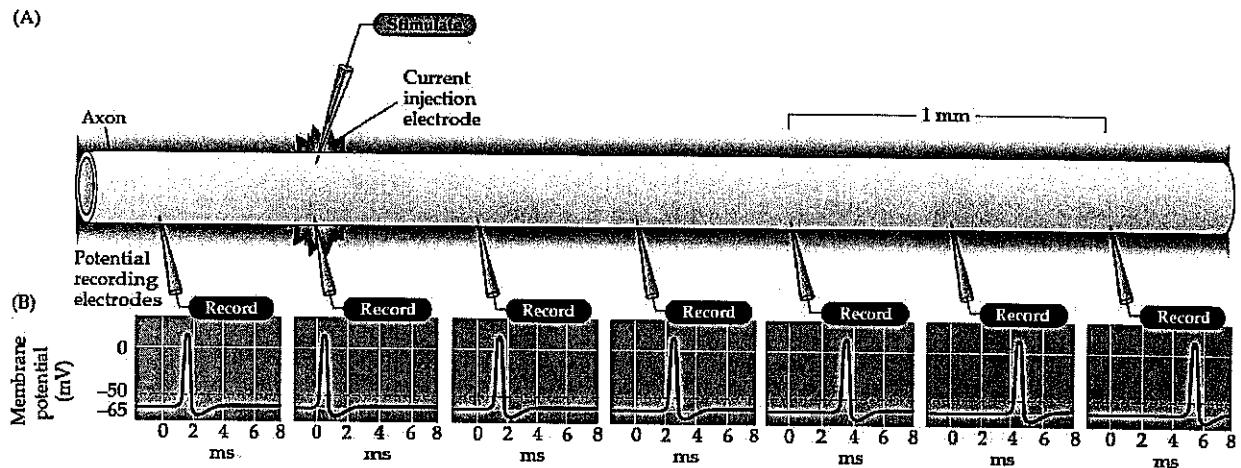


- These ions can cross the cell membrane of the nerve fiber that has a selective permeability through the channels. Na^+ channels; K^+ channels; Cl^- channels.
- The Potential difference between the inside and the outside of a neuron at rest is -70mV = Resting Membrane Potential. (It is so called since it occurs when the neuron is not stimulated (At rest).
- The origin of the resting membrane potential is due to the unequal distribution of ions between the inside the outside the neuron and the selective permeability of the neuron's membrane.
- What maintains the resting membrane potential at -70 mV is the activity of the active transport system that requires energy in the form of ATP and called Na^+/K^+ ATPase or Pump.
- Protein molecule Y transporter carries Na^+ ion from the interior to the exterior side of the membrane. Once the Na^+ ion detached outside from molecule Y, this molecule undergoes structural changes to become transporter X that carries K^+ ions from the exterior to the inside. Once K^+ ion is detached from transporter X, it takes its initial form Y that attaches again to Na^+ ion. This conformational change of transporter X into Y requires energy in form of ATP.



Action potential

It is an electro-chemical energy obtained after an effective stimulation of the nerve fiber; it consists of a wave of depolarization that propagates along the nerve fiber.



- Phases of action potential and its ionic mechanisms:

Depolarization:

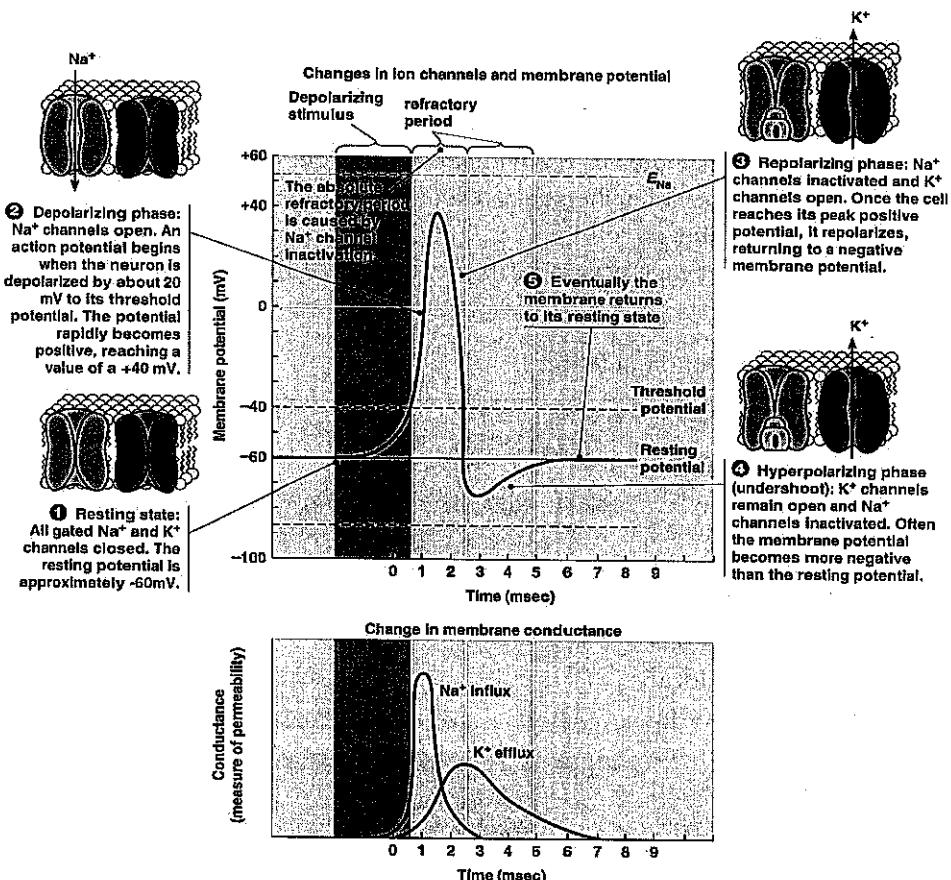
increase of the potential difference from -70 mV to $+30\text{ mV}$ due to the opening of Na^+ voltage dependent channels and massive entry of Na^+ ions.

Repolarization:

decrease of the potential difference from $+30\text{ mV}$ to -70 mV due to the opening of K^+ voltage dependent channels and the exit of K^+ ions.

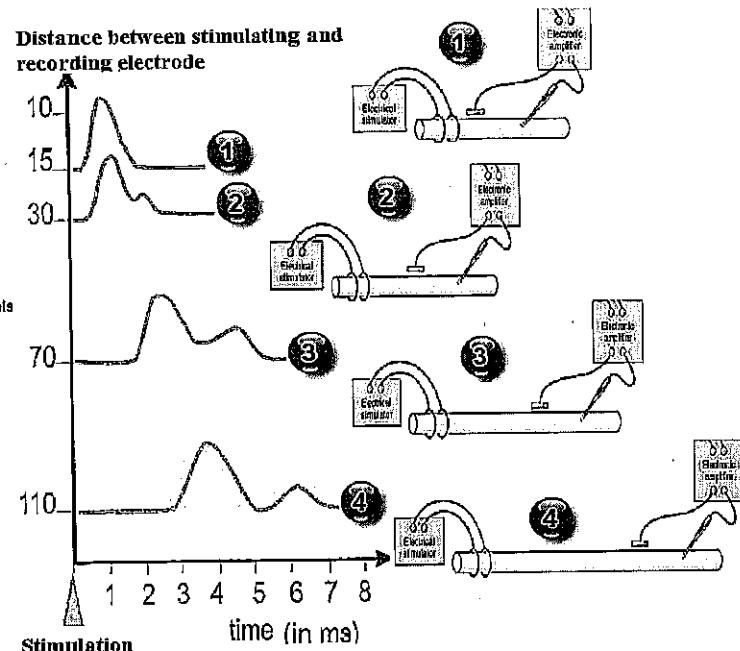
Hyperpolarization:

decrease of the potential difference from -70 mV to -80 mV due to the delay of the closure of K^+ voltage dependent channels and the exit of more K^+ ions.



- Properties of the action potential in a nerve fiber:
- Created starting from a stimulation threshold.
- Keeps constant amplitude with the increase of the intensity of the stimulation (law of all or none).
- Propagates along the nerve fiber saving the same amplitude and speed.
- Action potential's speed of propagation increases with the presence of myelin and with the increase in the fiber's diameter.

- Properties of the global potential (in a nerve):
 - Created starting from a stimulation threshold.
 - Increases its amplitude with the increase of the intensity of the stimulation (since the number of excited fibers increases) (law of summation) but it reaches a maximum amplitude (when all the fibers are excited).
 - In the nerve the nerve impulse is propagated by different speeds (nerve is made up of different nerve fibers: myelinated or not, of different diameters) that's why with distance along the nerve the global potential is separated.

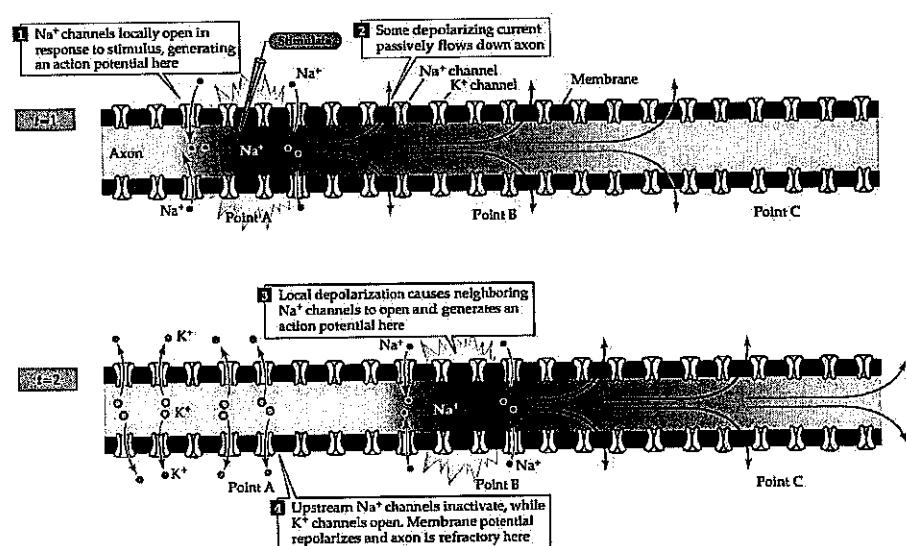


- Number of peaks indicates the number of different fibers conducting the nerve impulse.
- The time delay between the peaks reveals the difference in the speed of nerve impulse in different types of nerve fibers.
- The amplitude of each peak reveals the number of fibers transmitting the nerve impulse at certain speed.

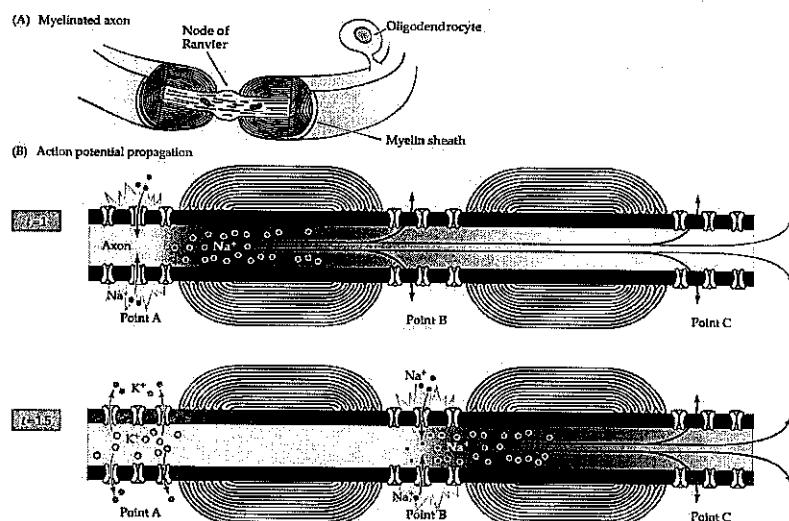
Propagation of the action potential:

When an action potential is created at a certain point of the nerve fiber,

due to the inversion of polarity the attraction between the positive and negative charges of the neighboring zones leads to the creation of local currents that lead to the depolarization of the next zone and make it more permeable for Na^+ ions, this will lead to the opening to the Na^+ voltage dependent channels and to the creation of an action potential that depolarizes the next zone and so on.



In a myelinated nerve fiber, the local current extends from one Ranvier node to the other where it leads to the creation of an action potential, thus the action potential jumps from one Ranvier node to the other, its conduction is said to be saltatory.



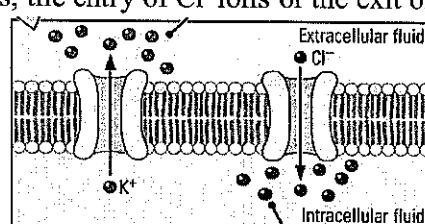
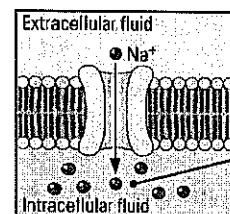
Genesis and Coding of the nerve message

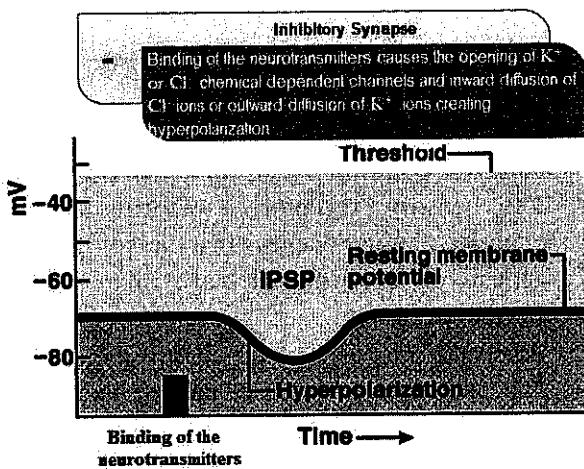
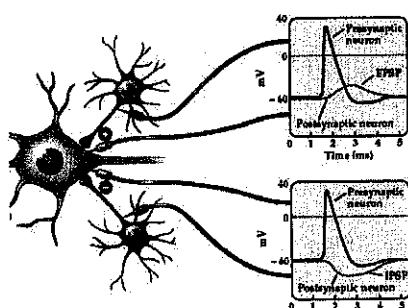
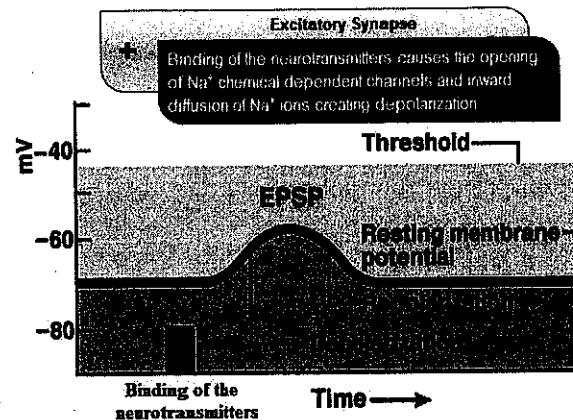
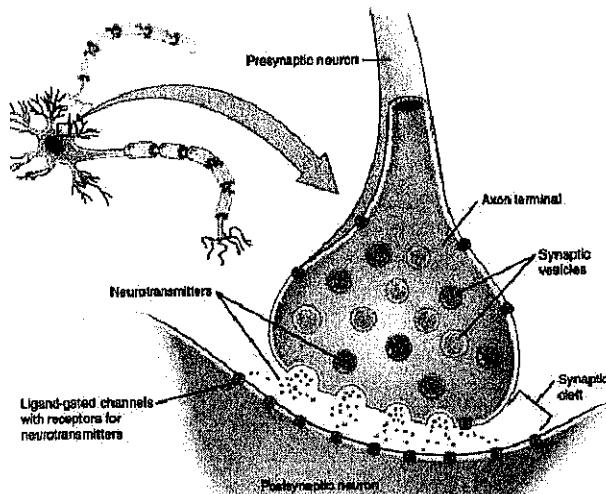
- Sensory receptors transform the environmental excitation into nerve impulse.
- The sensory receptor transforms the environmental excitation into receptor potential (electrochemical energy) that causes the genesis of generating potential that in turn triggers the genesis of the nerve message in the form of propagating action potentials saving the same amplitudes.
- When the intensity of stimulation increases the amplitude of the receptor potential increases and as a consequence the frequency of action potentials saving the same amplitude increases. Thus, the intensity of the stimulation is coded by the modulation of the amplitude of the receptor potential in the receptor, by the frequency of the action potentials in the nerve fiber and by the amplitude of the global potential at the level of the nerve

Transmission of the nerve message at the level of a synapse

After the arrival of an action potential to the terminal bud, there is an opening of the Ca^{2+} voltage dependent channels, then Ca^{2+} ions flow inside the terminal bud causing an exocytosis of the vesicles of neurotransmitters that are released in the synaptic cleft. Once released, the neurotransmitters bind to specific receptors, chemical dependent channels, located in the postsynaptic membrane; this will modify its membrane potential thus creating a postsynaptic potential (PSP). Later, the neurotransmitters are rapidly destroyed by a specific enzyme or recaptured by the terminal bud.

- If the channels are Na^+ chemical dependent channels, the entry of Na^+ to the postsynaptic cell through these channels by chemical and electrical gradients will lead to less electronegativity inside the membrane that causes a hypopolarization that is able to create an action potential if it reaches the depolarization threshold, this is the case of excitatory synapse.
- If the channels are K^+ or Cl^- chemical dependent channels, the entry of Cl^- ions or the exit of K^+ ions will lead to more electronegativity inside the membrane that causes a hyperpolarization that will make it hard for an action potential to be created. This is the case of an inhibitory synapse.



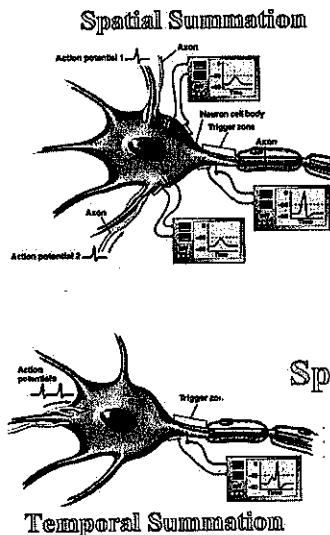


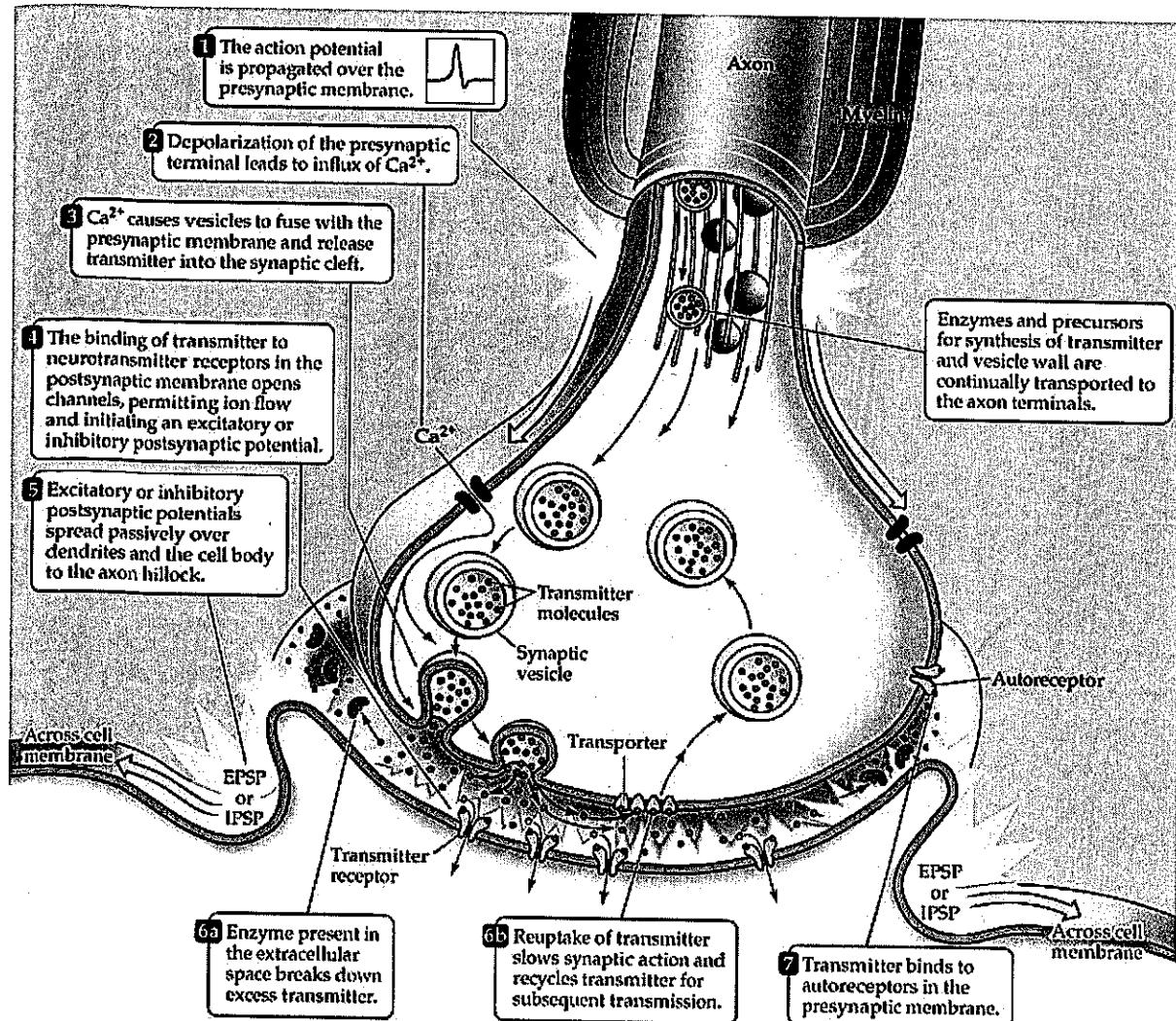
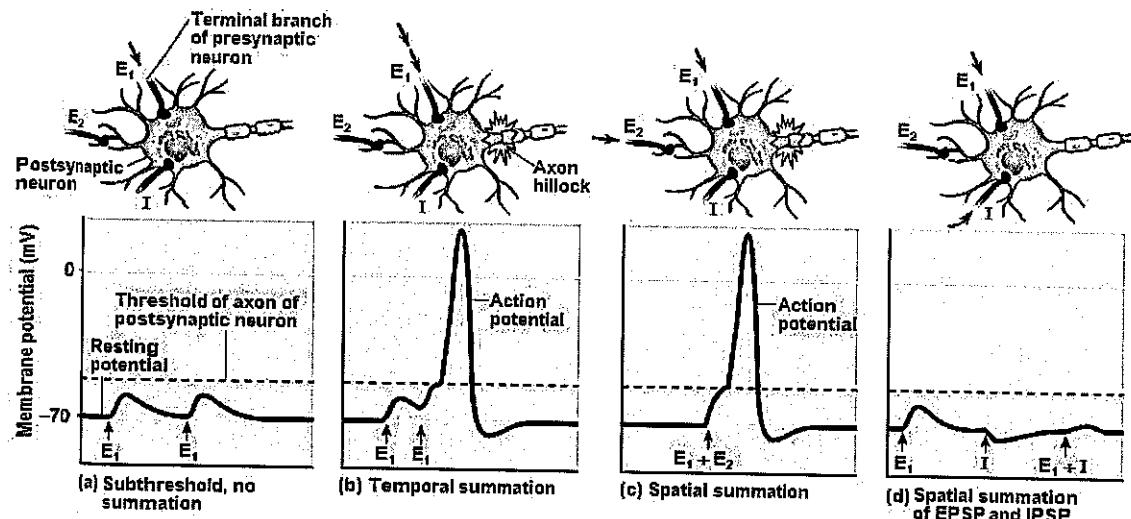
Integrating properties of nerve centers

Temporal summation is the summation of many PSPs (EPSP or IPSP) in the post synaptic neuron due to signals coming from the same presynaptic nerve fiber successively.

Spatial summation is the summation of many PSPs (EPSP and/or IPSP) in the post synaptic neuron due to impulses coming from different presynaptic nerve fibers simultaneously.

Integration corresponds to the algebraic sum of EPSP and IPSP received by the postsynaptic membrane by spatial and temporal summation. Hence after variable afferent information, the neuron elaborates again a new spatial-temporal efferent message.







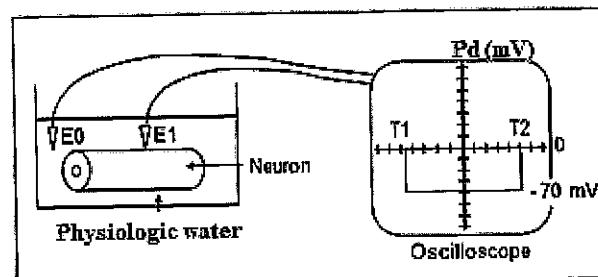
Function of neuron

Training exercises

EXERCISE 1 Resting potential

Microelectrodes E1 and E2 are used in order to measure the membrane potential of a nerve fiber. Document 1 shows the obtained results starting from time T1 where E1 is introduced inside the fiber till time T2 where E1 is taken out of it.

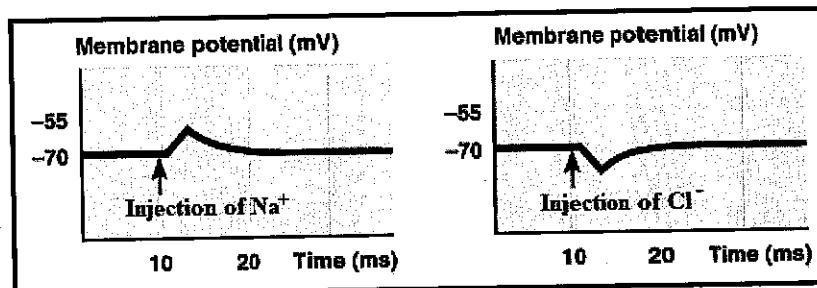
1. Pick out, from document 1, the value of the membrane potential.
2. Name the membrane potential obtained between T1 and T2.



Document 1

Na^+ or Cl^- ions are injected into the nerve fiber after insertion of the electrode E1 into it. The results of these injections are given in document 2.

3. Explain the obtained results.



Document 2

Measurements of ionic fluxes across the cell membrane shows that this latter is subjected continuously to equal inflow and outflow of Na^+ ions. After this fiber is deprived shortly of its chemical energy (ATP) the membrane potential shows the same variations observed in the case of the injection of Na^+ ions.

3. Show that the outflow of Na^+ ions is an active transport.

Solution:

1. -70 mv.
2. Resting potential.
3. The injection of Na^+ ions into the nerve fiber leads to the increase of the number of positive charges inside the fiber that will decrease the electronegativity of the internal side of the membrane, so the membrane potential will be less negative which leads to the increase of the membrane potential for 20 mv. This variation is rapidly returned to its normal state since the extra Na^+ ions are pumped outside the fiber and the concentration of Na^+ ions returns to its initial state.

The injection of Cl^- ions into the nerve fiber leads to the increase of the number of negative charges inside the fiber that will increase the electronegativity of the internal side of the membrane, so the membrane potential will be more negative which leads to the decrease of the membrane potential for 20 mv. This variation is rapidly returned to its normal state since the extra Cl^- ions diffuse rapidly to the extracellular medium because of the electrical gradient that repulses them from the internal negative medium to the external positive medium.

4. Since the inflow and the outflow of Na^+ ions are equal and the membrane shows an increase of its potential after being deprived of ATP, this means that the internal side of the membrane becomes more electropositive indicating that only the entry of Na^+ persists in this case so the outflow of Na^+ ions stops, thus the outflow of Na^+ ions needs energy in the form of ATP, subsequently, it is an active transport.

EXERCISE 2 Action potential

In order to record the response of a neuron to a stimulation, the experimental setup shown in document 1 is used.

- 1 Name the three phases a, b and c of this recording.
- 2 Explain the origin of the variation of the membrane potential during the phase a.

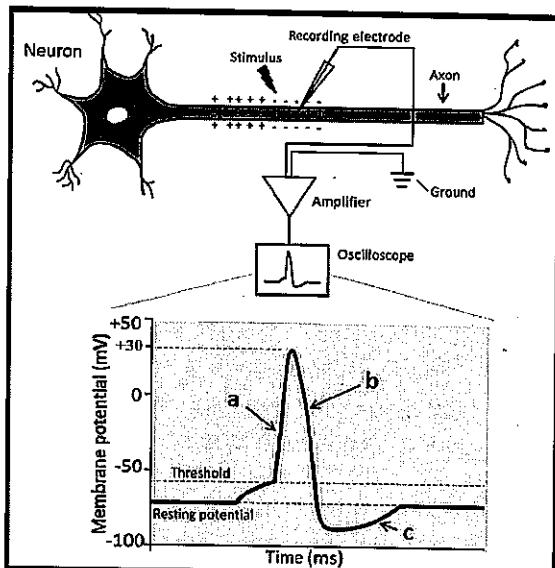
Local anesthetics are chemical substances that block locally the transmission of the nerve messages in order to block pain.

Document 2 shows the response of another nerve fiber to a stimulation in the normal state and in the presence of a local anesthetic.

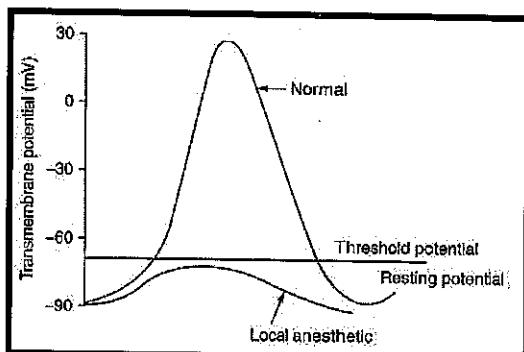
- 3 Compare the response of the fiber to the stimulation in the absence and in the presence of the local anesthetic.
- 4 Formulate a hypothesis to explain the effect of the local anesthetic.

The measurement of the inflow of Na^+ ions in the normal state and in the state of the application of the local anesthetic after effective stimulation shows very low inflow current in the presence of the anesthetic.

- 5 Explain the absence of action potential in the presence of anesthetic.



Document 1



Document 2

Solution

- a. Depolarization, b. Repolarization, c. Hyperpolarization.
- During the phase (a), the opening of Na^+ voltage dependent channels due to the stimulation leads to the entry of Na^+ ions due to chemical gradient (Na^+ concentration is higher outside) and to the electrical gradient (Na^+ ions are attracted by the negative charges inside the fiber), this entry of Na^+ ions leads to the decrease of the electronegativity of the internal medium that becomes positive after the massive entry of these ions, it increases from -70 mV to +30 mV.
- After the stimulation without anesthetic, the membrane potential shows an action potential of 120 mV of amplitude, while, it shows, in the presence of local anesthetic, a hypopolarization of amplitude 15 mV that is very small compared to that of the action potential.
- Hypothesis: Local anesthetic inhibits the opening of Na^+ voltage dependent channels.
- After application of anesthetic, there is a decrease of the inflow of Na^+ ions following a stimulation, this low inflow leads to the decrease of the electronegativity of the internal medium that leads in turn to the increase of the membrane potential for 15 mV only.

EXERCISE 3 Response to a stimulation

In this exercise, we consider that the amplitude of the artifact of stimulation does not depend on the intensity of the stimulation. Document 1 shows a nervous structure that is submitted to a series of stimulations. Document 2 shows the response of this nervous structure recorded by the oscilloscope 1 following this series of stimulations.

1. Indicate the stimulation(s) below threshold and that above threshold. Justify the answer.
2. Justify that the recordings given in this document are not sufficient to determine if the stimulated structure is a nerve or a nerve fiber.
3. Calculate the speed of the nervous message recorded by the oscilloscope 1.

The amplitude of the obtained response remains the same after we increase the intensity of the stimulation.

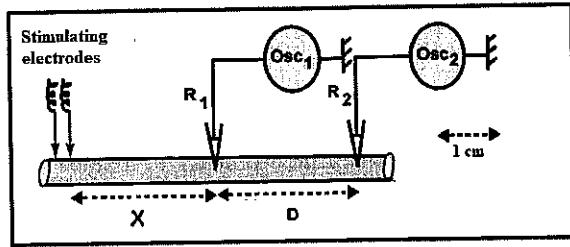
4. Identify the nervous structure used in this experiment.

The amplitudes of the action potentials obtained on the oscilloscope 2 are similar to those obtained on oscilloscope 1.

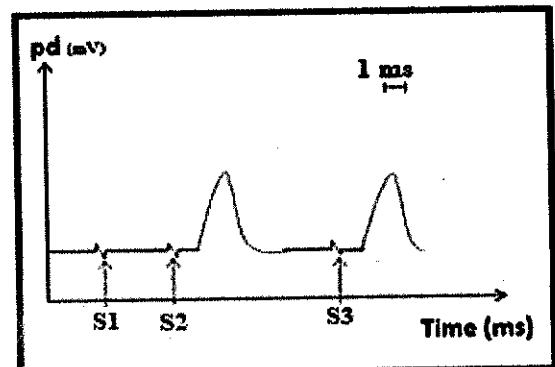
5. Draw out, starting from these results, two properties of the action potential.

Solution:

1. Stimulation S1 is below threshold since it does not create an action potential, while S2 and S3 are above threshold since each creates an action potential.
2. Since we do not know if these intensities are of increasing intensities or not, S2 and S3 may be of equal intensities, they will give, in the case of a nerve, responses of same amplitudes as well as in the nerve fiber, then we cannot determine if the stimulated structure is a nerve or a nerve fiber.
3. Speed = $\frac{\text{Distance}}{\text{Time}} = \frac{X}{1 \text{ ms}} = \frac{2 \text{ cm}}{1 \text{ ms}} = 2 \text{ cm/ms} = 20 \text{ m/s}$
4. The amplitude of the obtained responses remains the same after we increase the intensity of the stimulation, and since the isolated nerve fiber obeys the law of all or none and keeps the action potentials with same amplitudes whatever the intensity of the stimulation is, above the threshold; thus, this structure is a nerve fiber.
5. Action potential propagates in the nerve fiber.
Action potential keeps its amplitude constant with the propagation.



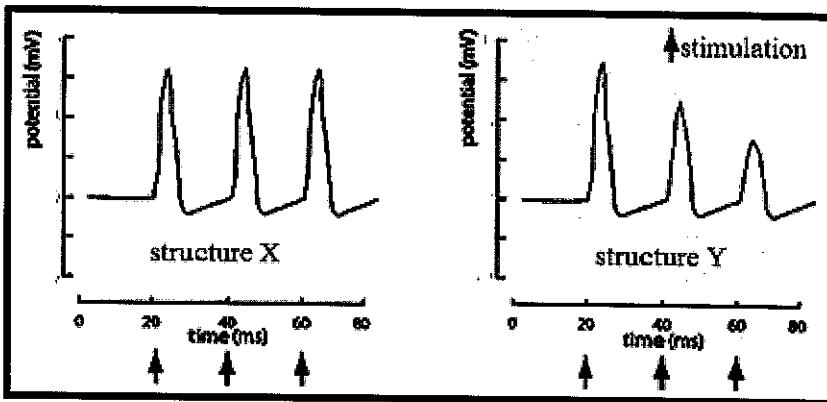
Document 1



Document 2

EXERCISE 4 An Isolated nerve fiber and a nerve

Document 1 represents two responses obtained after the same series stimulations done on two nervous structures X and Y, one of these two structures is a nerve fiber, the other is a nerve, they are not listed in order.

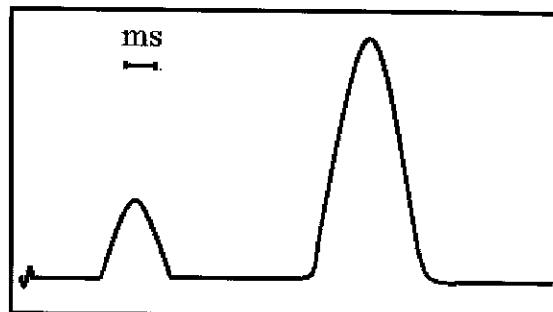


Document 1

1. Show that the structure Y is a nerve.
2. Explain the results obtained in the case of the structure Y.
3. Indicate the property of the structure X revealed by document 1.

In order to study structure Y in a more detailed way; we record its response to a stimulation similar to that made at 20 ms in document 1 but we move the recording electrode to a place where the distance to the stimulating electrode is 5 cm. Document 2 shows the obtained results.

1. Calculate the speeds of the nervous messages passing through the structure Y.
2. Explain the difference in the propagation of the nervous messages in the structure Y, knowing that all the fibers constituting it are of same diameter.



Document 2: Response of the structure Y at 5 cm

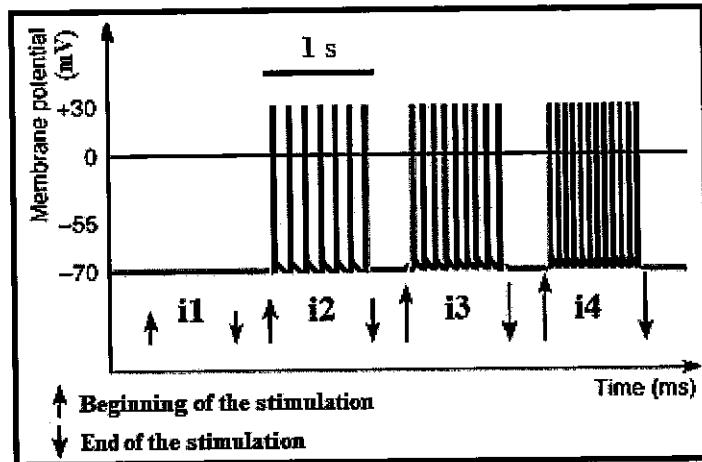
Solution:

1. Structure Y shows three responses that are of different amplitudes, and the nerve action potential amplitude depends on the number of excited fibers that depends on the intensity of the stimulation; then, the structure Y is a nerve.
2. The stimulations done in the case of the structure Y are of decreasing intensities, the first leads to the excitation of more nerve fibers that leads to a higher amplitude. This amplitude decreases with the decrease of the number of excited fibers that decrease in turn with the decrease of the intensity of the stimulation.
3. Law of all or none.
4. First response: Speed 1 = $\frac{\text{Distance}}{\text{Time}} = \frac{5 \text{ cm}}{2 \text{ ms}} = 2.5 \text{ cm/ms} = 25 \text{ m/s}$
Second response: Speed 2 = $\frac{\text{Distance}}{\text{Time}} = \frac{5 \text{ cm}}{9 \text{ ms}} = 0.555 \text{ cm/ms} = 5.55 \text{ m/s}$
5. Despite the same diameters of the fibers of the structure Y, they show propagation of nerve messages in two different speeds, this is due to that the fibers of higher speed are myelinated since myelin sheath accelerates the propagation of the nerve messages, while the fibers of lower speed are not myelinated.

EXERCISES Coding of the nervous message

In order to study the effect of the intensity of the stimulation on the nervous message created by a receptor, we apply a series of stimulations of increasing intensities (i1 to i4) on a sensory receptor for stretching located in a muscle. The results recorded at the level of the neuron related to this receptor are given in document 1.

1. Draw out, from the text, the studied problem.
2. Indicate the type of the fiber where the obtained messages are recorded.
3. Show, based on document 1, that the nervous message is created starting from a threshold of stimulation.
4. Analyze the obtained results.
5. Conclude the effect of the intensity of the stimulation on the nervous message.

**Document 1**

The stretching of the receptor located in the muscle, by an intensity above the threshold, leads to the contraction of this same muscle. This contraction increases in intensity with the increase of the intensity of stretching done on the muscle.

6. Explain, starting from the given and from document 1, the variation of the intensity of the contraction of the muscle with the increase of the intensity of the stimulation from i2 to i4.

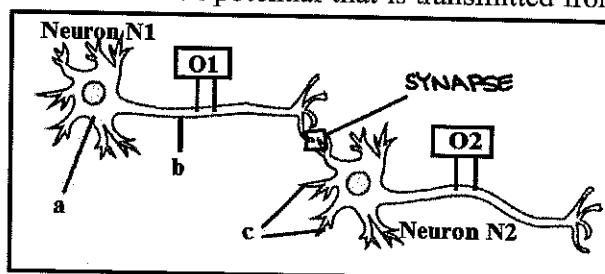
Solution:

1. What is the effect of the intensity of the stimulation on the nervous message created by a receptor?
2. Sensory fiber.
3. Since at i1 no action potentials are recorded, and they start to appear when the intensity of the stimulation increases to i2, thus action potentials are created starting from a minimal intensity of stimulation that is the stimulation threshold.
4. With the intensity i1 of stimulation, no action potentials are recorded; as the intensity of the stimulation increases to i2 then from i2 to i3, action potentials are obtained with a frequency increasing from 7 AP/s to 12 AP/s and by keeping the same amplitude equal to 100 mV.
5. The increase of the intensity of the stimulation amplifies the frequency of action potentials of the nervous message.
6. The increase of the intensity of stretching leads to the increase of the frequency of action potentials, and the intensity of the contraction of the muscle depends on the frequency of action potentials, thus the intensity of contraction of the muscle increases from i2 to i4.

EXERCISE 6 Transmission of the nervous message through a synapse

The transmission of the nervous message from one neuron to another is done through a special junction called synapse. This synapse can be of two types, the first allowing the transmission of an action potential and the second prevents the creation of an action potential that is transmitted from other neurons to the postsynaptic neuron of it.

1. Name the first and the second type of the synapse mentioned in the text.
2. List the steps of the transmission of the nervous message in a synapse.
3. Label the letters a, b and c in document 1.



Document 1

N°	Experiments	Results	
		O ₁	O ₂
1	Stimulation of N1	— —	— —
2	Stimulation of N2	— —	— —
3	Injection of the acetylcholine in the synaptic cleft the synapse.	— —	— —
4	Stimulation of the neuron N1 in a medium deprived of Ca ²⁺	— —	— —

Document 2

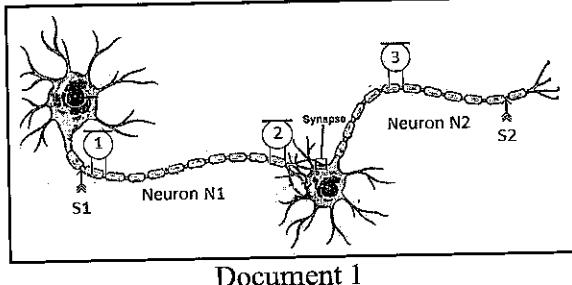
| : AP

Solution:

1. First type: Excitatory, second type: Inhibitory.
2. 1. Arrival of the nervous message.
2. Opening of Ca²⁺ channels and entry of Ca²⁺ ions to the terminal bud.
3. Release of neurotransmitters by exocytosis of vesicles.
4. Binding of neurotransmitters on the postsynaptic receptors.
5. Creation of a nervous message in the postsynaptic cell.
6. Degradation or recapture of neurotransmitters by the presynaptic terminal bud.
3. a. Cell body, b. Axon, c. Dendrites.
- 4.1 After the stimulation of the neuron N1 an action potential is observed in the neuron N1 as well as in the neuron N2, while after the stimulation of the neuron N2, an action potential is observed in the neuron N2 only.
- 4.2 We can conclude that the transmission of the nervous message in this synapse is unidirectional from the neuron N1 to the neuron N2.
5. After the injection of acetylcholine in the synaptic cleft, an action potential is observed in the neuron N2, since the acetylcholine binds on the postsynaptic receptors that are Na⁺ chemical dependent channels, their opening leads to the entry of Na⁺ to postsynaptic cell that decreases the electronegativity thus increasing the membrane potential to reach the threshold and creates an action potential in the postsynaptic cell.
In the absence of Ca²⁺ ions, the stimulation will not lead to the exocytosis of neurotransmitters that will not create a PSP. Thus, no action potential is obtained in O₂.

EXERCISE 7 Properties of the synaptic transmission?????????????????

In order to study some characteristics of the nerve message, we use the neuronal network shown in document 1. The table in document 2 shows the performed experiments and the obtained results.



Experiment	O1	O2	O3
Stimulation at S1	+30 mV -70 mV	+30 mV -70 mV	— 70 mV
Two close successive stimulations at S1	+30 mV -70 mV	+30 mV -70 mV	+30 mV -70 mV
Two close successive Stimulations at S2	— 70 mV	— 70 mV	+30 mV -70 mV

Document 2

1. Indicate if the stimulation S1 is below threshold or above threshold.
2. Interpret the results obtained at O1 and O2 for the stimulation S1.
3. Specify the type of the synapse that relates the neuron 1 to the neuron 2.
4. Explain the results obtained at the level of O3 for the two successive stimulations made at S1.
5. Justify the results of the two successive stimulations done at S2.
6. Explain the transmission of the nervous message from the neuron 1 to the neuron 2.

Solution:

1. Above threshold.
2. For the stimulation S1, we obtain an action potential of same amplitude equal to 100 mV at each of the two oscilloscopes O1 and O2, this means that the action potential propagates at the level of the nerve fiber while conserving its amplitude.
3. The synapse is excitatory since after two successive stimulations at S1, two action potentials of 100mV of amplitude are obtained at the level of the neuron N1 and one action potential of same amplitude is observed at the level of the neuron N2, indicating that an action potential is transmitted from N1 to N2.
4. After the two successive stimulations at N1, two action potentials of 100mV of amplitude are transmitted to the neuron N2, a temporal summation is made for the hypopolarizations obtained from the two action potentials, this summation leads to the increase of the membrane potential to a value above the potential threshold leading for the creation of an action potential.
5. When the two successive stimulations are done at S2, two action potentials are obtained at N2, on the contrary no action potentials are obtained at N1, since the transmission of the nervous message at the level of the synapse is unidirectional from the presynaptic neuron to the postsynaptic one because the neurotransmitters are only found in the presynaptic side and the receptors are on the postsynaptic membrane.
6. After the arrival of the nervous message to the presynaptic bud, Ca^{2+} voltage dependent channels are opened, they lead to the entry of the Ca^{2+} ions into the terminal bud, then Ca^{2+} ions lead to the release of the neurotransmitters to the synaptic cleft by exocytosis of the vesicles. The released neurotransmitters bind on the postsynaptic receptors that are chemical dependent channels for Na^+ leading for their opening and for the entry to Na^+ ions to the postsynaptic neuron that will lead to the decrease of the electronegativity inside this neuron, thus a hypopolarization is obtained. If this hypopolarization reaches the depolarization threshold, it will lead to the creation of an action potential in the postsynaptic neuron.

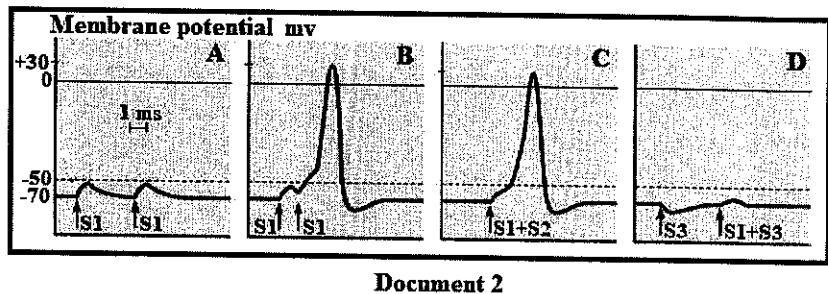
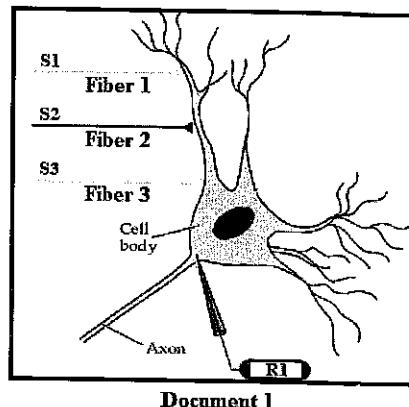
EXERCISE 8 Neuronal integration

Document 1 shows a neuronal circuit observed at the level of the spinal cord, the neuron schematized is a motor neuron responsible for the contraction of a given muscle.

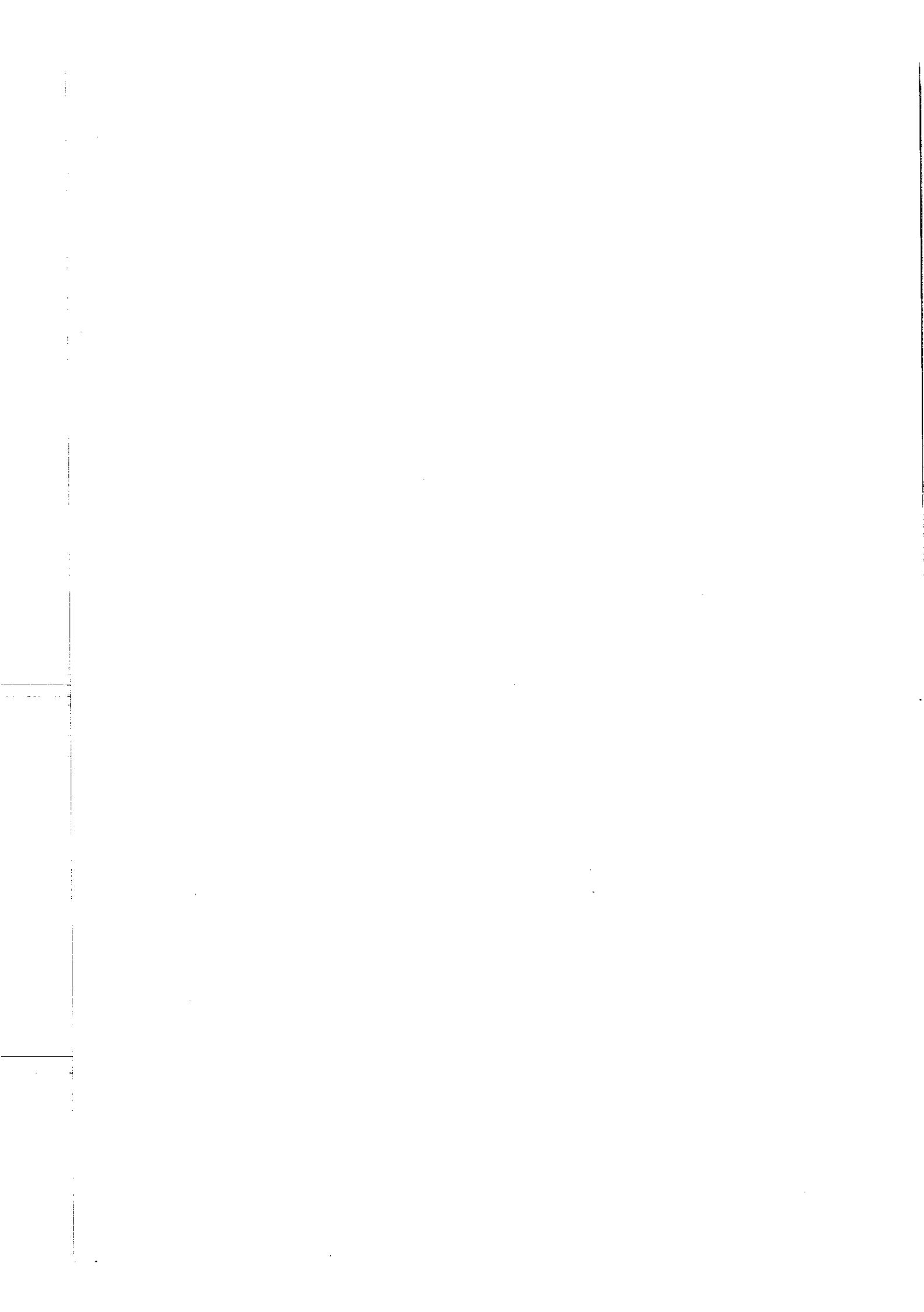
Four different experiments A, B, C and D are made by applying stimulations on some of the fibers 1, 2 and 3 connected to the motor neuron.

The results of these experiments are recorded by the electrode R1 and represented by document 2

1. Identify the type of each of the synapses relating the fibers 1 and 3 to the motor neuron.
2. Interpret the results of the experiments A and B.
3. Determine the type of the synapse connecting the fiber 2 to the motor neuron.
4. Specify the type of summation made by the motor neuron in the experiment C.
5. Justify the absence of an AP in the experiment D.

**Solution:**

1. The stimulation of the fiber 1 leads to a hypopolarization of amplitude 15 mV. Since hypopolarization is obtained following the transmission of the nerve message through an excitatory synapse, then, the synapse relating the fiber 1 to the motor neuron is excitatory. The stimulation of the fiber 3 leads to a hyperpolarization of amplitude 10 mV. Since hyperpolarization is obtained following the transmission of the nerve message through an inhibitory synapse, then, the synapse relating the fiber 1 to the motor neuron is inhibitory.
2. After two successive stimulations relatively far in time, we obtain two successive hypopolarizations of amplitude 15 mV separated by 3 ms, while when the two stimulations are closer in time, we obtain two successive hypopolarizations separated by 1 ms, the first of amplitude 15 mv, and the second allowing the creation of an action potential of 100mV of amplitude. This means that a summation of the two hypopolarizations is made when they are close enough in time.
3. In experiment C, we remark that the stimulations S1 and S2 lead to the creation of an action potential, and since the stimulation S1 alone does not lead to an action potential of amplitude 100mV, so the stimulation S2 helps the hypopolarization created by fiber 1 to reach the threshold, thus it is also a hypopolarization that indicates that the synapse connecting the fiber 2 to the motor neuron is excitatory.
4. The summation made in experiment 2 is spatial, since the two stimulations made in experiment C are on two different nerve fibers.
5. The stimulation S3 leads to a hyperpolarization of amplitude 10 mv, S1 leads to a hypopolarization of amplitude 15 mv. In experiment D, a spatial summation of the PSPs obtained by S1 and S3 leads to a hypopolarization of amplitude 5 mv that does not reach the threshold of stimulation and does not create an action potential.

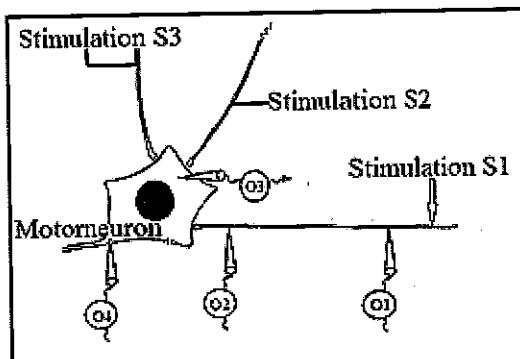


Function of neuron

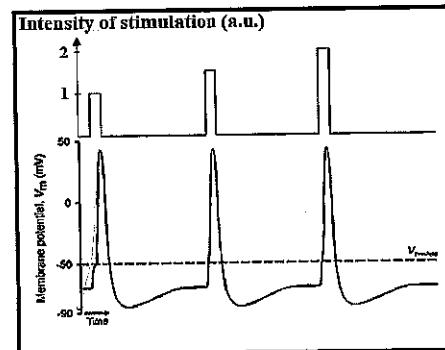
Solved exercises

EXERCISE 1 The properties of the neurons

At the point labeled by stimulation S₁ on the nerve fiber shown in document 1, we make three stimulations of increasing intensities. The results of these three above threshold stimulations are recorded on the oscilloscopes O₁ and O₂ indicated on the figure. The two oscilloscopes show the same results that are given by document 2.



Document 1



Document 2

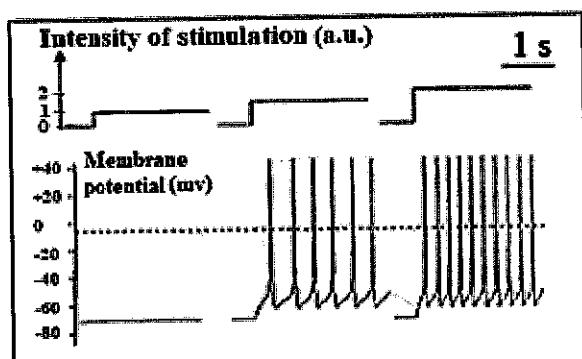
- 1.1 Analyze the results obtained by the oscilloscope 1.
- 1.2 What can you conclude?
2. Explain the mechanism that leads to the results obtained on the oscilloscope O₂.

Three stimulations of increasing intensities are now made on a receptor related to the same fiber stimulated above. The obtained results on the oscilloscopes O₁ and O₂ are represented by document 3.

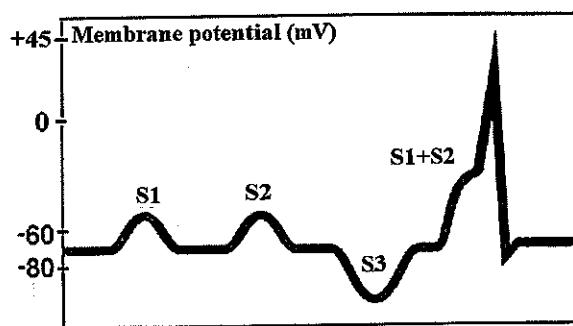
3. Represent, in a table, the different intensities of stimulations shown by document 3 with the corresponding responses obtained at the level of the nerve fiber.
4. Interpret the results obtained in document 3.

Document 4 shows the results obtained on the oscilloscope O₄ of many experiments where one or two stimulations, of stimulations marked in document 1, are made.

5. Determine the type of the synapses that lead to each of the obtained results by each of the stimulations S₁, S₂ and S₃.
6. Explain the result obtained after the simultaneous stimulations S₁ and S₂.



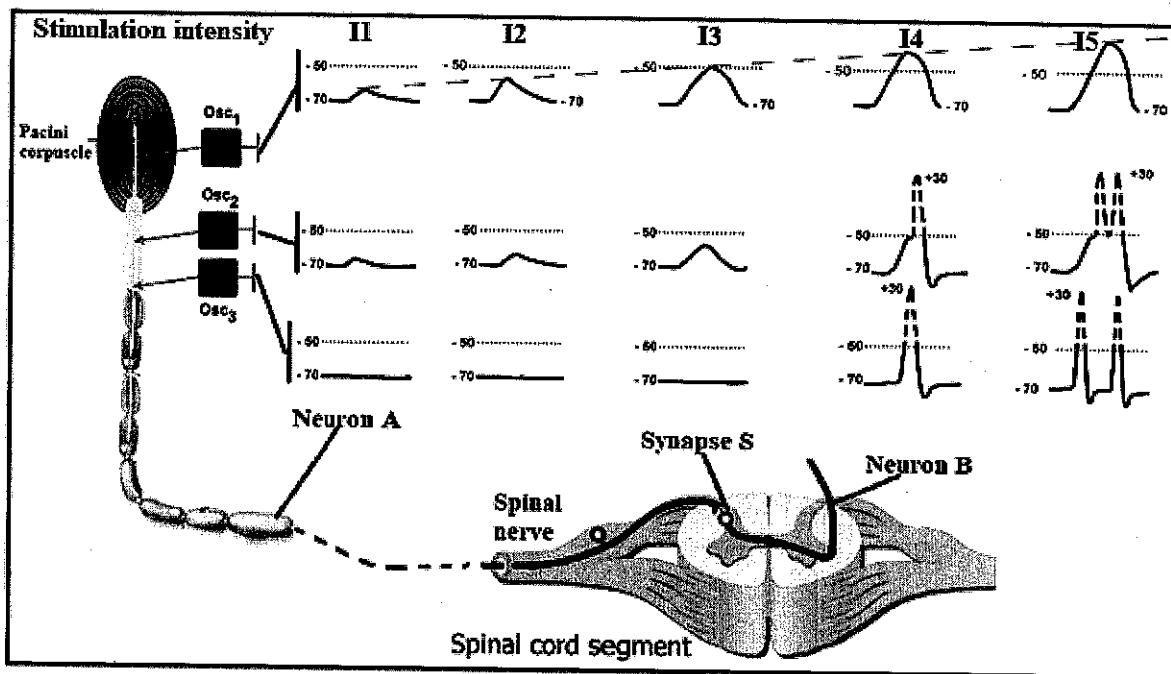
Document 3



Document 4

EXERCISE 2 Pacinian Corpuscle

Skin contains many sensory receptors. Pacinian corpuscles are the most obvious as they form large (~ 1 mm), onion-like structures in the dermis and hypodermis. Pacinian corpuscles contain a myelinated nerve ending in the central core. The outer layers are composed of flattened cells, collagen fibers and a lymph-like fluid. Pacinian corpuscles are sensitive to mechanical and vibratory pressure. In order to study the sensation feelings generated by this receptor, stimulations with increasing intensities are done on the receptor shown in document 1, the obtained recordings by the oscilloscopes 1, 2 and 3 are represented in the same document.

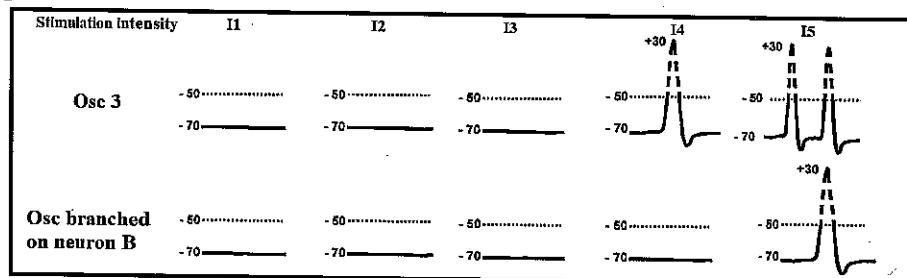
**Document 1**

1. Name each of the results given by oscilloscopes 1 and 2 in document 1 by response to the stimulations of intensities I1 and I5.
2. Interpret the results obtained on oscilloscope 1.
3. Show starting from the obtained results that not all variations of the membrane potential are transmitted through the nerve fiber.

The responses recorded by oscilloscope 3 are shown in document 2 in comparison with the responses recorded by an oscilloscope 4 branched on the neuron B.

4. Determine the type of the synapse S relating neuron A to neuron B.

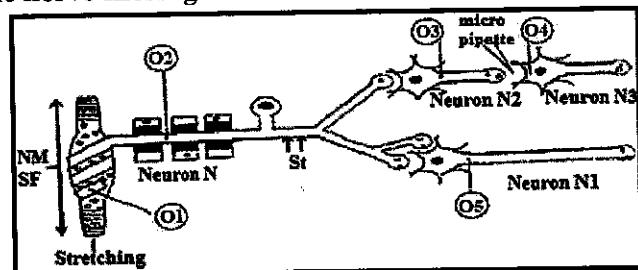
A type of summation is revealed by the results obtained on the oscilloscope 4.

**Document 2**

5. Specify the type of summation made by the neuron B and revealed by the results of oscilloscope branched on neuron B.
6. Explain, starting from all what preceded, that the sensations generated by this type of receptor begin starting from a threshold intensity of stimulation.

EXERCISE 3 Creation and transmission of the nerve message

We propose to study the mechanism of the creation and the transmission of the nervous message in a neuronal network. We use the experimental setup presented in document 1 and we realize many series of experiments.

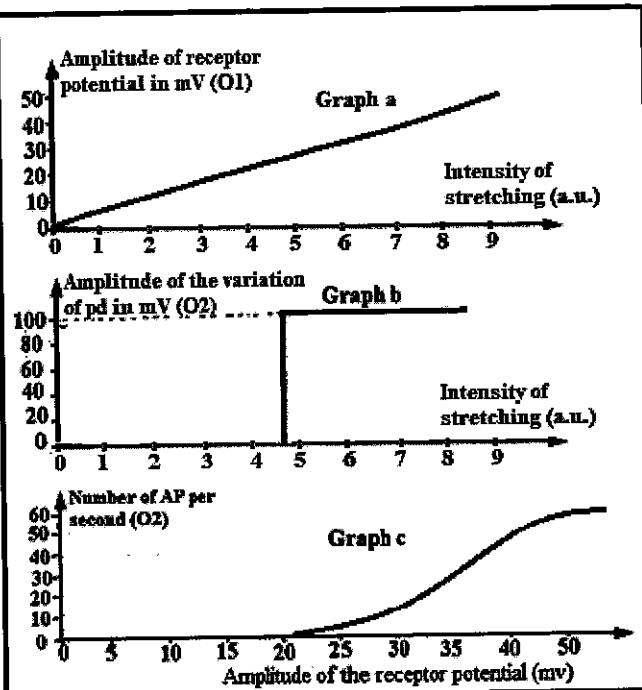


Document 1

1st series of experiment:

We subject the stretching receptor of a muscle (M) to stretching of increasing intensities. The curves in document 2 translate the recordings obtained at the level of the oscilloscopes O1 and O2.

1. Deduce, by referring to graph b, two properties of the action potential.
2. Determine, by referring to graph c, the coding of the intensity of stretching.
3. Show, starting from what precedes, that the receptor is responsible for the coding of the intensity of stimulation in the nerve fiber.



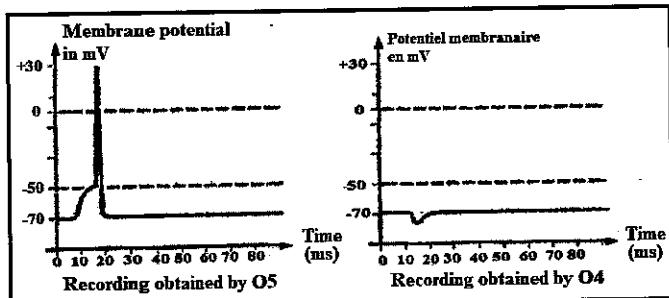
Document 2

2nd series of experiments:

Experiment 1: we make one effective stimulation at St (doc. 1), we obtain at O5 the recording given in document 3.

Experiment 2: we make two effective stimulations at St (doc 1), we obtain on O4 the recording given in document 3.

1. Identify the nature of each of the synapses N-N1 and N2-N3.

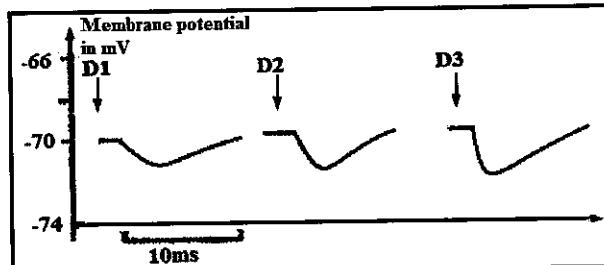


Document 3

3rd series of experiments:

In the absence of any stimulation, we realize a microinjection at the level of the synaptic cleft of the synapse N2-N3, of the corresponding neurotransmitter with increasing concentrations D1, D2 and D3. We obtain the results represented by document 4.

2. Determine the coding of the nervous message at the level of the synaptic cleft.

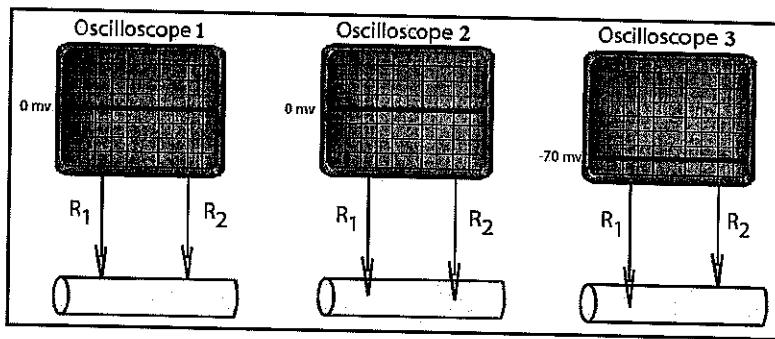


Document 4

EXERCISE 2 Resting potential

In order to measure the potential difference that corresponds to the resting potential, three oscilloscopes are used as shown in document 1.

1. Justify the values obtained on each of the three oscilloscopes 1, 2 and 3.



Document 1

Searching for the mechanisms used by the cell to keep its resting potential constant some experiments are done.

Experiment 1:

A nerve fiber is deprived of its chemical energy (ATP) by the injection of cyanide. The membrane potential is soon lost by the fiber that records 0 mV.

2. Show, starting from experiment 1, that the resting potential is an active process.

Experiment 2:

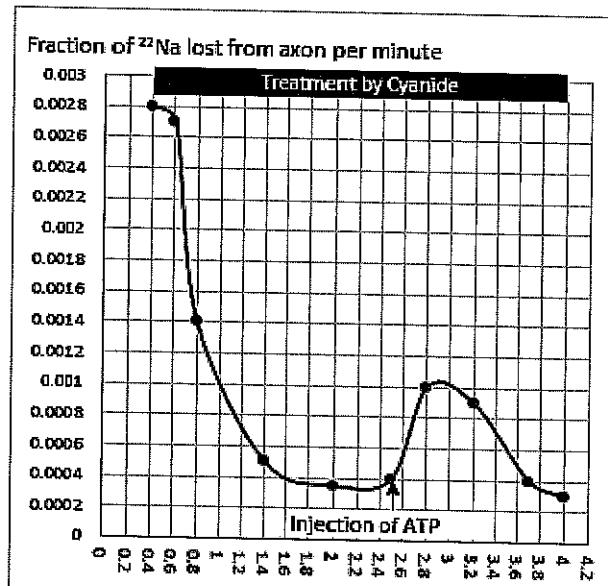
The use of radioactive Na⁺ (²²Na⁺) allows to monitor the flow of Na⁺ ions through the cell membrane of the nerve fiber.

Measurements of inflow and outflow of Na⁺ ions, in the absence or in the presence of cyanide are done.

Inflow of Na⁺ ions remains constant before and after the addition of cyanide.

The outflow of Na⁺ ions is measured before and after the addition of cyanide, the results are shown in document 2.

3. Represent the given in document 2 in a table.
- 3.1. Analyze the results shown in document 2.
- 3.2. Conclude the effect of ATP on the outflow of Na⁺ ions.
- 3.3. Specify if each of the inflow and the outflow of Na⁺ is passive or active.



Document 2

We know also that deprivation of the nerve fiber from ATP stops the inflow of K⁺ ions but it does not affect the outflow of them.

6. Explain starting from all what preceded the mechanisms used by the cell to keep its resting potential constant.

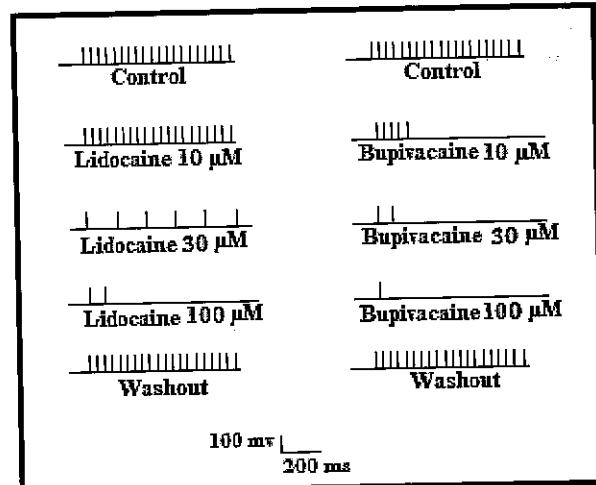
EXERCISE 5 Action of some anesthetics

Bupivacaine and lidocaine are two pharmaceutical substances used in the medical field. They have effects on the transmission of the nervous messages and subsequently on the sensations and the motor activities.

In order to study the effect of these two substances on the nervous messages; many experiments were done.

In the first experiment, bupivacaine and lidocaine are injected into the skin of an individual having an inflammation, then the frequency of action potentials in the nociceptive fibers is recorded in different experimental conditions in the area of injection of these substances. Out of this area, the frequencies are not affected. The experimental conditions and the obtained results are represented in document 1.

1. Show starting from the results in document 1 that Lidocaine and bupivacaine are local anesthetics.
2. Determine, out of lidocaine and bupivacaine, the most efficient anesthetic.
3. Justify that the actions of lidocaine and bupivacaine are short-term local actions.

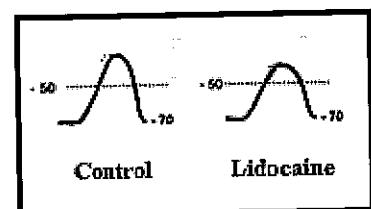
**Document 1**

In order to study the mode of action of lidocaine, two hypotheses are proposed, the first concerns the nociceptor while the second concerns the nociceptive nerve fiber.

4. Formulate the two hypotheses mentioned above.

Aiming to validate the hypothesis that concerns the nociceptor, the receptor potential of the nociceptor is recorded in a control case and in the case of injection of 100 μM of lidocaine. The results are shown in document 2.

5. Validate the hypothesis that concerns the receptor.

**Document 2**

The possible action of lidocaine on the nerve fiber is determined by the measure of the intensity threshold of the stimulation of the nerve fiber while increasing the concentration of lidocaine added into the medium. The results are shown in document 3.

Concentration of lidocaine (a.u.)	0	10	30	100
Threshold of stimulation of the nerve fiber in a.u.	50	60	80	150

Document 3

- 6.1 Analyze the obtained results.

- 6.2 Conclude the effect of lidocaine on the sensitivity of the nerve fiber to stimulations.

Further studies aim to determine the action of lidocaine on the voltage dependent Na^+ channels. For that, the current of Na^+ ions is measured in the absence and in the presence of lidocaine. Document 4 shows the obtained results.

7. Based on all what preceded, explain the mode of action of lidocaine.

**Document 4**

EXERCISE 6 Mutation and Paralysis

Caenorhabditis elegans is a small nematode worm whose nervous system is well known. It constitutes a model animal to study the functioning of the neuromuscular synapse. Synapse studies are performed on worms with a mutation in the unc-13 gene and complete paralysis of the muscles. These studies lead to find the cause of this paralysis.

Experiment 1: By an experimental apparatus, electrical stimulations of motor neurons innervating muscles in a wild worm and an unc-13 mutant worm were done and the electrical phenomena in their muscles was recorded. The setup and the obtained results are represented in document 1.

- 1.1. Indicate the steps numbered from 1 to 5 in document 1.
- 1.2. Propose two hypotheses that explain the dysfunction of the motor plates in unc-13 mutant worms.
- 1.3. Compare the results obtained and shown in document 1.
- 1.4. Draw out the cause of the muscles' paralysis.

Document 2 illustrates the contents of presynaptic vesicles and the electrical response of a muscle fiber during nicotine injection into the synaptic cleft in wild worm and mutant unc-13 worm.

	Wild worm	Mutant worm unc-13
Content of presynaptic vesicles	Acetylcholine	Acetylcholine
Nicotine injection * in the synaptic cleft	Contraction of the muscle cell	Contraction of the muscle cell

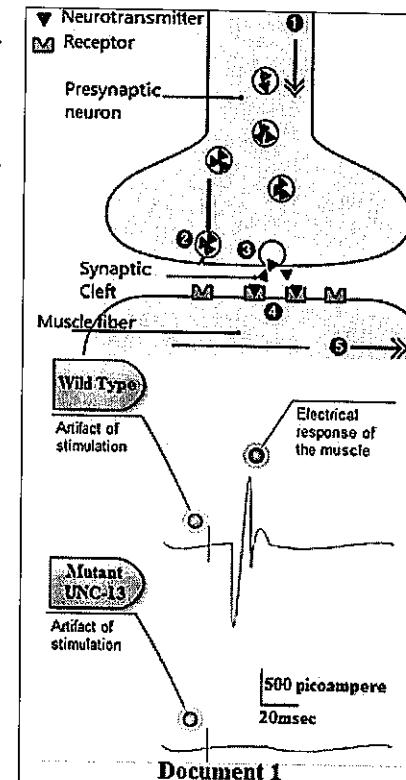
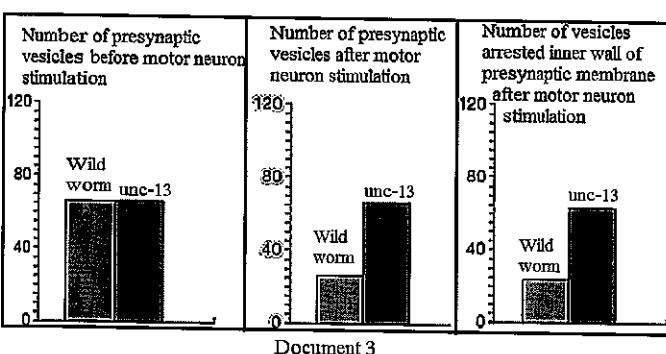
Document 2

* Nicotine is a molecule with a three-dimensional structure close to that of acetylcholine.

2. Justify that the paralysis in the mutant worm is related to step 3 in document 1 during synaptic transmission.

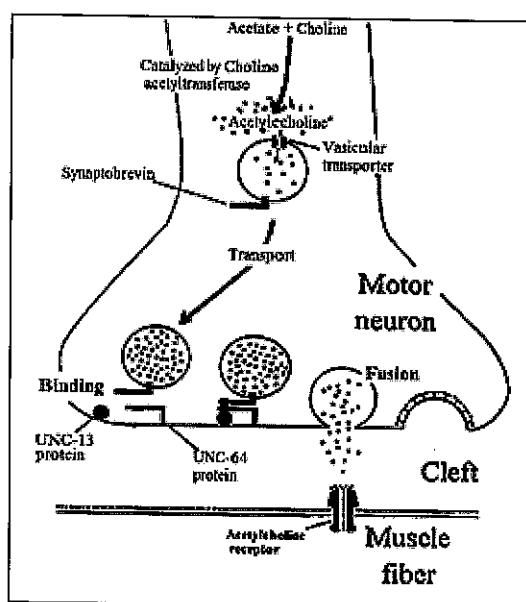
Experiment 2: The number of vesicles in synaptic terminations of the presynaptic neurons after motor neuron stimulation was measured. The results are present in document 3.

3. Determine, referring to document 3, the effect of the mutation in UNC-13 gene.



Document 4 represents some phenomena implicated in the contraction of muscles in the cholinergic synapse, after the arrival of the nerve impulse in the presynaptic terminal.

4. Describe the events illustrated in document 4.
5. Explain, referring to what preceded, how the mutation in UNC-13 gene is the origin of the worm paralysis.



Document 4



Function of neuron

Solved exercises solution

Exercise 1 The properties of the neurons

- 1.1. With the increase of the intensity of the stimulation from 1 to 2 a.u. we obtain action potentials of constant amplitudes equal to 115 mV.
 - 1.2. We can conclude that the action potential amplitude in the nerve fiber is conserved whatever the intensity of the stimulation above the threshold.
 2. The action potential propagates in the nerve fiber, in fact, when an action potential is created, it leads to the inversion of the polarity of the membrane in a certain point of the nerve fiber, the attraction between the charges of the site of action potential with the neighboring zones leads to the creation of local currents that depolarize the next zone and make it more permeable for Na^+ ions thus creating an action potential and so on.
 3. Table showing the variation of the responses obtained at the level of the nerve fiber as a function of the intensity of the stimulation.
- | Intensity of stimulation in a.u. | 1 | 1.5 | 2 |
|----------------------------------|---|-----|-----|
| Amplitude of AP (mV) | 0 | 100 | 100 |
| Frequency of AP/s | 0 | 3 | 5 |
4. At 1 a.u. of the intensity of the stimulation, no action potentials are recorded, on the contrary, when the intensity of the stimulation increases to 1.5 a.u. a frequency of 3 AP/s is recorded all with same amplitude equal to 115 mV, this frequency increases to 5 AP/s with the increase of the intensity of the stimulation from 1.5 a.u. to 2 a.u. but the amplitude remains the same. This means that the AP start to be created starting from an intensity threshold and the intensity of the stimulation is coded by the frequency of action potentials and not by amplitude.
 5. Synapses 1 and 2 create EPSP of same amplitudes of 15 mV respectively after the stimulations S1 and S2, then they make the membrane potential closer to the threshold of stimulation, so they are excitatory synapses. On the contrary, Synapse 3 creates an IPSP of amplitude of 20 mV after the stimulation S3, then it makes the membrane potential far from the threshold of stimulation, so it is inhibitory synapse.
 6. After the simultaneous stimulations S1 and S2, the two EPSP of the synapses 1 and 2 make spatial summation that permits the resulted EPSP to reach the threshold and create an action potential of amplitude 100 mV.

Exercise 2 Pacinian Corpuscle

1. Oscilloscope 1: receptor potential for the two stimulations.
Oscilloscope 2: Local hypopolarization for the stimulation of intensity I1. Action potentials for the stimulation of intensity I5.
2. The amplitude of the receptor potential increases with the increase of the intensity of the stimulation from an amplitude of 5 mV at the intensity I1 to reach an amplitude of 35 mV at the intensity I5 while passing the threshold at the intensity I3 of stimulation, this means that the amplitude of the receptor potential is amplified by the increase of the intensity of stimulation allowing it to attain the threshold at a certain intensity.
3. The results show that the small variations of the membrane potential that are observed following the stimulations of intensities I1, I2 and I3 are recorded only at the oscilloscope 2, the oscilloscope 3 shows resting potential for these stimulations. On the contrary, the action potentials observed after the stimulations of intensities I4 and I5 are recorded on both oscilloscopes 2 and 3, so the hypopolarizations are not transmitted while the action potentials are transmitted from the site of the placement of the recording electrode of oscilloscope 2 to that of the oscilloscope 3, thus not all the variations of the membrane potential are transmitted through the nerve fiber.
4. After the stimulation of intensity I5, the oscilloscope 3 shows two successive action potentials of 100 mv of amplitude, while the oscilloscope branched on neuron B shows only 1, this indicates that the synapse that relates the neuron A to the neuron B is able to transmit the action potentials despite the attenuation of their number, so it is an excitatory synapse.
5. Two successive action potentials of amplitude 100 mV originating from the same fiber of neuron A submit the summation in order to create an action potential of amplitude 100 mV in the neuron B, thus this is a temporal summation.
6. We remark that the receptor succeeds in creating action potentials starting from a threshold intensity of stimulation of intensity I3, but at its level, the action potential is not transmitted to the neuron B in order to be directed to the superior nerve centers, this transmission begins with the intensity I5 that creates two action potentials in the neuron A that are submitted to a temporal summation allowing the creation of an action potential in neuron B, thus, the feelings generated by this receptor begin with a threshold intensity I5 of stimulation.

Exercise 3 Creation and transmission of the nerve message

- The graph b shows a maximum and constant amplitude of 100 mV that appears beyond the intensity 4.7 a.u., so the action potential obeys the law of all or none and has a stimulation threshold.
- Since the number of action potential recorded by O2 increases from 0 to 60 AP/s when the amplitude of the receptor potential increases from 20 mv to 60 mv and this latter increases from 0 to 50 with the increase of the intensity of the stimulation from 0 to 9 a.u., then the intensity of stretching is coded by the modulation of frequency at the level of the nerve fiber.
- The Increase of the intensity of the stretching provokes an increase of the amplitude of the receptor potential which increases the frequency of action potential at the level of the nerve fiber; therefore, the increase of the intensity applied on the receptor leads to the increase of the frequency of action potential at the level of the nerve fiber, thus the neuromuscular spindle is responsible for the coding the message in the fiber.
- Since O5 records an A.P of amplitude 100mV after stimulation in St, and since the excitatory synapse is responsible for the transmission of the action potentials then the synapse N-N1 is excitatory. Since O4 records a hyperpolarization of 3mv after stimulation in St and the inhibitory synapse is responsible for the creation of a hyperpolarization in the postsynaptic side, then the N2-N3 is inhibitory synapse.
- The injection of a concentration D1 of neurotransmitters at the level of N2-N3 causes hyperpolarization of amplitude 1mv, this hyperpolarization increases in amplitude with the increase of the injected concentrations to attain 3mv with concentration D3. This shows that the nervous message is coded by the modulation of neurotransmitter concentration in the synaptic cleft.

Exercise 4 Resting potential

- For the oscilloscope 1, the two electrodes are placed in the extracellular side of the cell membrane; for the oscilloscope 2, the two electrodes are placed in the intracellular side of the cell membrane, in these two cases, the two electrodes of the oscilloscopes are placed in two mediums of same potential (same charge) then the potential difference is null. On the contrary, for the oscilloscope 3, the recording electrode R1 is placed in the intracellular side while the recording electrode 2 is place in the extracellular side, these two sides have between them a potential difference since they have different charges, the extracellular side is positive, while the intracellular side is negative; this potential difference is of -70 mV recorded by oscilloscope 3.
- Since when the fiber is deprived of its chemical energy (ATP) the membrane potential becomes null, so the fiber is consuming energy in order to keep its resting potential constant, thus the resting potential is an active process.
- Table showing the variation of lost ^{22}Na from axon per minute as a function of time and the cyanide treatment with injection of ATP.

Time (hours)	0.4	0.6	0.8	1.4	2	2.5	2.8	3.2	3.7	4
Fraction of ^{22}Na lost from axon/min	0.0028	0.0027	0.0014	0.0005	0.00035	0.0004	0.001	0.0009	0.0004	0.0003
Injection of ATP										
Treatment by Cyanide										

- After the beginning of the treatment by Cyanide, the fraction of ^{22}Na lost from axon decreases from 0.0028/min at 0.4 hour to 0.0035/min at 1.6 hour, while it remains constant till 2.4 hour, time of injection of ATP. Starting from 2.4 hours to 2.9 hours it increases to become 0.001/min, on the contrary, it decreases again to reach 0.0003/min at 4 hours.
- Therefore, ATP amplifies the outflow of Na^+ ions.
- Inflow of Na^+ is passive, since it does not need energy, since after the addition of Cyanide that blocks the synthesis of ATP, the inflow of Na^+ ions remains constant. Outflow of Na^+ ions is active, since it needs energy in the form of ATP, in fact, it decreases upon treatment by Cyanide from 0.0028 to 0.00035/min, and reincreases after the injection of ATP to become 0.001/min.
- The concentration of Na^+ and K^+ ions are high respectively in the extracellular medium and the intracellular medium, Na^+ flows to the inside passively as K^+ flows outside according to the concentration gradients for both, and according to the electrical gradient for Na^+ since intracellular medium is negatively charged. Na^+/K^+ pump consumes energy in the form of ATP to pump Na^+ outside and K^+ inside the fiber in order to keep the concentrations of Na^+ and K^+ constant that keeps the resting potential constant.

Exercise 5: Action of some anesthetics

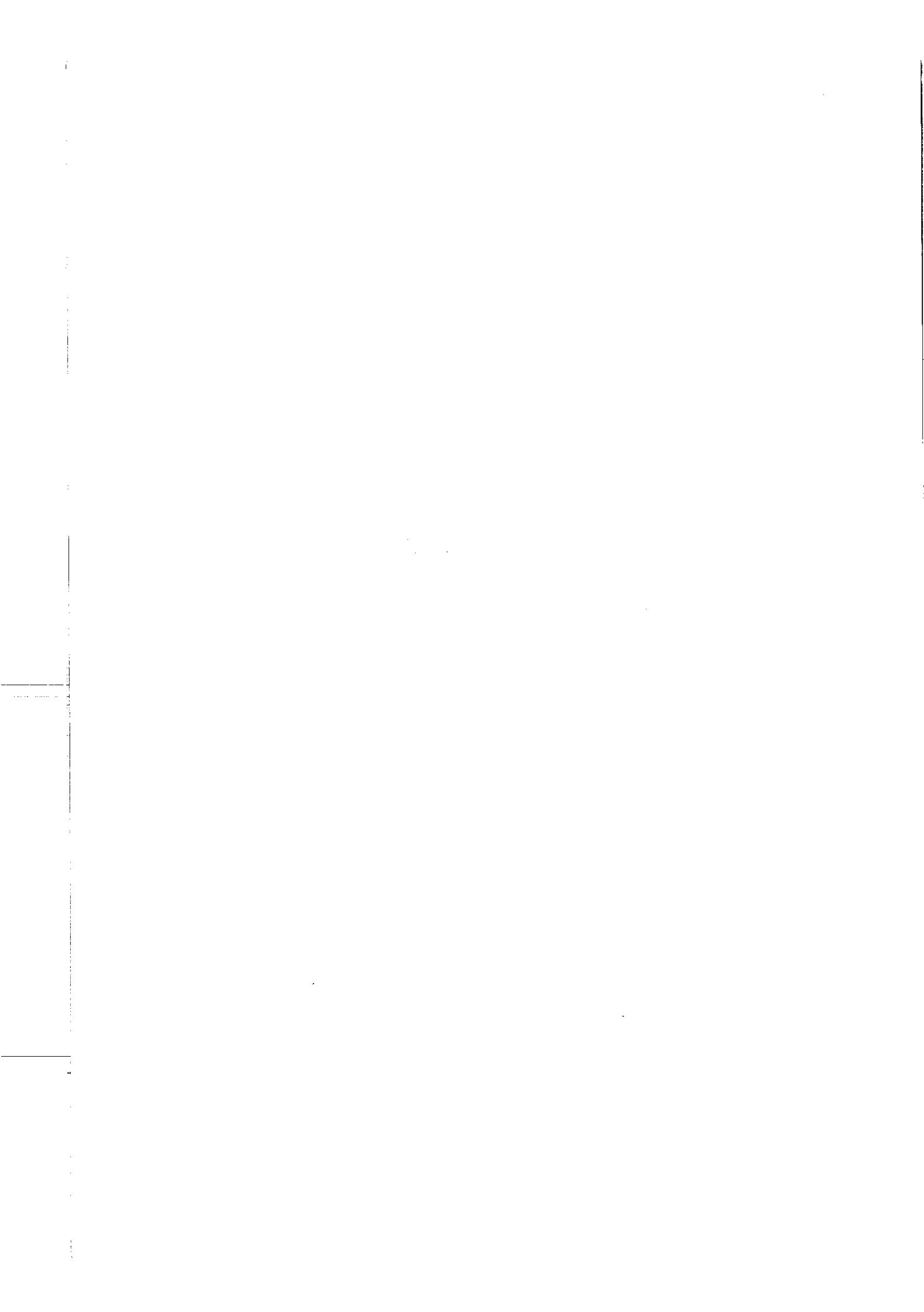
1. In the case of inflammation, the control individual shows a frequency of 5 AP/200 ms all of same amplitude equal to 100 mv and for a time equal to 800 ms, after application of 100 μM , two action potentials during 100 ms are obtained for lidocaine, whereas only one action potential is obtained with bupivacaine, always with same amplitude.
This indicates that both lidocaine and bupivacaine inhibit the pain message, so both are anesthetics and since, out of the area of injection of them the frequencies are not affected, so they are local anesthetics.
2. The message remains the same after applying 10 μM of lidocaine but after applying 10 μM of bupivacaine, it becomes shorter in time of 200 ms while conserving the same frequency of 5 AP/200 ms.

When the dose of lidocaine increases to 30 μM we obtain along the same time of 800 ms a lower frequency equal to 1 AP/200 ms, while for bupivacaine, two action potentials only are obtained along a shorter time of 100 ms, the amplitude remains the same for both. After application of 100 μM , two action potentials during 100 ms are obtained for lidocaine, whereas only one action potential is obtained with bupivacaine. Thus, bupivacaine anesthetic action starts with lower dose than that of lidocaine and reaches a lower frequency after the increase of the dose for both to 100 μM , therefore, bupivacaine is most efficient than lidocaine.

3. Lidocaine and bupivacaine do not damage the nociceptive fibers, since after washout, the frequencies of AP in both cases return to their initial states.
4. Hypotheses: Lidocaine reduces the receptor potential.
Lidocaine blocks Na^+ voltage dependent channels.
5. The control case shows a receptor potential of 35 mV of amplitude that decreases to 30 mV after applying lidocaine, so lidocaine reduces the amplitude of the receptor potential which validates the hypothesis formulated above.
- 6.1 Without lidocaine the threshold of stimulation of the nerve fiber is of 50 a.u., it increases after applying a concentration of 10 a.u. of lidocaine, and increases to 150 with the increase of the concentration of applied lidocaine to 100 a.u.
- 6.2 We can conclude that lidocaine reduces the sensitivity of the nerve fiber.
7. Lidocaine reduces the amplitude of the receptor potential thus reduces the frequency of action potentials of pain message. On the other hand, lidocaine inhibits the opening of Na^+ voltage dependent channels at the level of the nerve fiber thus reduces the entry of Na^+ to the fiber, this will lead to the decreases of the frequency of action potentials in the nociceptive fibers. The decrease of the frequency of the action potentials in the nociceptive fibers caused by the action of lidocaine on both, the receptor and the nerve fiber leads to the anesthetic action of lidocaine.

Exercise 6 Mutation and paralysis

- 1.1. Arrival of action potential. 2- Migration of synaptic vesicles. 3-Exocytosis. 4-Binding of neurotransmitter of receptors. 5-PSP formation.
- 1.2. Hypothesis: UNC-13 mutants have abnormal receptors of neurotransmitters.
Hypothesis: UNC-13 mutants have abnormal neurotransmitters.
Hypothesis: Exocytosis is prevented in UNC-13 mutants.
- 1.3. Upon stimulating the motor neuron, an electrical response was recorded in the motor neuron of the wild type with amplitude 100 pico-ampere per 20 ms, but in the UNC-13 mutants no response was generated.
- 1.4. Absence of a generation of a response in the muscle.
2. Since both the wild worm and the mutant worm have acetylcholine in their presynaptic neurons and their muscles contract after injecting Nicotine, molecule with a three-dimensional structure close to that of acetylcholine, in the muscle cleft, then the cause of paralysis in UNC-13 mutants is the inability to release the acetylcholine in the cleft or its exocytosis (step 3).
3. Before the stimulation of the motor neuron, the number of the presynaptic vesicles was equal 60 in the wild and unc-1 mutant worm, while after the stimulation of the motor neuron, the number of vesicles in the wild worm decreased to 30 but in the mutant remained constant 60, moreover, the number of the vesicles arrested in the inner membrane of the motor neuron after stimulation was 30 in the wild worm less than it in the unc-13 mutant worm. Then the unc-13 mutation inhibits the exocytosis of synaptic vesicles.
4. Acetate and choline are catalyzed by choline acetyltransferase into acetylcholine that are stored in the synaptic vesicle by the vesicular transporter. The synaptic vesicle transported to the synaptic knob where the vesicular synaptobrevin bind with unc-13 and unc-64 where fusion took place and the acetylcholine released in the cleft to bind on the acetylcholine receptors in the membrane of the muscle fiber.
5. The mutation in the unc-13 gene caused the formation of abnormal unc-13 protein, this latter is unable to assemble with unc-64 and synaptobrevin, as a consequence the synaptic vesicles do not fuse with membrane, no exocytosis of acetylcholine will be realized in the cleft, in this case, no response will be generated in the muscles causing paralysis.

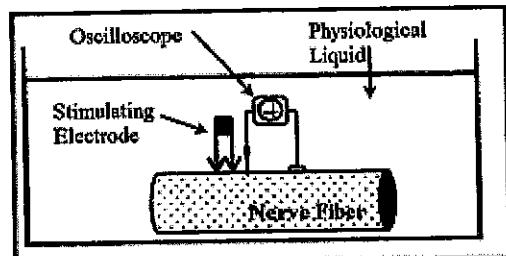


Function of neuron

Non-solved exercises

Exercise 1 Scorpion Venom and Paralysis of Preys

The toxin in scorpion venom can cause death in some mammals by causing disruption in the functioning of the nervous system. An attempt is made to understand the effect of these toxins on certain aspects of the activity of nerve fibers and their ability to transmit nerve impulses. For this reason, experiments were conducted on identical fibers F1 and F2 using the experimental device represented in document 1.



Document 1

Experiment 1: Effective stimulation is carried out on the F1 nerve fiber and recordings in two different situations.

- First situation: Under normal physiological conditions.
- Second situation: in the presence of a scorpionic toxin added to the physiological fluid.

Document 2 shows the recordings obtained.

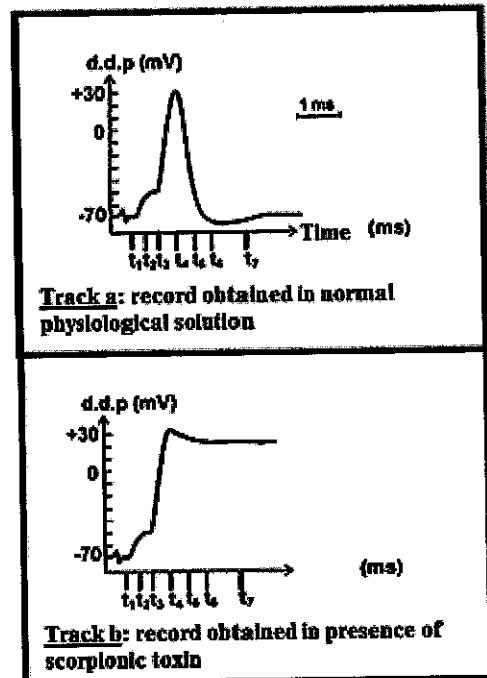
- Formulate two hypotheses that explains the obtained results.

Experiment 2: The nerve fiber F2 is placed in the physiological solution that is stimulated efficiently, the ionic channels A and B opening per unit surface area of the nerve fiber membrane is recorded. The results are represented in document 3.

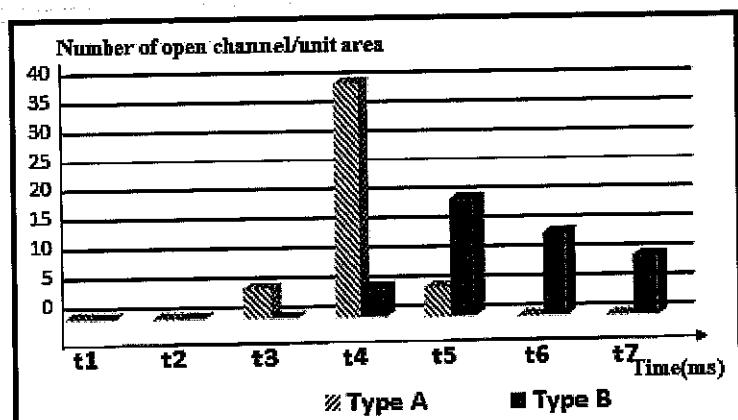
- Tabulate the obtained results.
- Identify each of the two types of channels.

Experiment 3: Radioactive scorpionic toxin that is detected on channels level only, is added to physiological solution in the presence of nerve fiber F2. Effective stimulation is carried on F2 and the number of type A channels opened is counted. The results are presented in document 4.

- Determine, based on document 3 and 4, the mode of action of the scorpion toxin.
- Explain how the scorpion paralyzes its preys.



Document 2



Document 3

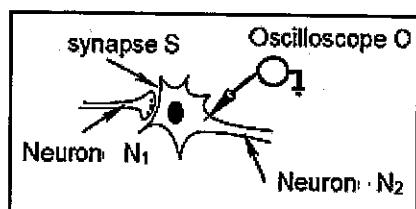
Time(ms)	t1	t2	t3	t4	t5	t6	t7
Number of type A channels opened/unit area	0	0	5	38	37	36	36

Document 4

Exercise 2 Aspects of Synaptic Transmission

It is proposed to study the mechanism of the transmission of the nervous message at the level of a synapse S shown in document 1.

Microscopic observations are made at the synapse S, figures A and B in document 2 show its structural state in two different situations.



Document 1

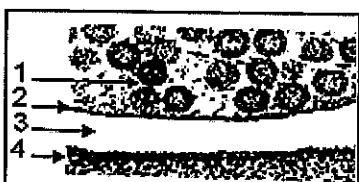


Figure A



Figure B

Document 2

1. Specify the state of the synapse S shown by each of the figures A and B. justify your answer.
2. Indicate the conditions necessary for the transition from state A to state B.

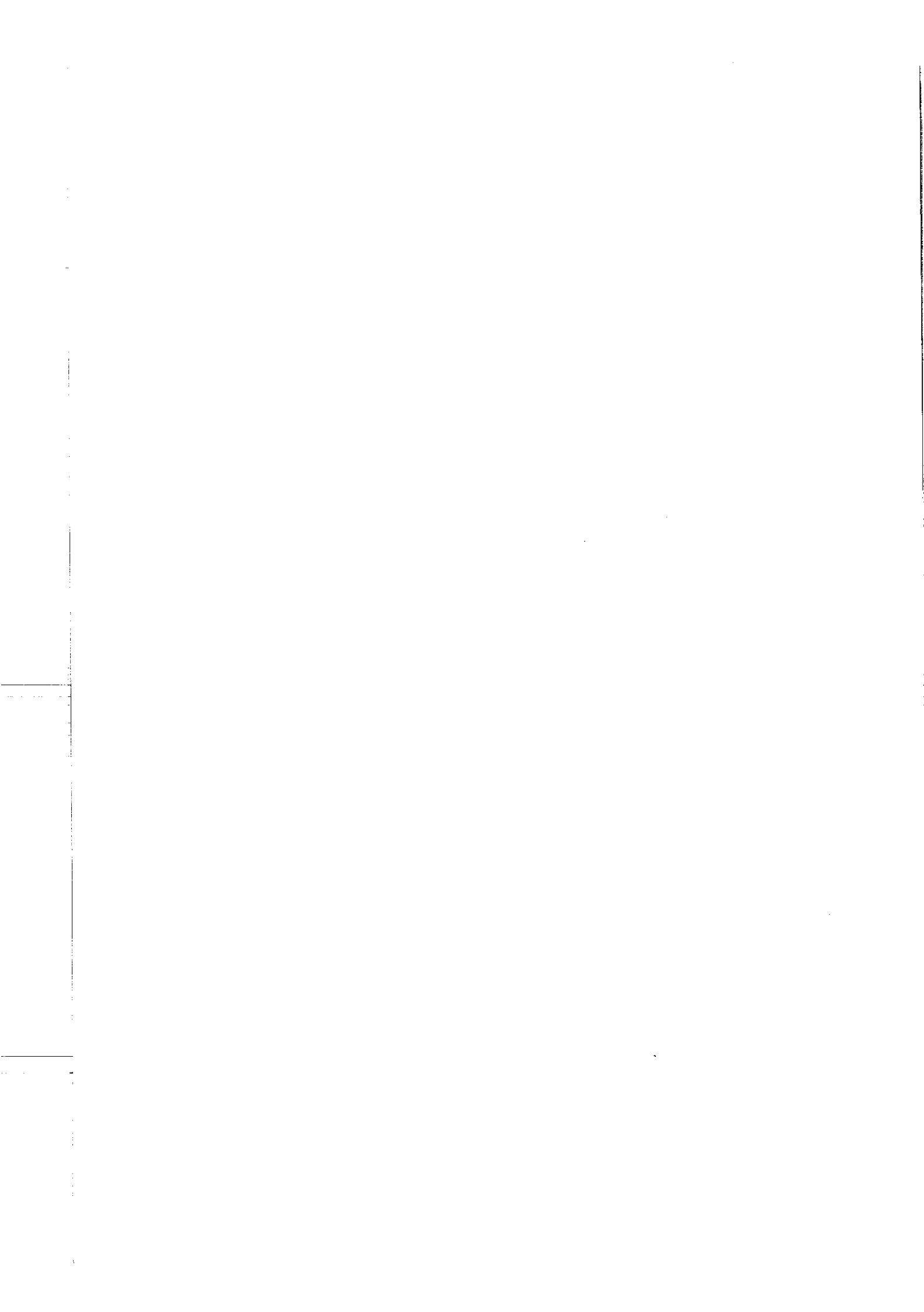
The experimental device in document 1 is used to carry out the following experiments:

Experience 1: The injection of calcium ions (Ca^{++}) into the synaptic knob shows the structural state of Figure B in document 2 and gives rise to excitatory postsynaptic potential (EPSP) at the level of the oscilloscope O.

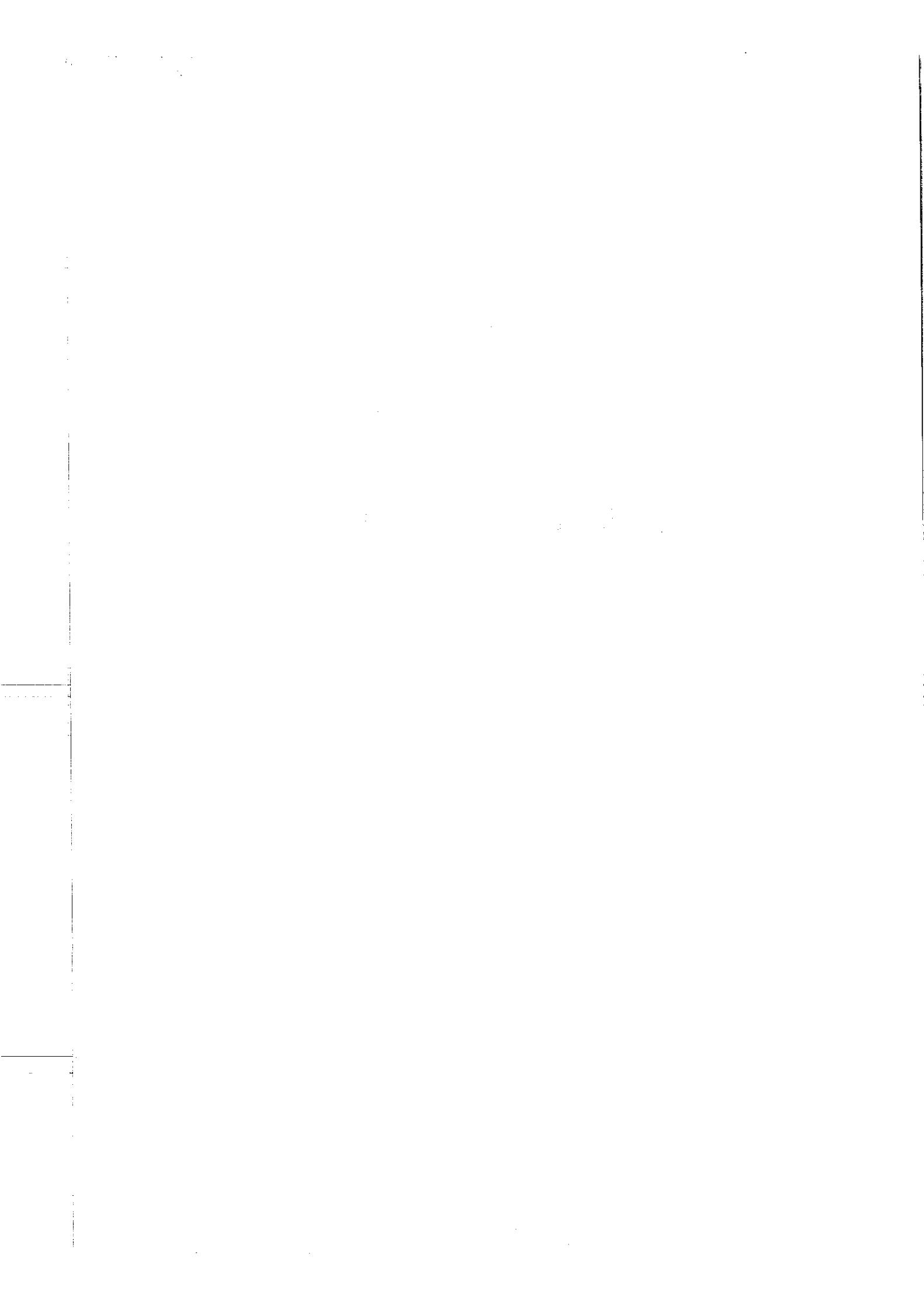
Experience 2: Direct injection of a neurotransmitter (acetylcholine) into element 3 of figure A, shows the structural state of figure A and gives rise to EPSP at the level of the oscilloscope O.

Experience 3: Experiment 2 is repeated, but the Na^+ and K^+ chemical dependent channels are blocked at the postsynaptic membrane, this shows the structural state of figure A and the absence of EPSP at the level of the oscilloscope O.

3. Interpret the results of the experiments 1, 2 and 3.
4. Schematize, based on the above and your knowledge, the successive stages of the mechanism of synaptic transmission.



Ch. 6 Myotatic reflex



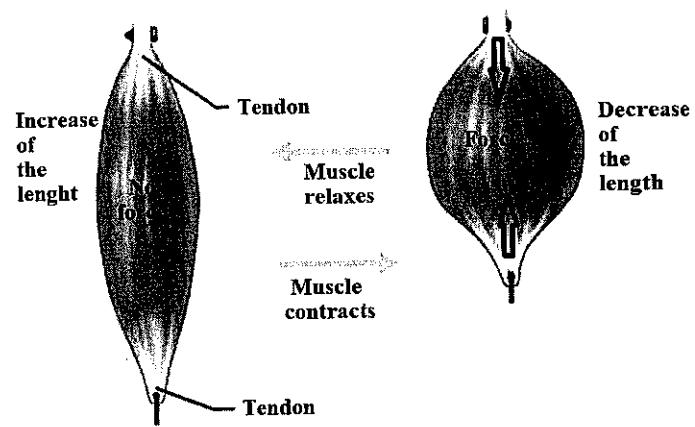
Myotatic reflex

Course abstract

1. The muscle

The muscles are responsible for all the movements done by the body. Those that are responsible for the movements of the bones are called skeletal muscles. Each one of them is connected from its two extremities to two bones by tendons.

The muscles are able to exert forces on the bones by an activity called contraction. The contraction leads to the decrease of the length of the muscle. In the absence of a contraction, the muscle is said to be relaxed and it does not exert any force.

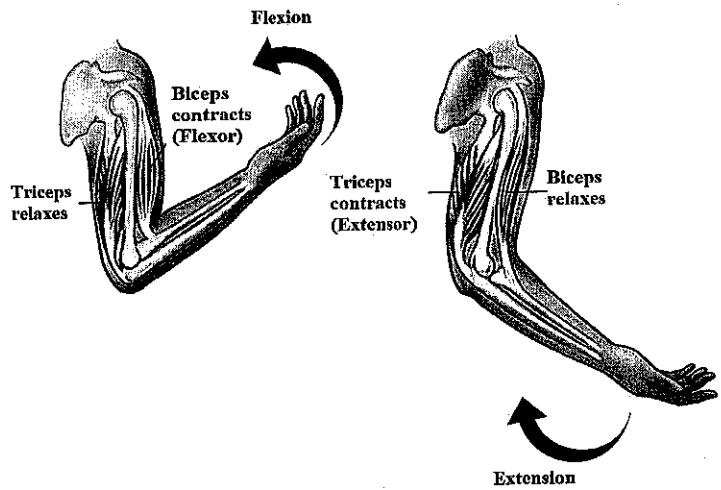


2. Roles of the muscles

When a muscle contracts, it can move one of the two bones related to it, if this movement is an extension of a part of the body, this muscle is said to be extensor, in this case the opposite muscle is relaxed to allow the extension to be done.

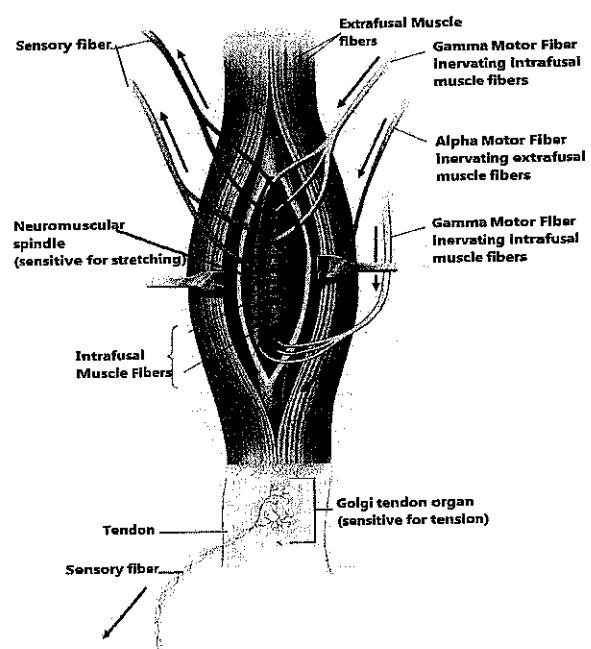
Oppositely, if the done movement is a flexion of a part of the body, the muscle is said to be flexor, in this case the opposite muscle is relaxed to allow the flexion to be done.

Flexor and extensor muscles are said to be antagonistic.



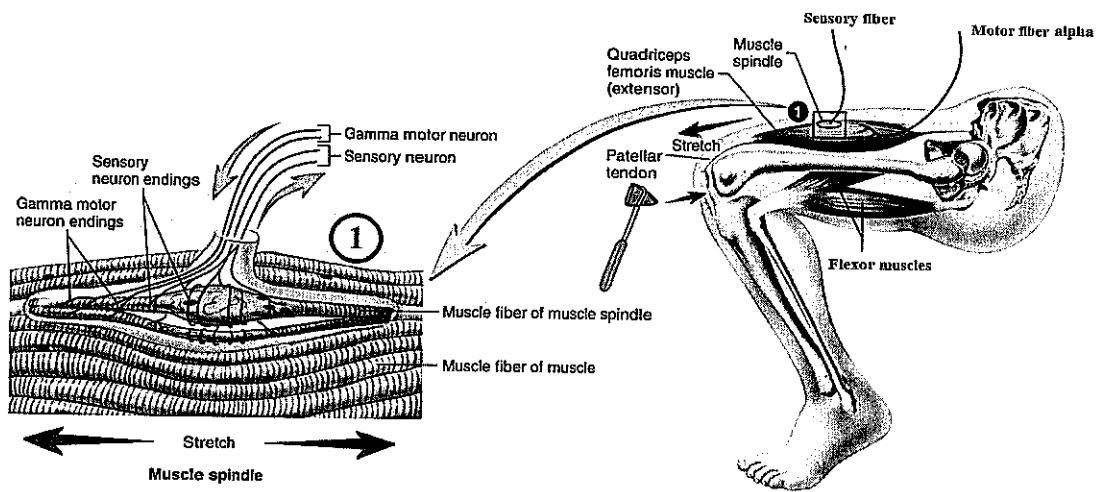
3. Skeletal muscle anatomy

The adjacent figure shows the different structures included in a skeletal muscle.



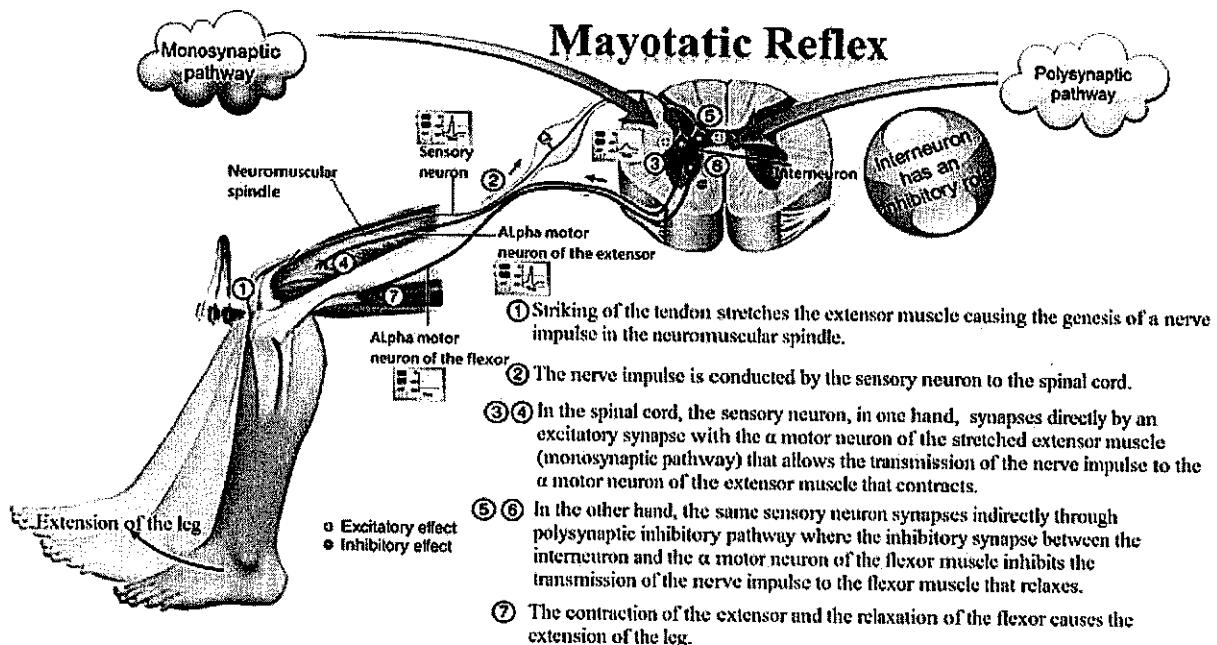
4. The stretching of a muscle

A muscle can be stretched by exerting, on one or both of its extremities, a force of traction that tends to increase its length. This stretching can be done by hitting its tendon by a hammer. The receptor of stretching that will be stimulated in this case is a structure of the muscle called muscle spindle.



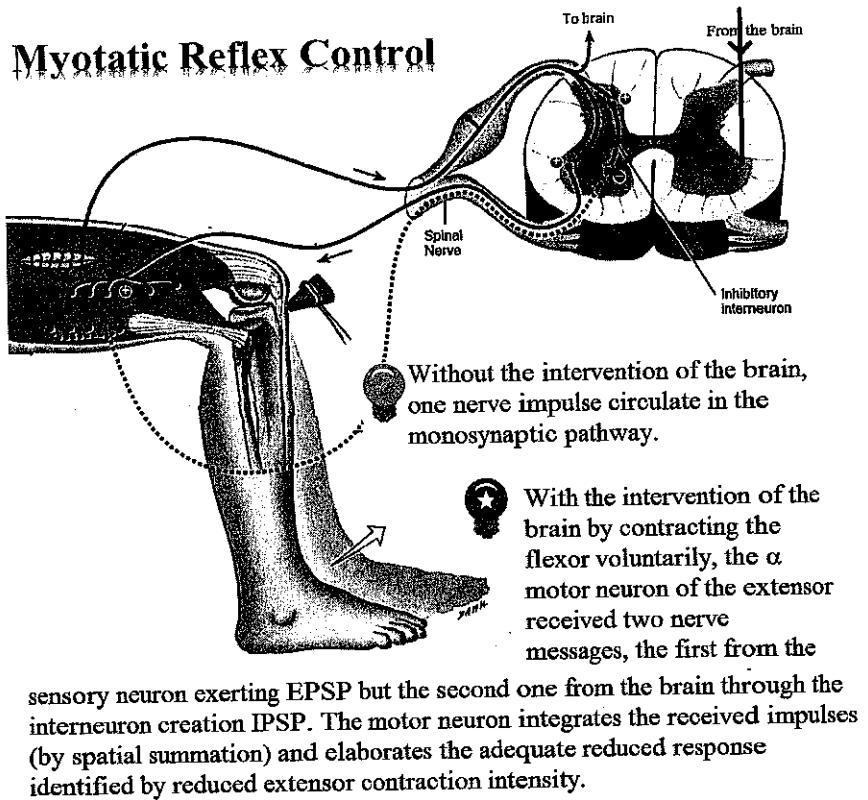
5. Myotatic Reflex

Myotatic reflex is the contraction of the muscle in response to its own stretching where the muscle is the sensory and effector organ.



Myotatic reflex

- Monosynaptic pathway triggers the contraction of the stretched muscle, but the polysynaptic pathway triggers the inhibition of its antagonistic muscle. The interneuron inhibits the transmission of the nerve impulse.
- Neuromuscular spindles in muscles are sensory for stretching while Golgi tendon organs are sensitive for tension.
- Myotatic reflex can be controlled and even inhibited by the intervention of the brain voluntarily where the motor neurons play the integration role.

Myotatic Reflex Control

Myotatic reflex

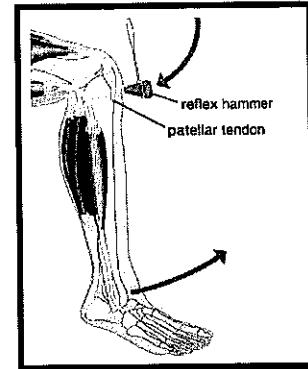
Training exercises

EXERCISE 1 Elements of the myotatic reflex

The myotatic reflex is the contraction of a muscle due to its own stretching.

A simple clinical test can be performed in order to verify the state of the myotatic reflex (adjacent figure). This test consists on hitting gently the tendon of the extensor muscle of the leg or the foot by a hammer then observing the amplitude of the performed movement.

1. Determine the movement that will be done after hitting the tendon of the extensor muscle of the leg.



Some clinical observations are made about this reflex:

- Any lesion of the nerve innervating a muscle will suppress the involuntary and the voluntary extensions performed by it.
- The paralysis of the extrafusal fibers of a skeletal muscle leads to the disappearance of the reflex.
- Any traumatism of the spinal cord at the level of the connection of the nerve corresponding for a muscle will suppress the reflexes made by it.
- The degeneration of a structure found within the muscle called neuromuscular spindle leads to the disappearance of the reflex while the voluntary movements remain possible.

2. Draw out, starting from the clinical observations, the elements involved in a myotatic reflex.
3. Indicate the role of the neuromuscular spindle and that of the spinal nerve in this reflex.

A person having disorders in myotatic reflexes due to degenerations in the neuromuscular spindles will show unbalance and problems in walking as well as a modification in the body posture.

4. What can you deduce concerning the importance of the myotatic reflex?

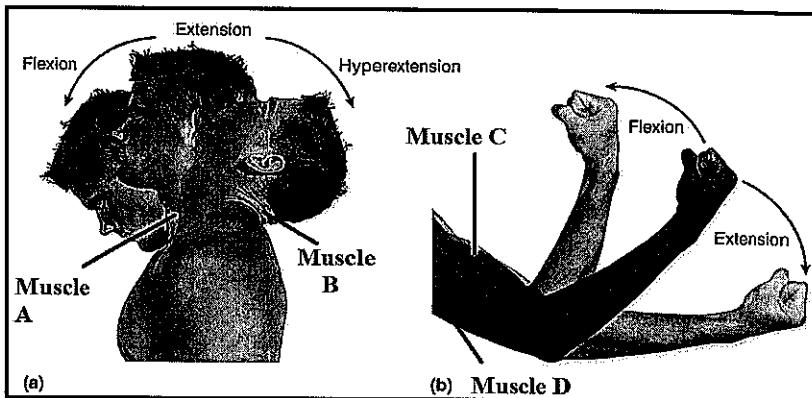
Solution:

1. After hitting the tendon of the extensor muscle of the leg or the foot, this muscle will contract, and since the contraction of the extensor muscle is responsible for the extension then hitting the tendon of this muscle leads to an extension of the foot.
2. The elements involved in the myotatic reflex are:
 - The nerve innervating the muscle.
 - The extrafusal muscle fibers of the muscle involved in the reflex.
 - Region of the spinal cord at the level of the connection of the nerve innervating a muscle.
 - The neuromuscular spindle.
3. The neuromuscular spindle is the receptor for stretching of the muscle. The spinal nerve conducts the sensory and the motor messages corresponding for the reflex.
4. The degenerations in the neuromuscular spindles shows unbalance, problems in walking and modification in the body posture, we can deduce that the myotatic reflex is responsible for balancing including those involved in walking, and for maintaining the body posture.

EXERCISE 2 Roles of the muscles

Document 1 shows two voluntary movements made by each of the head (a) and the forearm (b).

1. Indicate in the body, the location of each of the muscles A, B, C and D.
2. Name the part of the body that is moved by the activity of each of these muscles.
- 3.1. Label the muscles A, B, C and D as extensor or flexor. Justify your answer.
- 3.2. How can we qualify the two muscles A and B relative to each other? Justify.



Document 1

Gravity tends to pull the head down. Myotatic reflex corrects always the position of the head.

2. List in order the elements used by the body to correct the pulling down of the head.

After forward acceleration in a car, head is submitted to a forced hyperextension. During less than one second this hypertension is corrected toward the normal position of the head by the activity of the muscles and the nervous system.

3. Explain the activities of the muscles and the nervous system during the correction of the position of the head after forward acceleration.

Solution:

1. Muscle A is located in the anterior side of the neck, muscle B in the posterior side of the neck. Muscle C is located in the anterior side of the arm, muscle D in the posterior side of the arm.
2. Muscles A and B lead to the movement of the head. Muscles C and D lead to the movement of the forearm.
- 3.1. Muscles A and C are flexor muscles since the contraction of each of them leads to a flexion of a given part of the body. Muscles B and D are extensor muscles since the contraction of each of them leads to a flexion of a given part of the body.
- 3.2. A and B are antagonist muscles. Since they have opposite roles, A is a flexor muscle while B is an extensor muscle.
4. Neuromuscular spindle of the muscles of the neck, extensors of the head, sensory fibers of the spinal nerve corresponding for these muscles, spinal cord, motor fibers of the spinal nerve corresponding for the same muscles, extrafusal muscle fibers of the extensor muscles of the head.
5. A forward acceleration of the body leads to a hyperextension of the head that stretches the muscles of the neck, flexors of the head, this stretching leads to a sensory message that propagates towards the spinal cord by sensory nerve fibers, where it is transmitted by excitatory synapses to the motor neurons of the same muscles. These latter, send motor messages through the motor nerve fibers to the muscles at the origin of the sensory messages. Then the contraction of the flexor muscles of the head leads to the flexion of the head that correct the hyperextension caused by the forward acceleration.

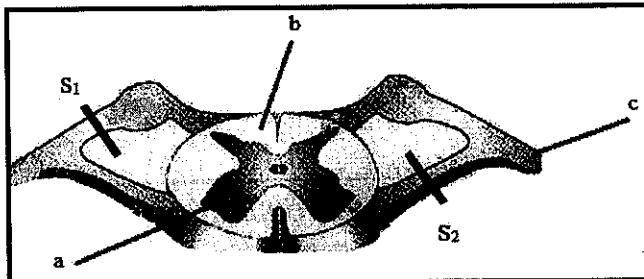
EXERCISE 3 Role of the spinal nerve and its roots

On the roots of the spinal cord of a dog, two sections S1 and S2 are made as shown in document 1.

1. Label document 1.
2. Name the root on which each of the two sections S1 and S2 are made.

The results, on the state of the innervated organs, of the two sections realized on this part of the spinal cord are represented in the table below:

3. Interpret the results of these experiments.
4. Indicate the state of the animal after the section of the nerve indicated by the letter c.
5. Specify the result of the stimulation made on each of the central and peripheral end of the dorsal root after the section S1 is made.



Document 1

Experiment	Section	Results
1	S3	Loss of the sensation Normal motricity
2	S2	Loss of the motricity Normal sensation

Document 2

After a car accident, a man loses its sensibility and motricity in the inferior members of its body.

6. Formulate two hypotheses to explain the state of this man.

Solution:

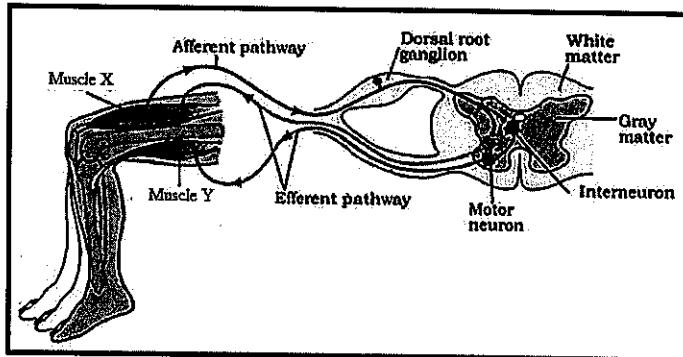
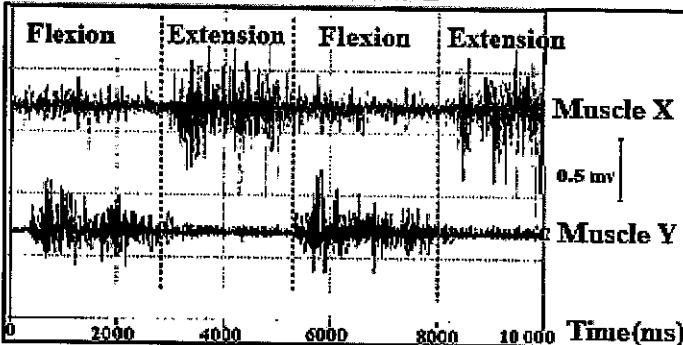
1. a: gray matter, b: white matter, c: spinal nerve.
2. The section S1 is realized on the dorsal root and the section S2 is realized on the ventral root.
3. The section of the dorsal root leads to the loss of the sensations and to a normal motricity, in contrary the section of the ventral root leads to the loss of the motricity and to normal sensations. This means that the dorsal root is sensory and the ventral root is motor.
4. Loss of the motricity and of the sensibility.
5. The stimulation of the central end of the dorsal root leads to pain sensations and to the contraction of the corresponding muscles; the stimulation of the peripheral end does not lead to any reaction, since in the dorsal root the direction of the nervous message is centripetal from the periphery to the nerve center. Then, the message created by the stimulation of the peripheral end is not able to complete its pathway toward the center while the one created in the central end can do it.
6. Hypotheses: The two roots of the spinal nerve are sectioned.
The spinal nerve is sectioned.

EXERCISE 4: Neuronal circuit of myotatic reflex

A person, sitting on a chair, is asked to perform alternating flexions and extensions. During this activity the electrical signals of two muscles X and Y of the thigh are recorded. The recordings are shown in Document 2.

1. Specify for each of the two muscles X and Y if it is an extensor or a flexor muscle.
2. Justify that the two muscles X and Y are qualified as antagonistic.

In absence of any voluntary action; hitting the tendon of muscle X leads to an extension of the leg; the electric activities of the two muscles X and Y are similar to those obtained during the voluntary extension (document 2).

**Document 1****Document 2****Solution:**

1. During flexion that lasts for 2500 ms, muscle X shows an activity of maximal amplitude of 2 mV, while muscle Y shows higher activity of maximal amplitude reaching 4 mV, on the contrary, during extension that lasts for 2500 ms, muscle X shows an activity of 4 mV of maximal amplitude while muscle Y remains at rest, so muscle X contracts during extension while muscle Y contracts during flexion, therefore, muscle X is an extensor muscle while muscle Y is a flexor muscle.
2. The two muscles X and Y are qualified as antagonistic since when one of them contracts the other relaxes.
3. The stretching of muscle X by hitting its tendon leads to a sensory message that propagates towards the spinal cord by sensory nerve fibers, where it is transmitted by an excitatory synapse to the motor neuron of the same muscle and through an inhibitory neuron to the motor neuron of muscle Y. This will lead to a motor message directed toward muscle X leading to its contraction and to the inhibition of the messages directed toward muscle Y leading to its relaxation. Thus, spinal cord coordinates the activity of the two muscles X and Y.
4. We conclude that superior nerve centers inhibit the myotatic reflex of a muscle.

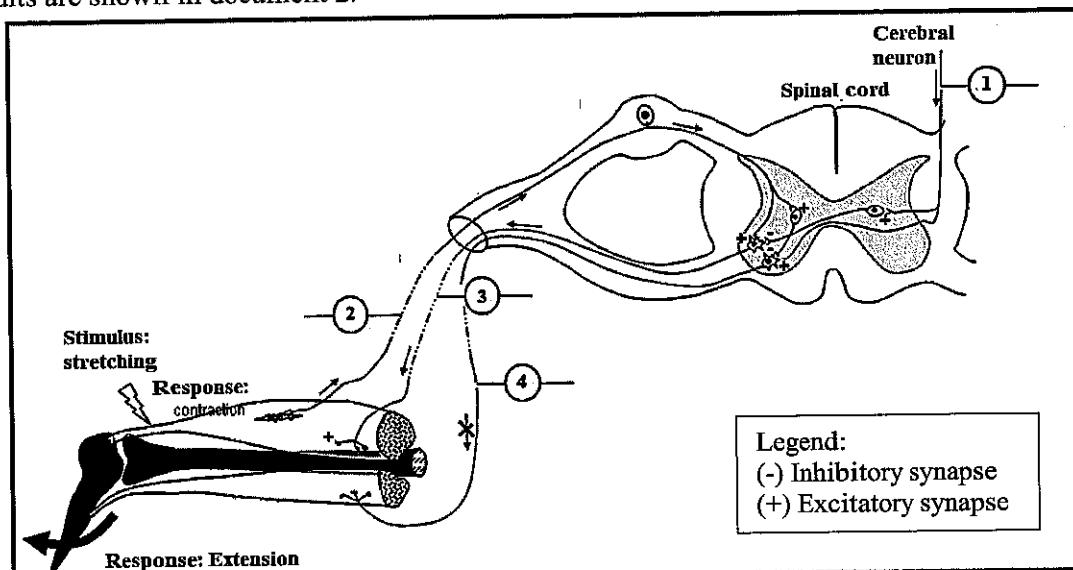


Myotatic reflex solved exercises

EXERCISE 1: Control of the myotatic reflex

In an Achillean reflex, hitting the Achillean tendon leads to the contraction of the extensor muscle and to the relaxation of the flexor muscle of the foot, this leads to a movement observed at the level of the foot. In order to study the role of the spinal cord in this movement, we perform the following experiments and recordings.

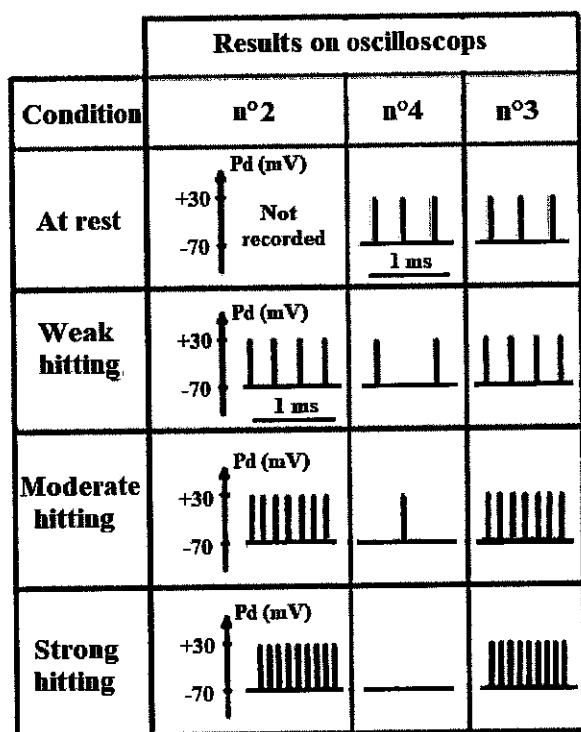
We stimulate the Achillean tendon by applying a series of hits of increasing intensities; at the same time, we record the obtained electric activities by the oscilloscopes n°2, n°3 and n°4; the obtained results are shown in document 2.

**Document 1**

1. Specify the movement of the foot mentioned in the above text.
2. Explain the presence of nervous messages on the oscilloscopes without any stimulation and without any voluntary contraction.
3. Interpret the obtained results.
4. Explain the role of the spinal cord in the variation of the intensity of contractions of the extensor and the flexor muscles following hitting.

The nervous motor fiber issued from the encephalon is responsible for the contraction of the flexor muscle.

5. Explain the effect of the voluntary contraction of the flexor muscle on the frequencies of AP obtained by the oscilloscopes 3 and 4 in the case of moderate hitting. Justify the answer.

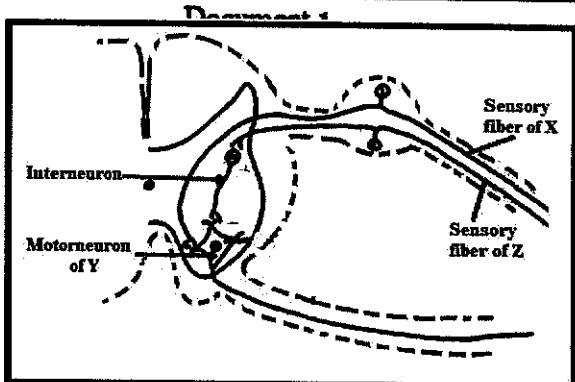
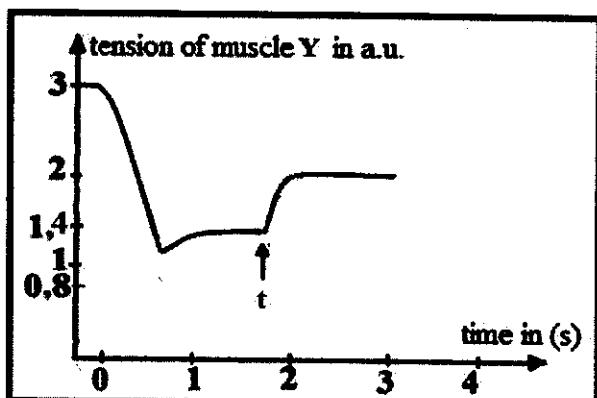
**Document 2**

EXERCISE 2 An inhibition of the myotatic reflex

In order to study the role of the spinal cord in the control of the myotatic reflex; we perform the following experiment on three muscles X, Y and Z of a man's thigh, the muscle Y was contracted at the beginning of the experiment.

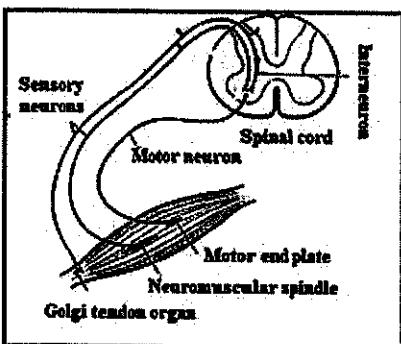
At $t = 0$ we stretch weakly the muscle Z of the thigh and we keep it stretched till the end of the experiment, and then at time t we stretch muscle X. Document 1 shows the results of the recording of the tensions obtained at the level of the muscle Y. The weak stretching made on the muscle Z leads to an extension of the foot. The stretching made at time t leads to the contraction of the muscle X.

1. Justify that the above given proves the presence of a myotatic reflex.
2. Determine the role of the muscle Y.
3. Explain, by referring to document 2, the role of the motor neuron of muscle Y in the variations shown in document 1.

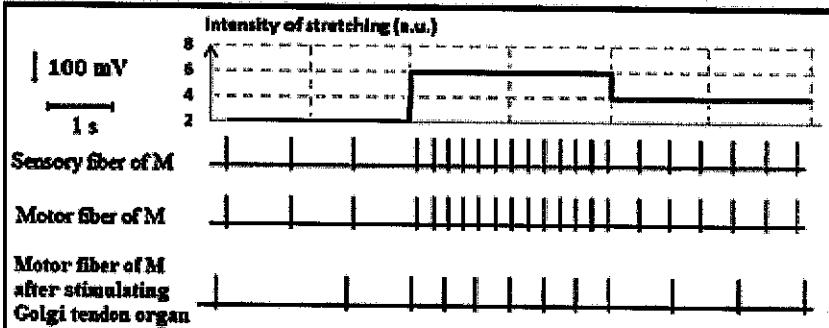


Document 2

Golgi tendon organ (Document 3) is a sensory receptor for tension; it is located in the tendon of the muscle. A stimulation of Golgi tendon organ of a muscle M is done at the same time of stretching muscle M by the intensities shown with the corresponding results in document 4.



Document 3



Document 4

4. What can you deduce starting from the results in document 4?

Very high intensities of contraction done by a given muscle or very high tensions exerted on it, may lead to lesions at the level of the muscle tissue.

5. Explain the role of Golgi tendon organ in the protection of the muscles from lesions under very high tensions.

EXERCISE 3 Sensation and nervous integration

Sensation begins in the receptors that are connected to afferent fibers transmitting nervous messages to the corresponding nerve centers. In some cases, sensory messages pass through several nervous centers where they undergo successive treatments before being sent to other centers or to the effector organs.

We want to study how is the nervous message generated in a receptor. Using the device in document 1, the potential differences are recorded in response to the application of a pressure of increasing intensity on a pressure receptor, these recordings were made at the level of the receptor and the afferent fiber.

- What can draw out from the results in document 1?

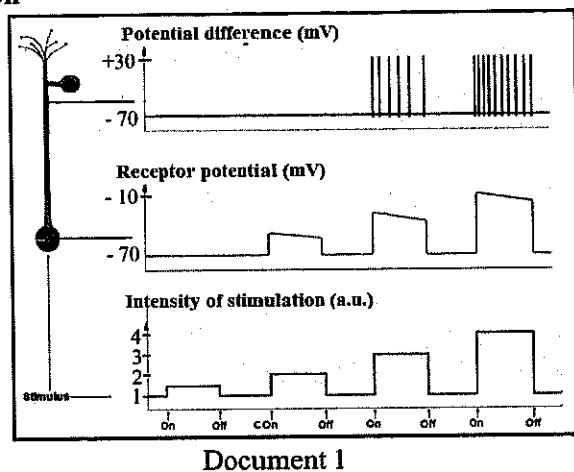
At a high frequency of AP at the level of the sensory fiber, the message will be considered as painful, it results in a protective reflex. The response of this reflex is shown in document 2.

- Determine, out of the extensor and the flexor muscles of the foot, the muscle that contracts and the muscle that relaxes during the response of the reflex shown in document 2.

Document 3 shows the experimental device used to study the role of the spinal cord in a myotatic reflex with or without a painful message.

On this device, three separate experiments are carried out. In the first experiment, the muscle M is stretched without any painful message. During the second and the third experiment, the muscle M is stretched by a stretching of the same intensity as that of the first experiment and at the same time subjected to an application of a strong pressure leading to foot pain. This experiment is done with or without benzodiazepine that act on some synapses involved in the reflex.

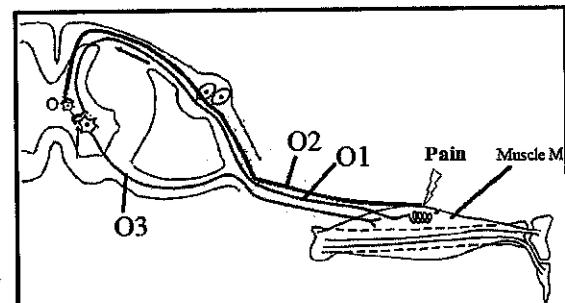
The nervous messages are recorded during these three experiments and shown by the document 4.



Document 1



Document 2



Document 3

Experiment	Experimental intervention	Frequency of AP / s		
		O1	O2	O3
1	Stretching of muscle M without pain message	5	0	5
2	Stretching of muscle M with pain message	5	3	2
3	Stretching of muscle M with pain message and applying benzodiazepine	5	3	0

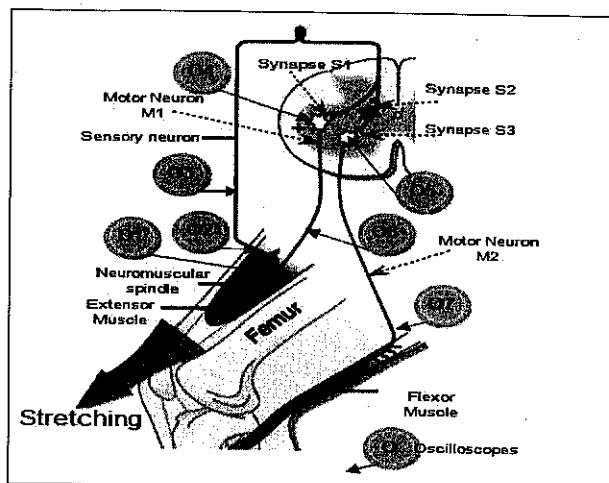
Document 4

- Interpret the results obtained in the first and second experiment.
- Explain the role of the spinal cord in the difference of the frequency of the nervous message at the level of the oscilloscope between the first and the second experiment.
- Specify the movement of the foot in the second experiment compared to the first experiment. Benzodiazepine acts at the level of the synapse relating the interneuron to the motoneuron of muscle M.
- Specify the action of the benzodiazepine at the level of this synapse.
- Formulate a hypothesis explaining the mode of action of benzodiazepine at the level of this synapse.

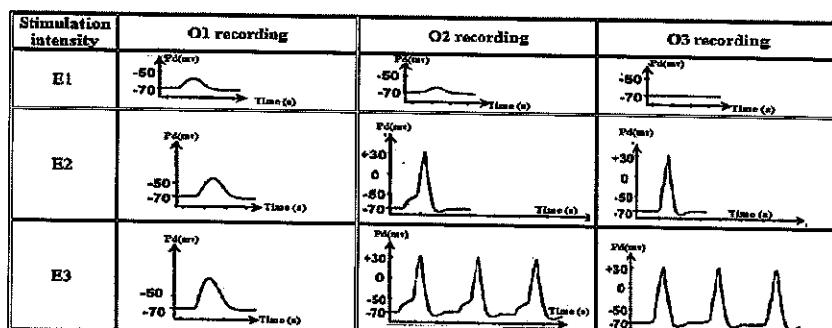
EXERCISE 4 Nervous Pathways during Myotatic Reflex

Some aspects of myotatic reflex are studied during stretching the extensor quadriceps femoral muscle as shown in the experimental setup in document 1.

Document 2 represents the experimental results upon applying stretching with increasing intensities E1, E2 and E3 on the neuromuscular spindle of the extensor muscle.

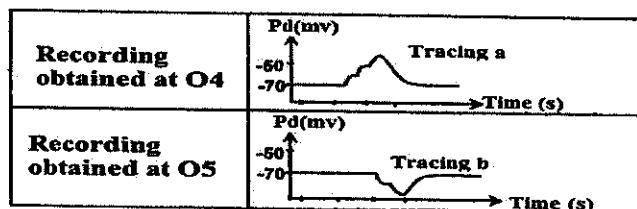
**Document 1**

1. Specify the characteristics of the receptor potential and the action potential.
2. Determine how the nerve message is coded in the receptor and along the nerve fibers.

**Document 2**

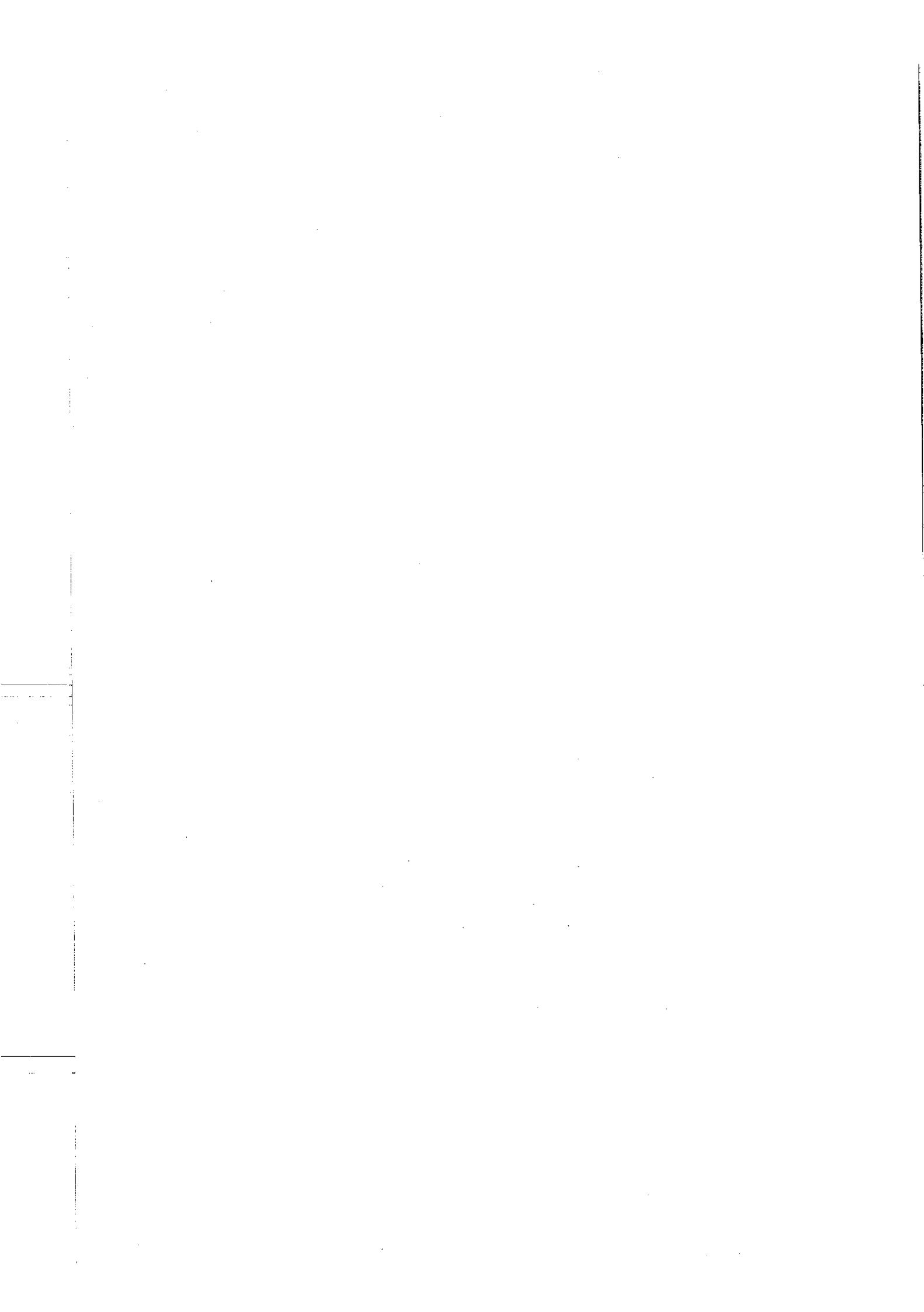
Document 3 shows the electrical activity recorded at Oscilloscope O4 and O5 after stretching the neuromuscular spindle by intensity E3.

3. Identify the nature of the synapse S1.
4. Explain the mechanism behind tracing b.
5. Draw the recordings obtained at O6 and O7 and the state of M1 and M2 muscles in case of applying E3.

**Document 3**

Upon stretching the neuromuscular spindle with intensity E3, the nerve fiber, originated from the brain, indirectly connected to M1 and causing the contraction of the flexor muscle, was efficiently stimulated. The extensor muscle did not contract.

6. Explain the obtained results.



Myotatic reflex

Solved exercises solutions

Exercise 1 Control of the myotatic reflex

1. The movement observed at the level of the foot is an extension, since the extensor muscle of the foot contracts and its contraction is responsible for the extension.
2. At rest, the muscles are in a state of slight permanent contraction, they are not totally relaxed. This tension, called muscular tonicity, is maintained by motor nerve messages directed to the muscle, these motor messages are observed on the oscilloscopes 3 and 4.
3. At rest, motor fibers of the extensor and flexor muscles show each a frequency of 3 AP/s. This means that at rest, motor messages are directed to the muscles.
After weak hitting, a frequency of 4 AP/s is recorded in the sensory fiber of the extensor muscle, the frequency of AP in the motor neuron of this muscle increases to 4 AP/s, on the contrary, it decreases in the motor neuron of the flexor muscle to 2 AP/s. with the increase of the intensity of hitting from weak to strong, the frequency of AP increases in the sensory fiber to 9 AP/s as well as in the motor neuron of the same muscle, on the contrary it decreases to be null in the motor neuron of the flexor muscle. This means that stretching the muscles, amplifies the frequency in the sensory and in the motor fiber of it, and attenuates till it blocks the frequency of AP in its antagonistic.
4. Hitting leads to the stretching of the neuromuscular spindle of the extensor muscle, which leads to an increasing frequency of AP with the increase of the intensity of stretching caused by hitting, this frequency is conducted to the spinal cord where it is transmitted to the motor neuron of the same muscle by an excitatory synapse (EPSP), leading to an increase of the frequency of AP in the motor neuron of the extensor muscle that increases its contraction intensity; in the same time, the sensory message is transmitted to the motor neuron of the flexor through an inhibitory neuron (IPSP), this will inhibits the motor message of the flexor muscle leading to attenuation of its intensity of contraction till it is totally relaxed. Thus the spinal cord coordinates the activity of the two antagonist muscles by contracting the stretched muscle and relaxing its antagonist.
5. The voluntary contraction of the flexor muscle leads to the increase of the frequency of AP on oscilloscope 4 and its decrease on oscilloscope 3, since the motor fiber corresponding for the flexor muscle is connected to the motor neuron of the flexor muscle by an excitatory synapse, the spatial summation of the EPSP of this synapse with the IPSP of the synapse transmitting the messages originating from the sensory fiber of the extensor leads to the increase of the frequency of AP in the motor fiber of the flexor. On the contrary, the voluntary motor neuron of the flexor is connected with the motor neuron of the extensor by an inhibitory synapse; the spatial summation of the IPSP generated by it with the EPSP generated by the sensory fiber of the extensor leads to the decrease of the frequency of the AP in the motor neuron of the extensor muscle.

Exercise 2 An inhibition of the myotatic reflex

1. The given proves the presence of a myotatic reflex since stretching the muscle X at time t leads to the contraction of the same muscle, thus a contraction of a muscle is obtained following its own stretching.
2. After stretching muscle Z; muscle Y, that is originally contracted, shows a decrease in its tension from 3 to 1.1 a.u. during 0.6 s, thus upon stretching muscle Z, muscle Y relaxes, and since the observed movement in this case, is an extension of the foot then muscle Y is a flexor muscle that relaxes during extension.
3. Stretching muscle Z leads to a decrease of the tension of muscle Y from 3 to 1.1 a.u. since the sensory fiber of muscle Z is connected to the motoneuron of muscle Y by an inhibitory neuron, the generated IPSP by this interneuron is submitted to a spatial temporal summation with the EPSP that caused the contraction of this muscle, this summation leads to the decrease of the frequency of the AP directed toward the muscle, thus muscle Y is partially relaxed and its tension decreases.

After stretching muscle X, the sensory message of it is transmitted to the motor neuron of Y by an excitatory synapse, the spatial temporal summation of the EPSP generated by this synapse with the EPSP that caused the initial contraction of Y with the IPSP generated by the sensory fiber of Z leads to the increase of the frequency of AP in the motor neuron of Y leading to the increase of its intensity of contraction which explains the incease of its tension from 1.4 a.u. to 2 a.u. Thus the motor neuron integrates the various excitatory and inhibitory afferent messages in order to create a spatial temporal efferent message.

4. After stretching muscle M, we observe a frequency of AP in the sensory fiber of M that is equal to the frequency obtained in the motor fiber of it for the different given intensities of stimulation; at 2 a.u. of intensity of stretching 1 AP/s is obtained in both of them, but in the case of the stimulation of Golgi tendon organ it decreases in the motor neuron to less than 1 AP/s, this frequency increases to 4 AP/s in the sensory fiber and the motor fiber with the increase of stretching intensity to 6 a.u., also it increases but to lower value of 2 AP/s in the case of the stimulation of Golgi tendon organ. On the contrary it decreases to 2 AP/s with the decrease of stretching intensity to 4 a.u., similarly it decreases but also to lower value of 1 AP/s when the intensity of stretching decreases to 4 a.u. We can deduce that the activity of Golgi tendon organ inhibits the myotatic reflex.
5. Under very high tensions exerted on the muscles, the muscle will respond by a reflex that leads to the contraction of the muscle, this contraction will increase more the tension exerted on it and may lead to lesions in its tissue. Golgi tendon organ inhibits this reflex and prevents the muscle from remaining under the effect of high tensions thus protecting it from damage.

Exercise 3 Sensation and nervous integration

- 1 From the results in document 1 we can conclude that:
 - The receptor has a threshold at which it starts recording a receptor potential.
 - The sensory fiber has a threshold at which it begins to show AP.
 - The stimulation intensity is coded in modulation of the amplitude of the receptor potential in the receptor and in modulation of frequency at the level of the nerve fiber.
 - 2 The response of the reflex shown in document 2 is a flexion, this is why the muscle responsible for the flexion contracts, it is the flexor muscle, and the antagonist muscle to the flexor muscle relaxes, it is the extensor muscle.
 - 3 Following the stretching of the muscle M without a pain message, the sensory fiber of the muscle M shows a frequency of 5 AP / s which is equal to the frequency obtained in the motor fiber of the same muscle and no AP is observed at the level of the sensory fiber transmitting pain, this shows that the sensory nervous message caused by the stretching of the muscle is excitatory for the motor fiber of the same muscle. On the other hand, following the stretching of the muscle with a pain message, the frequency of AP remains the same equal to 5 PA / s at the level of the sensory fiber originating from the muscle M, and there is a frequency of 2 AP / s At the level of the sensory fiber transmitting the pain but the frequency at the level of the muscle motor fiber is smaller equal to 2 AP / s, this shows that the pain message inhibits the motor message of the muscle M and that the motoneuron integrates the two sensory messages.
 - 4 During the stretching of the muscle M without a pain message, the motoneuron of the muscle M receives from the muscle an excitatory nerve message transmitted by an excitatory synapse, which leads to the same number of AP in the motor fiber. After stretching the muscle M with pain message, the motoneuron of the muscle M receives two messages, one excitatory and the other inhibitory from the nociceptive sensory fiber through an inhibitory interneuron, the spatial summation of these two messages results in the decrease of the AP frequency from 5 to 2 AP / s. Then the spinal cord integrates the two afferent messages in order to modulate the frequency of the efferent message.
 - 5 The movement made by the foot in the second experiment is a less important extension than the stretching alone because the frequency of the motor message of the extensor muscle which results in the contraction of the extensor muscle leads to an extension and when the frequency of this message decreases, the corresponding muscular contraction decreases, which leads to a less important extension.
- 6.1.** The action of the benzodiazepine is amplificatory at the level of this synapse since this synapse is inhibitory and the benzodiazepine inhibits more the obtained nervous message obtained at the level of the motoneuron of the muscle M.
- 6.2.** Hypothesis: the benzodiazepine increases the exocytosis of the neurotransmitters at the level of the synapse relating the interneuron to the motoneuron of the muscle M.

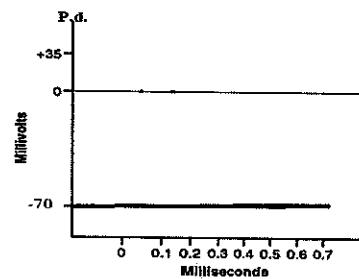
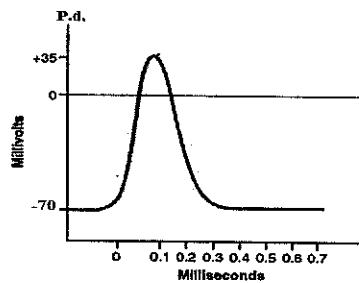
Exercise 4 Nervous Pathways during Myotatic Reflex

1. Since when the intensity of stretching increased, the amplitude of the receptor potential increased recorded in O1 only, then the receptor potential is local depolarization whose amplitude increases with the increase of the intensity of stimulation.

Since the number of action potentials saving the same amplitude increased with the increase of the stimulation intensity, then action potentials propagate along the nerve fiber saving the same amplitude and frequency.

2. The amplitude of the receptor potential increased from 10 to 30 mV when the intensity of stimulation increased from E1 to E3, then the nerve message is coded by the modulation of the receptor potential amplitude. However, the frequency of action potentials increased saving the same amplitude when the intensity of stimulation increased from E2 and E3, then the nerve message is coded by the modulation of frequency of action potentials.
3. Since the arrival of the action potential, recorded by O3, caused hypopolarization in the membrane potential difference of M1 recorded by O4. Thus, synapse S1 is excitatory synapse.
4. The arrival of the nerve impulse by the interneuron causes the release of neurotransmitters, their binding on their receptors present in the postsynaptic membrane leads to the opening of K^+ channels (outward diffusion of K^+ ions) or the opening of Cl^- channels (inward diffusion of Cl^- ions), this makes the membrane potential difference of the motor neuron M2 more electro-negative (hyperpolarization) recorded by O5.

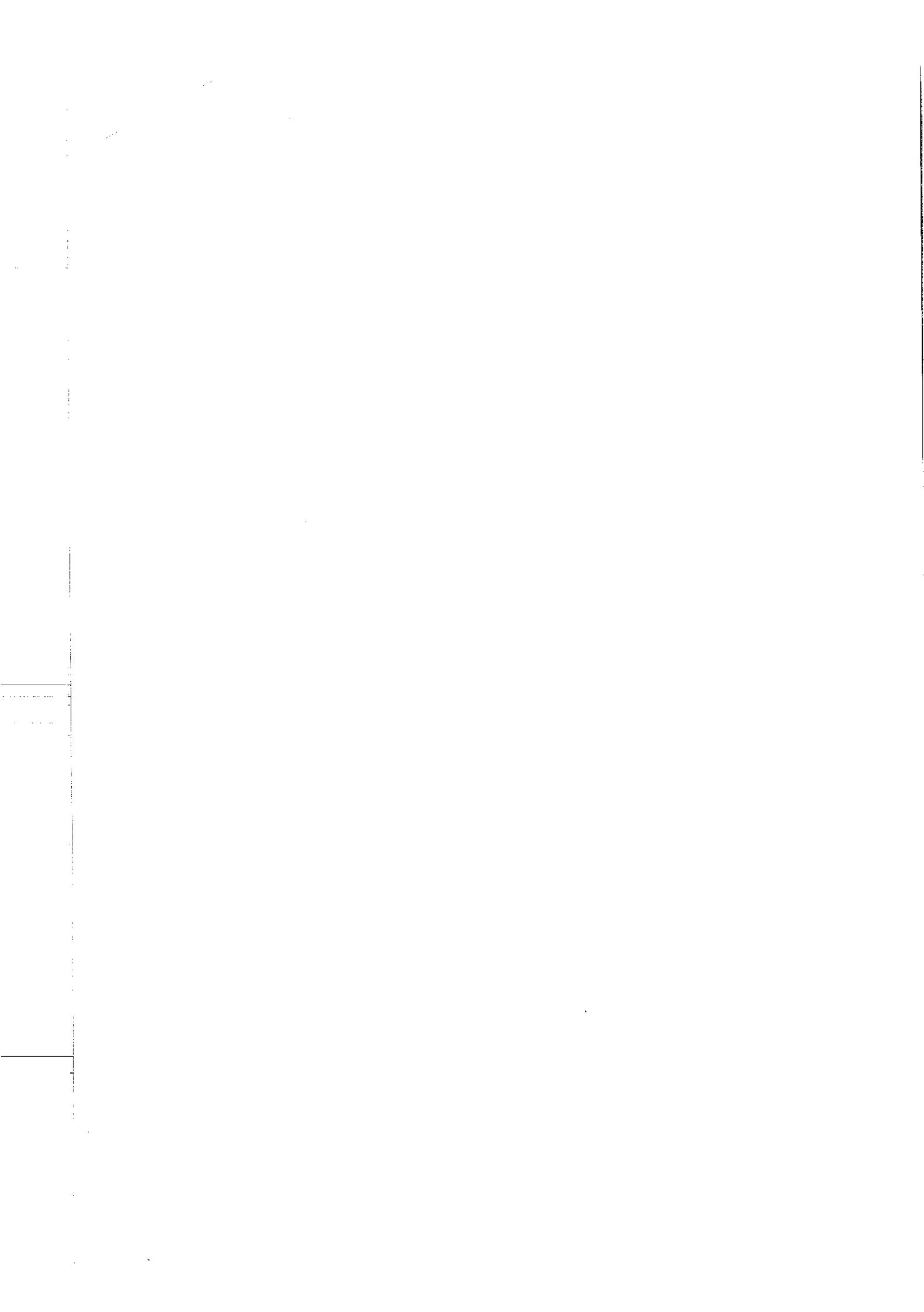
5.



Recording in O6
M1 contracted

Recording in O7
M2 relaxed

6. The motor neuron M1 received impulses from sensory neuron creating EPSP, at the same time received impulses from brain through an interneuron creating IPSP. By spatial summation, M1 membrane potential did not cross the threshold of depolarization where no action potential is generated, then the extensor muscle did not contract.



Myotatic reflex

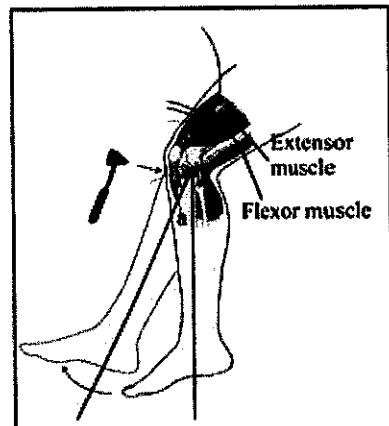
Non-solved exercises

EXERCISE 1 Activity of muscles during myotatic reflex

Hitting the patellar tendon of a sitting person leads to the movement of the leg shown in the document 1. In this document the angle (α) represents the angle between the original position of the leg and the maximal position of it when it extends.

1. Show that the obtained reaction of the leg results from a myotatic reflex.

The measurement of the angle (α), following many stimulations by hitting the patellar tendon with increasing intensities, leads to the results given in the table in document 2. This document shows also the results of the same hitting intensities repeated in the case of the voluntary flexion of the leg.

**Document 1**

Intensity of hitting/N	1	3	4	5	6
Angle (α) in degree in absence of voluntary flexion	0	5	10	15	20
Angle (α) in degree in the presence of voluntary flexion	0	0	0	5	10

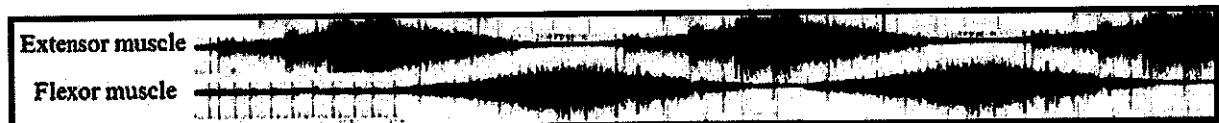
Document 2

2. Represent graphically the obtained results in document 2.
3. Interpret the obtained results in document 2 in the absence of voluntary flexion.
4. What can you deduce starting from the results of the measurements done in the presence of voluntary flexion of the leg?

In order to study the nervous messages at the origin of this reflex; we branch 4 oscilloscopes 1, 2, 3 and 4 respectively on: the sensory fiber of the extensor muscle, the motor fiber of the flexor muscle issued from the brain, the motor fiber in the spinal nerve of the extensor muscle, and the motor fiber in the spinal nerve of the flexor muscle.

5. Schematize the nervous circuit where the 4 oscilloscopes are branched by showing the synaptic connections in the spinal cord of all the given fibers.
6. Explain the role of the spinal cord in the variation of the results of the table in document 2.

We decide for a person to make successive extensions and flexions of his leg. At the same time we record the electric activities of these two muscles. The results are given in the document .

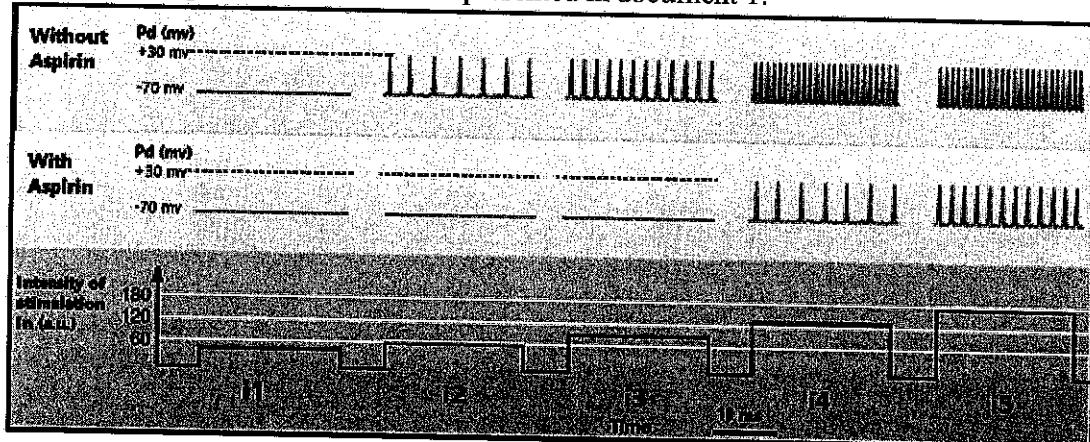
**Document 3**

7. Justify, by referring to document 3, that the extensor and the flexor muscles are antagonistic.

EXERCISE 2 Pain reflex and myotatic reflex

A pain reflex is a reflex caused by an intense pain perceived by a nociceptor. It leads to the flexion of the part of the body on which the stimulation is done.

We apply on a nociceptor located in the leg many stimulations of increasing intensities, and we record the obtained nervous messages at the level of the nociceptive neuron before and after the application of aspirin on the nociceptor. The results are represented in document 1.



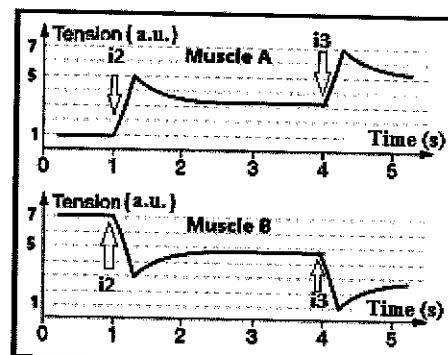
Document 1

1. Interpret the results obtained at the level of the nociceptive neuron in the absence of aspirin.
2. Explain the obtained results after the application of aspirin.

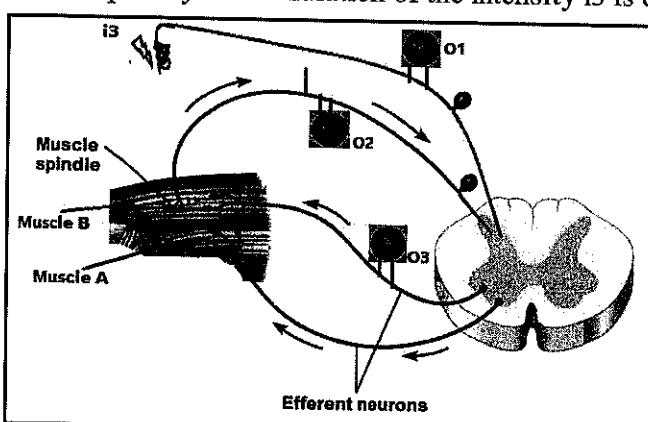
In another experiment, we record, at the same time of the stimulations of intensities i_3 and i_4 done without aspirin, the tension of two muscles A and B of the leg, the results are given by document 2.

3. Identify the roles of the two muscles A and B.

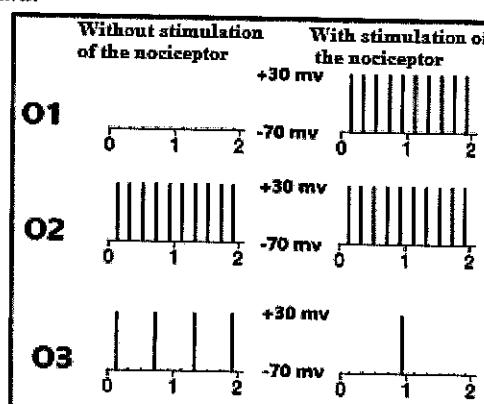
A neuromuscular spindle located in the muscle B is stretched by a moderate stretching with or without the stimulation of the nociceptor by the intensity i_3 . The recordings obtained by the oscilloscopes branched on the nociceptive neuron, the sensory neuron originating from the neuromuscular spindle of muscle B and the motor neuron of the muscle B are given by document 4. The tension of the muscle B after stretching and stimulating the nociceptor by the stimulation of the intensity i_3 is of 3 a.u.



Document 2



Document 3

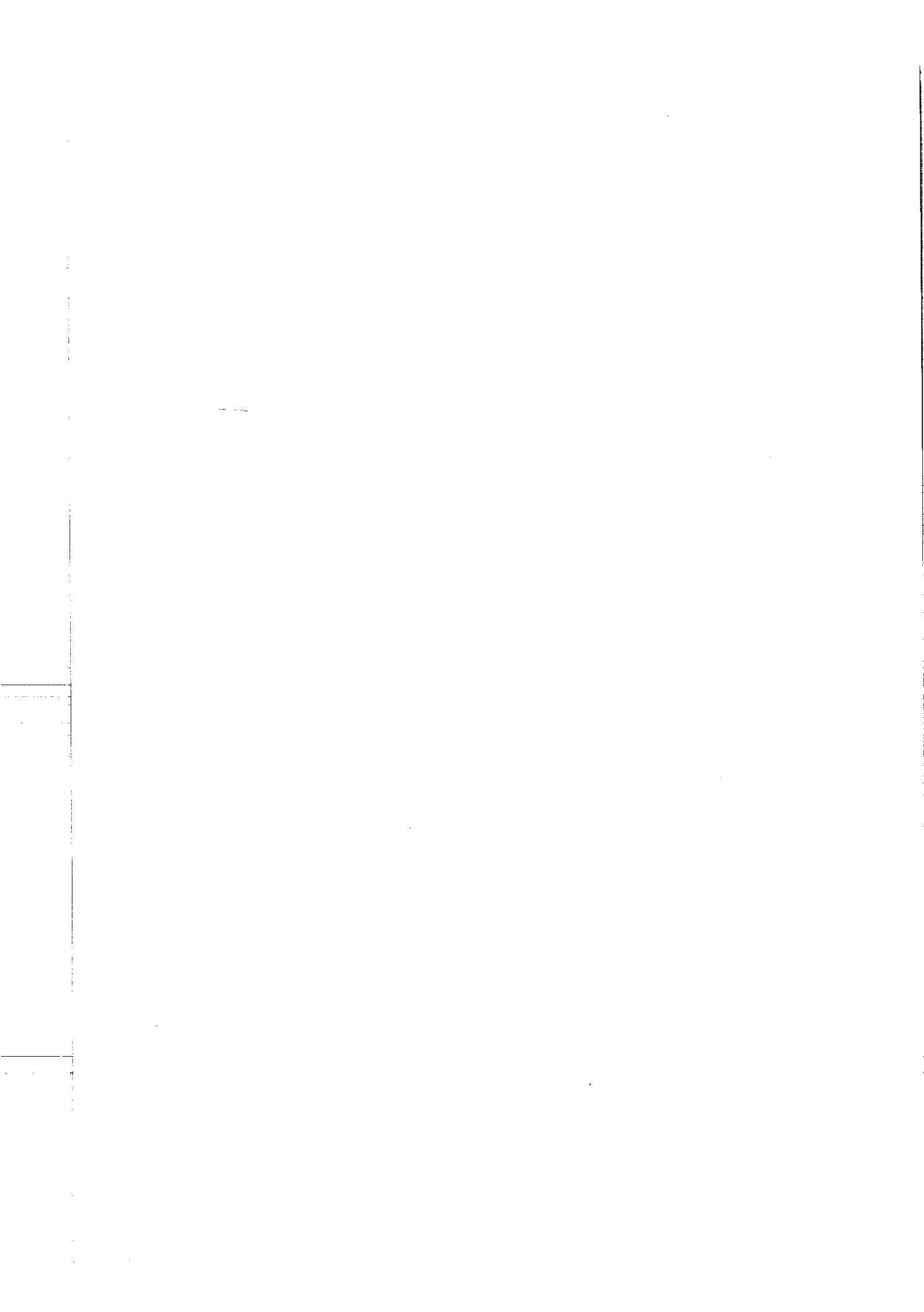


Document 4

4. Justify that the response of the muscle in this case is the result of a myotatic reflex.
5. Explain the role of the spinal cord in the variation of the responses given by document 4.



Ch. 7 Neurotransmitters and medical applications



Neurotransmitters and medical applications

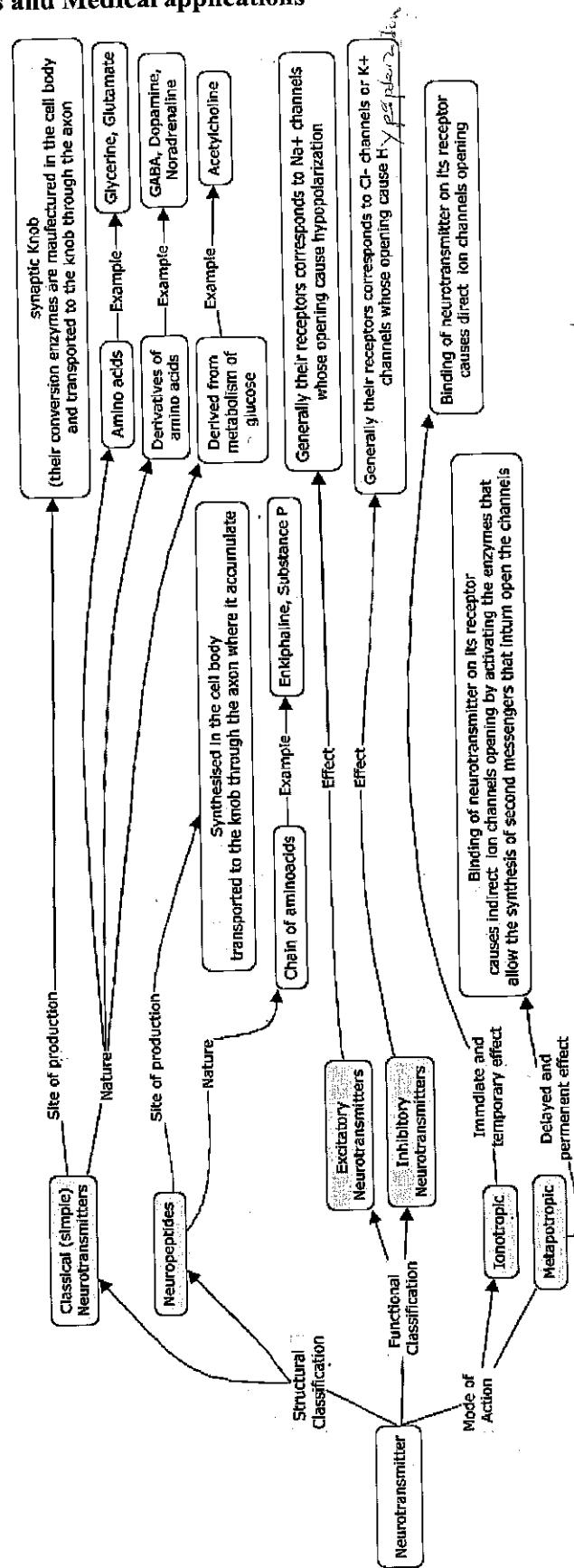
Course abstract

Neurotransmitters and Medical applications

1. Types of neurotransmitters

The adjacent map shows the different types of neurotransmitters according to their:

- Nature.
- Function.
- Mode of action.



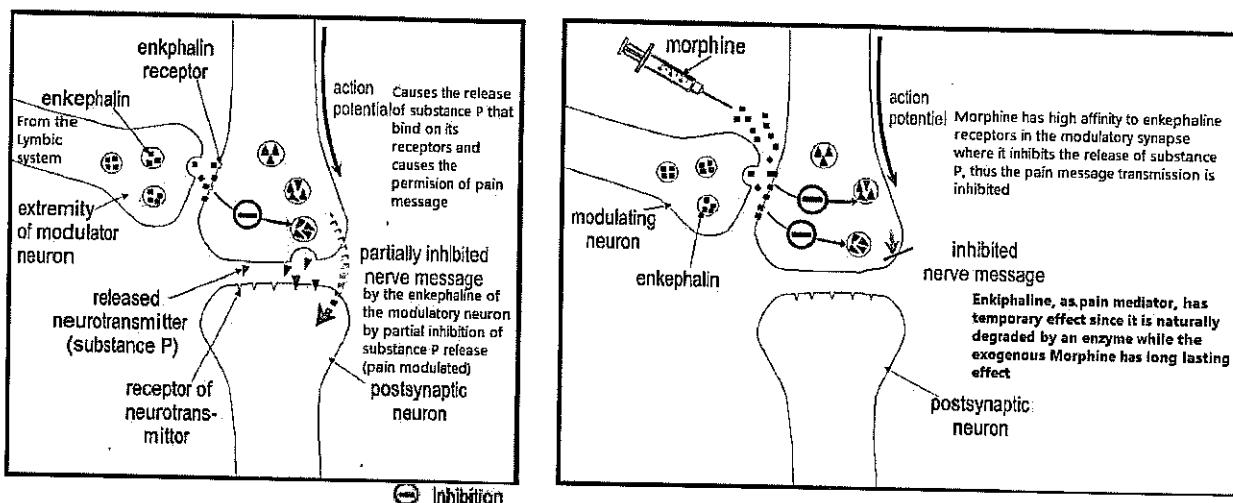
2. Nervous system disorders related to some neurotransmitter deficiencies

Some Disorders of the Nervous System

Disease	Cause	Symptoms	Average age of attack
Alzheimer	Deficiency in acetylcholine	Aphasia <small>speech</small> Apraxia <small>movement</small> Agnosia <small>sensation</small>	Above 65
Parkinson	Deficiency in dopamine and increase in ACH	Akinesia <small>slow movement</small>	Above 50
Huntington chorea	Increase in dopamine and deficiency in ACH and GABA	Hyperkinesia Muscular hypotrophy	Between 30-50

3. Analgesic effects of some chemical substances

Some analgesics have local action by stopping the production of prostaglandins, at the injury site, the substance released by damaged cells and accelerate the frequency of nociceptors, thus increasing the pain sensation.



4. Action of drugs on nervous system

A drug is a chemical substance that, when administered to a person, modifies its sensations by acting on the nervous system. A drug leads after a certain time of its use to a drug addiction.

4.1 Stages of addiction:

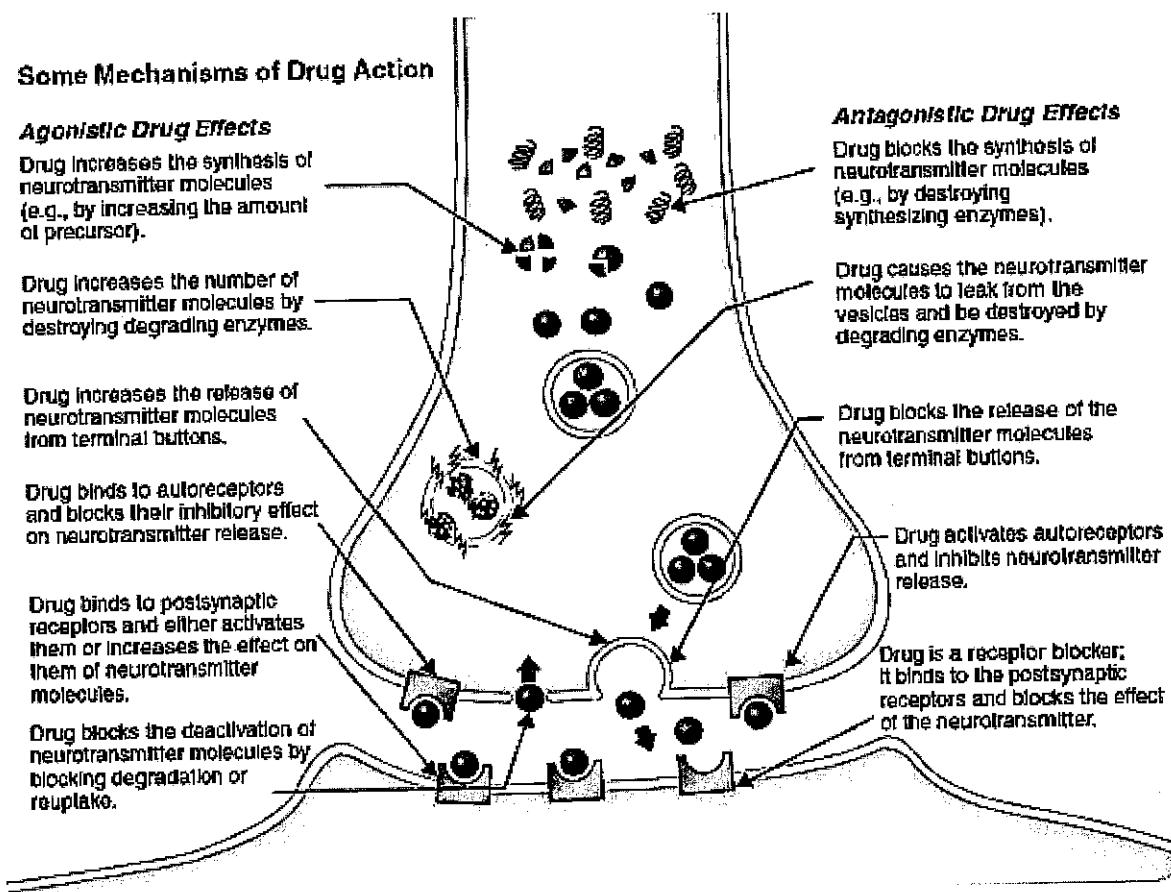
1. Psychic dependency: the pleasant state that the drugs provide encourages the addict to ask for more.
2. Tolerance: is when the same amount of drug starts to have less effect due to the adaptation of the body, as a result of tolerance the addict starts to increase the dose to get the same pleasant state.
3. Physical dependency: It is the biological adaptation to the drug where the addict can no longer live without it. It is translated by depression or pain, according to the type of drug the addict is using. (withdrawal syndrome).

4.2.Mode of action of some drugs

The table below shows examples of the actions of some drugs on the nervous system

Substance	Affected step of synaptic transmission	Mode of action
Curare	Fixation on neurotransmitter receptors	Blocking the receptors of acetylcholine
Cocaine	Recapture of neurotransmitter by presynaptic membrane	Inhibits the recapture of dopamine
Amphetamine	Release of neurotransmitter by exocytosis	Increases the liberation of neurotransmitter
Benzodiazepine	Fixation on neurotransmitter receptors	Facilitate the fixation of GABA

4.3.Levels and modes of action of drugs on the synaptic transmission

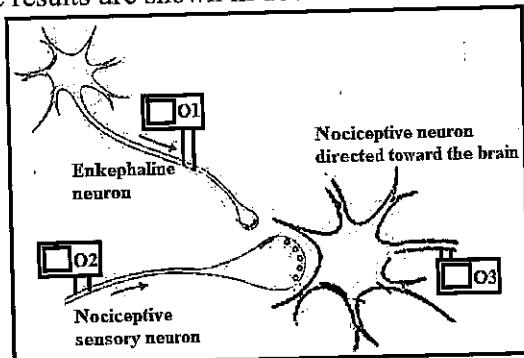


Neurotransmitters and applications

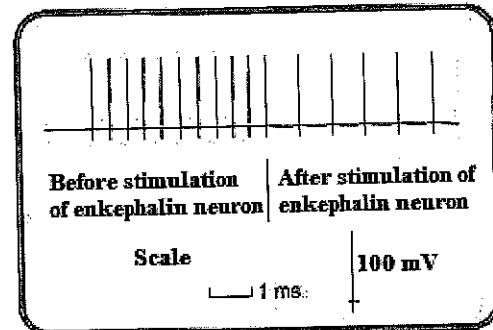
Training exercises

EXERCISE 1 Action of morphine on pain message

Document 1 shows the neuronal network involved in the transmission of the pain message. We want to study the action of enkephalin neuron on the transmission of pain message. The pain message is recorded by the oscilloscope O3 before and after the stimulation of enkephalin neuron. The results are shown in document 2.



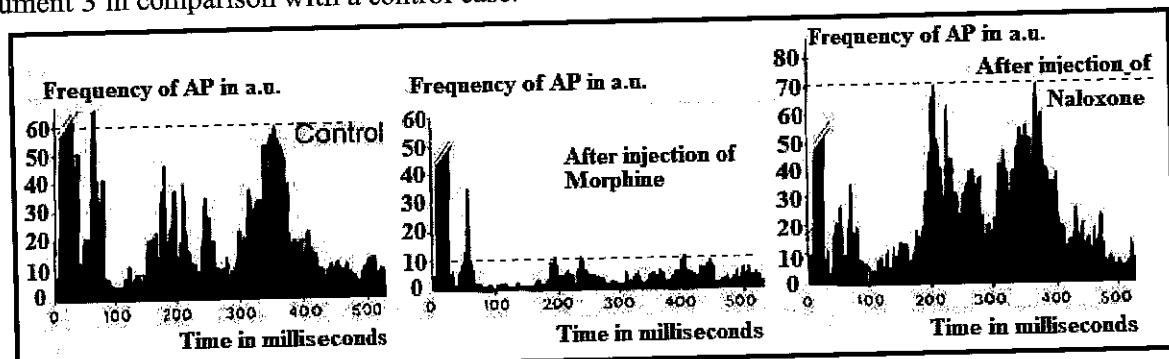
Document 1



Document 2

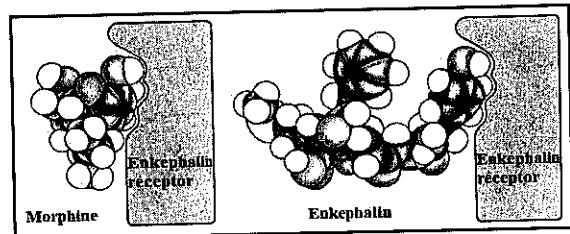
1. Interpret the obtained results.
2. Explain the mode of action of enkephalin neuron on the transmission of pain message.

We study the action of two different substances on the transmission of slow pain obtained after 100ms from the stimulation of a nociceptor. The obtained results given by these two substances are given in document 3 in comparison with a control case.



Document 3

- 3.1 Compare the maximal frequency of slow pain obtained in the two cases of application of Morphine and Naloxone to that of the control case.
 - 3.2 Conclude the action of each of Morphine and Naloxone on the transmission of pain message. Knowing that Morphine and Naloxone act at the level of enkephalin synapse.
 - a. Specify if the action of each of Morphine and Naloxone is inhibitory or amplifying on the enkephalin synapse.
 - b. Formulate two hypotheses to explain the mode of action of each of Morphine and Naloxone at the level of enkephalin synapse.
- Document 4 represents the structure of the molecules of Morphine and enkephalin.
4. Explain, based on document 4, the action of Morphine on the transmission of pain message.



Document 4

Solution:

1. Before stimulation of the enkephalin neuron, the oscilloscope O3 records 4 AP/ms of amplitude 100mv. While, after stimulation of the enkephalin neuron, this number decreases to the half of 2AP/ms with the same amplitude. This indicates that the enkephalin neuron attenuates the transmission of the pain message.
2. After the stimulation of the enkephalin neuron, this neuron releases the enkephalin in the synaptic cleft then the enkephalin molecules bind to the postsynaptic receptors and prevents partially the release of the substance P, mediator of the pain, this inhibition of the release of the substance P attenuates the transmission of pain message.
- 3.1. The frequency of slow pain after 100ms of nociceptor stimulation is 10AP after morphine injection 6 times smaller than that obtained in control case 60PA. On the contrary, this frequency is greater 70PA after the injection of naloxone compared to the control case.
- 3.2. Morphine attenuates the transmission of the message of pain; naloxone amplifies the transmission of the message of pain
4. Morphine enhances the action of the enkephalin synapse because morphine such as enkephalin reduces the transmission of the pain message.
Naloxone inhibits the action of the enkephalin synapse because it increases the AP frequency of the pain, unlike enkephalin.
5. Hypothesis 1: Morphine binds to receptors while exerting the same action on the nociceptive neuron.
Hypothesis 2: Naloxone prevents the release of enkephalin.
6. Document 4 shows that morphine has a configuration similar to that of enkephalin, follows the injection of morphine, exogenous origin, it binds rapidly to the enkephalin receptor and prevent completely the release of the substance P, mediator of pain. Then morphine inhibits totally the pain sensation.

EXERCISE 2

When a person absorbs a certain dose of Ritalin for several tens of days, the pleasure sensations caused by this absorption start to weaken, this will push the user to increase the dose of Ritalin in order to reach the desired effect.

In order to study the mode of action Ritalin, an experiment is performed on a dopamine neuron. In this experiment, we measure the frequency of action potentials in a presynaptic dopamine neuron as well as the number bound neurotransmitters on the postsynaptic receptors in two cases, the first, without application of Ritalin, and the second with its application. Moreover, the frequency of AP is measured in the postsynaptic neuron in the two cases indicated above. The results of the measurements are given in document 1.

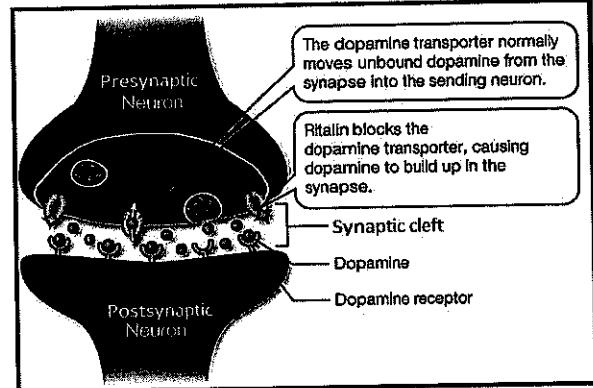
	Without Ritalin	With Ritalin
Frequency of AP in the presynaptic dopamine neuron in a.u.	20	20
Amount of bound dopamine on the postsynaptic receptors in a.u.	50	80
Frequency of AP in the postsynaptic cell	15	30

Document 1

1. Pick out from the above text the information that indicate that Ritalin is a drug.
- 2.1. Compare the obtained results.
- 2.2. Conclude the effect of Ritalin on dopamine synapse.
3. Formulate two hypotheses about the mode of action of Ritalin.

Document 2 shows the mode of action of Ritalin on a dopamine synapse.

4. Explain, based on document 2, the increase of the amount of dopamine bound of the postsynaptic receptors in the presence of Ritalin.

**Document 2****Solution:**

1. For several tens of days, the pleasure sensations caused by this absorption start to weaken, this will push the user to increase the dose of Ritalin in order to reach the desired effect.
- 2.1. The frequency of AP in the presynaptic dopamine neuron is the same 20 u.a. with and without Ritalin but the frequency of AP in the postsynaptic neuron with Ritalin is 30 u.a. double that its absence 15 u.a. Similarly, for the amount of bound dopamine on the postsynaptic receptors that is 80 u.a. with Ritalin larger than its absence 50 u.a.
- 2.2. Ritalin enhances the activity of the dopamine synapse.
3. Hypothesis 1: Ritalin facilitates the binding of dopamine to its receptors
Hypothesis 2: Ritalin prevents the recapture of dopamine.
4. Document 4 shows the presence of dopamine transporters that carry the non-bound dopamine molecules on postsynaptic receptors to the presynaptic button but in the presence of Ritalin these transporters will be blocked which increases dopamine in the synaptic cleft and hence the number of dopamine bound to its postsynaptic receptors.

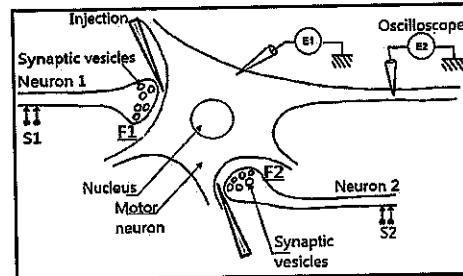
Neurotransmitters and applications

Solved exercises

EXERCISE 1 Anxiety

Anxiety is a state of psychic discomfort frequently accompanied by muscular convulsions. These convulsions correspond to sudden contraction of the skeletal muscles of the body.

To better understand the state of anxiety, we realized a series of experiments. Document 1 shows the experimental set up and document 2 shows the experiments and the obtained results.



Document 1

- 1.1** Indicate the type of each synapse (F1 and F2) involved in this neuronal circuit. Justify your answer.

- 1.2** Determine the role of neuron 2.

- 1.3** Specify the role of the motor neuron.

Document 3 shows the effects of the injections of GABA and acetylcholine neurotransmitters in the absence of stimulation, as well as, the ionic concentrations on both sides of the membrane of the motor neuron in the absence of stimulation.

- 2**. Identify the neurotransmitter implicated in the synaptic transmission at F1 and F2.

- 3**. Show that GABA and acetylcholine are antagonistic substances.

- 4**. Deduce from document 4 the type of GABA receptors.

Picrotoxin is a substance that causes anxiety.

We can experimentally reproduce the situation of synapses associated with anxiety. The picrotoxin was injected in synaptic cleft F1.

Picrotoxin is capable of binding to GABA membrane receptors present on the membrane of the motor neuron. The results are represented in document 4.

- 5**. Deduce the effects of the picrotoxin on GABA synapse and its manifestations in the muscular activity.

Document 5 represents the action of benzodiazepines in mammals. Many substances used in medicine as drugs specifically bind to membrane receptors.

Benzodiazepines (such as Valium) are tranquilizers (used against anxiety) that bind specifically to membrane receptors in GABA.

- 6**. Explain, based on what preceded, how benzodiazepines are efficient substances in reducing the symptoms of anxiety.

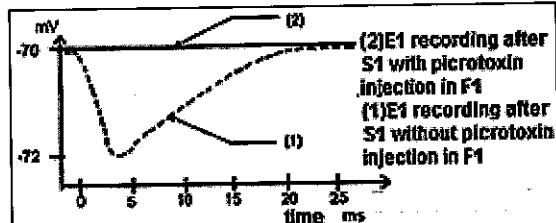
Experiments	Recording E1	Recording E2	Muscle contraction
Experiment: stimulation at S1			-
Experiment2: stimulation at S2			+
Experiment3: simultaneous stimulations at S1 and S2			-

*----: Threshold of depolarization (+presence -absence)

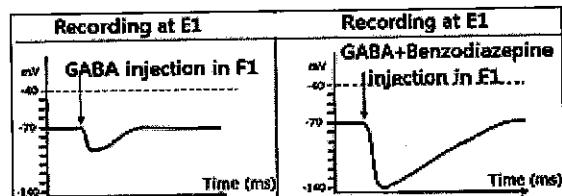
Document 2

operation	injection of GABA in F1	injection of GABA in F2	injection of acetylcholine in F1	injection of acetylcholine in F2
recording at E1	mV -70 -72	mV -70 -72	mV -70 -72	mV -70 -72
before injection of GABA in F1				
	Ions (mmol/L) Na ⁺ K ⁺ Cl ⁻	extracellular medium 440 22 400	intracellular medium 50 400 40	
after the injection of GABA in F1				
	Ions (mmol/L) Na ⁺ K ⁺ Cl ⁻	extracellular medium 440 22 50	intracellular medium 60 400 390	

Document 3



Document 4



Document 5

EXERCISE 2 Mode of action of curare

Curare is a poison that causes deadly paralysis. Studies are done to explain how curare provokes paralysis.

Claud Burnard isolated two frog gastrocnemius muscles with their sciatic nerves, Claude Bernard wrote:

"In a petri dish A that contained solution of curare, the nervous stem of the muscle and the muscle itself were placed outside the solution. The electric stimulation of the nerve immersed in curare provoked a muscular contraction".

"In the other preparation B, the muscle immersed in curare but its nerve was placed outside the solution. The electric stimulation of the muscle didn't provoke any muscular contraction. In contrary, the direct electric stimulation of the muscle provoked its contraction" (Document 1)

- E.** Interpret the results of these experiments.

Micro-electrodes were used to record the membrane potential of excitable cells, neurons and excitable fibers allowed Fatt and Katz to study the response of muscular fibers following the stimulation of their motor nerves.

A. Fatt and Katz isolated frog muscles with their motor nerves and they placed the preparations in a physiological liquid. They isolated from these muscles many fibers and localized their motor end plates. Then they inserted a micro-electrode E1 in the muscular fiber at the level of the motor end plate and a reference electrode in the physiological liquid. The two microelectrodes were related to an oscilloscope O1. (Document 2a).

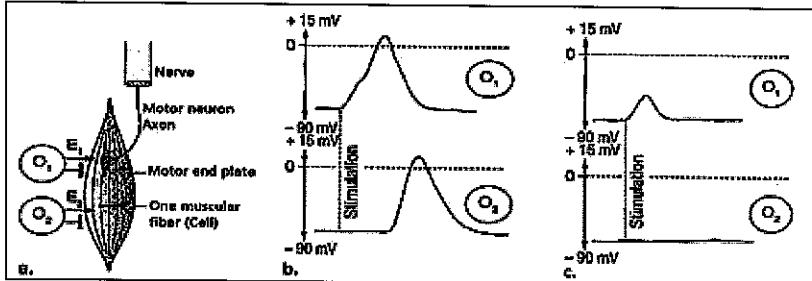
The same setup was realized with a second microelectrode E2 inserted in the muscular fiber, outside the motor end plate and far away by 2.5 m, and related by O2. Document 2b indicates the membrane potential recorded following to one electric stimulation in its motor nerve.

B. Scientists repeated the same experiment, but this time, with a muscle placed in a physiological liquid containing curare. Document 2-c indicates the obtained recordings at the level of the motor end plate E1 and at a distance E2 following the stimulation of its motor nerve.

- E.** Compare the results of the experiments.

- E.** What can you conclude concerning the mode of action of the curare?

With identical setup used by Fatt and Katz, Nustuk studied the variation of the membrane potential of a muscular fiber, not by direct stimulation of its motor nerve, but in response to acetylcholine. Document 3 indicates the obtained results in E1 and E2 in document 2.



Document 2

a, b and c: Muscle fiber was placed in physiological liquid without curare.

d: Muscle fiber was placed in physiological liquid with curare.

a: Deposition of acetylcholine at the surface of the fiber at the level of the motor end plate.

b: Injection of acetylcholine inside the fiber at the level of the motor end plate.

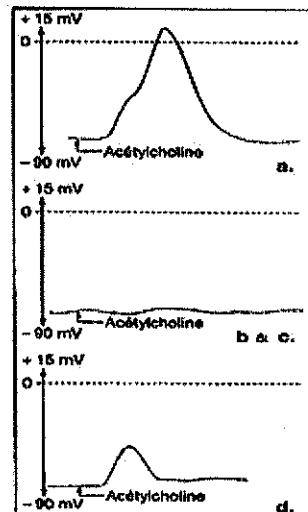
c: Deposition of acetylcholine at the surface of the fiber far away from the motor end plate.

d: Deposition of acetylcholine at the surface of the fiber at the level of the motor end plate.

- E.** Analyze the results of the cases a, b and c.

- E.** What can you conclude concerning the site of acetylcholine action?

- E.** Deduce, referring to the experiments a and d, the site and the mode of action of curare.



Document 3



Neurotransmitters and medical applications

Solved exercises solutions

Exercise 1 Anxiety

- 1.1.F1 is an inhibitory synapse, since S1 produces hyperpolarization (IPSP) of 10mV at the motor neuron membrane of F1. But S2 causes a depolarization (EPSP) of 35 mV at the same membrane then F2 is excitatory.
- 1.2.Upon stimulating neuron 1, hyperpolarization was observed with amplitude 10mV in the motor neuron, however depolarization with amplitude 15mV was observed in the motor neuron upon stimulating neuron 2. But by stimulating the two neurons 1 and 2 simultaneously, a slight depolarization was recorded in the motor neuron with decreased amplitude 5mV. Thus neuron 1 has modulatory role (inhibitory).
- 1.3.Since the motor neuron receives many impulses, integrates them and elaborate adequate responses, then the motor neuron has integration role.
2. Injection of GABA in F1 and F2 produces hyperpolarization of the postsynaptic membrane of F1 only; but the injection of acetylcholine in F1 and F2 causes a depolarization of the postsynaptic membrane of F2 only. Then GABA is the neurotransmitter of synapse F1 while acetylcholine is the neurotransmitter of synapse F2.
3. Hyperpolarization of the postsynaptic membrane of F1 recorded upon injecting GABA in F1, but the injection of acetylcholine in F2 causes a depolarization of the postsynaptic membrane of F2. Then GABA and acetylcholine are antagonistic.
4. Before the injection of GABA, the extracellular medium in F1 is richer in Na^+ ($440_{\text{out}} > 50_{\text{in}}$) and Cl^- ($400_{\text{out}} > 40_{\text{in}}$) by around 10 folds, while the intracellular medium is richer in K^+ ($22_{\text{out}} < 400_{\text{in}}$) by 20 folds. However, after the injection of GABA in F1, it was noted that the intracellular medium becomes richer in Cl^- ($390_{\text{in}} > 50_{\text{out}}$). Thus, GABA receptors correspond to Cl^- channels.
5. After stimulating neuron 1 hyperpolarization was recorded by E1 in the motor neuron with amplitude 2mv without injection by picrotoxin in F1, while upon injecting picrotoxin, and under the same conditions, the resting membrane potential remained constant at -70 mV, this means that picrotoxin inhibits the inhibitory effect of neuron 1. Thus, with picrotoxin, the nerve message is no longer blocked by GABA, causing anxiety (muscle contractions increase).
6. GABA in an inhibitory neurotransmitter, benzodiazepine injection reinforces the inhibitory effect of GABA by increasing the inward diffusion of Cl^- and in consequence increasing the amplitude of the hyperpolarization in the motor neuron. In this case the membrane potential will deviate away from the threshold of depolarization and thus reducing the muscle contractions, the symptoms of anxiety.

Exercise 2 Mode of action of curare

1. Muscular contraction is observed following the electric stimulation of the nerve placed in the presence of curare. In contrary, no muscular contraction is observed when the muscle itself is placed in the presence of curare while the muscle can be directly excited. This shows that curare inhibits the transmission of the nerve message at the level of the neuromuscular junction.
2. The resting potential of the muscle fiber is always -90mV with and without curare. But in doc-2b following a stimulation, at the neuromuscular junction and as a distance of 2.5 mm, the O1 and O2 recorded two muscular AP spaced in time but of amplitude 105mv. But in doc. 2c, in amplitude there is muscular depolarization of 40 mV recorded by O1, but no AP of the fiber recorded by O1 at the end plate, which does not spread where no response is recorded by O2 that remained remains-90mV.
3. Therefore, curare attenuates the depolarization of the muscle fiber membrane potential and consequently the generation of an AP following the stimulation.
4. Acetylcholine deposited at the neuromuscular junction causes the generation of a muscle AP (a), whereas the injection of acetylcholine into the muscle fiber at the neuromuscular junction (b) or deposited on the surface of the fiber outside of the driving plate (c), has no effect.
5. Thus, ach has the same action of the nerve stimulation and it acts only at the level of the motor end plate.
6. Since, in "d", acetylcholine deposited on the surface of the endplate in the presence of curare has no effect while, in "a" without curare, a response has observed. So, curare inhibits muscle contraction by inhibiting the action of acetylcholine at the motor endplate.



Neurotransmitters and medical applications

Non-solved exercises

EXERCISE 1 Botox mode of action

Botulism is a serious disease that causes paralysis of skeletal and smooth muscles. It becomes fatal when it affects the muscles of vital organs. This disease is caused by toxins called Botulinum toxins, secreted by bacteria: Clostridium Botulinum. These bacteria are found mainly in meat foods that are undercooked or poorly preserved.

In addition, Botulinum toxins are used in therapeutic medicine, to treat certain diseases in aesthetic medicine, to erase the traces of aging. There are currently 7 types of Botulinum toxins, of which 4 types are the cause of botulism in humans: Botulinum toxins A, B, E and F. These toxins are enzymes (proteases) that act on proteins at different levels. In order to understand the mode of action of these toxins, the following experiments were performed.

1. List the steps from 1 to 7 in Document 1.
2. Formulate three hypotheses concerning the mode of action of Botulinum toxins.

Document 2 shows the effects of low dose injections of Botulinum toxins (A, B, E and F) on muscle activity.

1. UA: Arbitrary Unit.
2. Motor neuron stimulations in all experiments are performed before diffusion of the toxin to the extracellular medium.

3. Specify the cell structure (s) target (s) of Botulinum toxins.

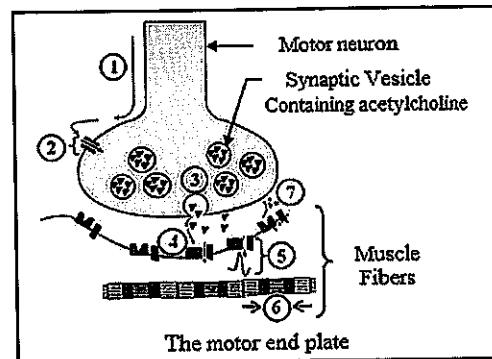
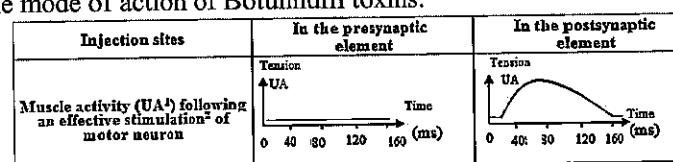
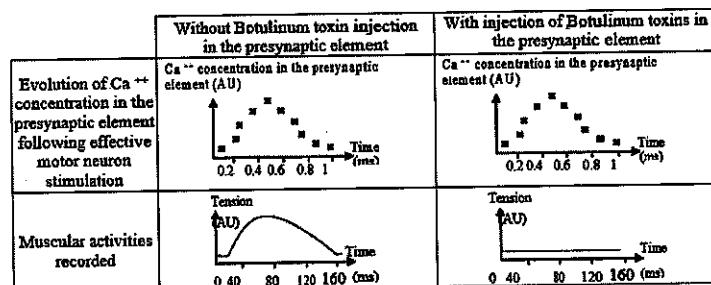
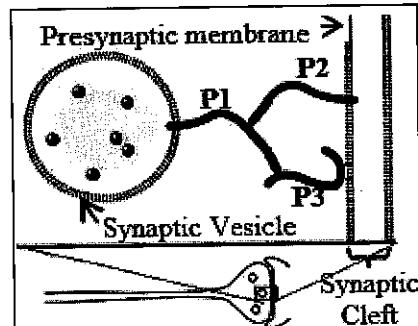
Document 3 shows the effects of low dose Botulinum toxin injections (A, B, E and F) on Ca^{2+} ion flux and muscle activity.

4. What additional information do you provide with the data from Document 3? Justify your answer.

Document 4 shows the neuromuscular synapse. Phenomenon 3 in document 1 involves 3 proteins: P1 (synaptobrevin): synaptic vesicle membrane protein. P2 (syntaxin) and P3 (SNAP protein): two proteins of the presynaptic plasma membrane.

These proteins interact and bind to each other, thus allowing the progression of the phenomenon 3 of the document 1.

Botulinum toxins cut protein, the ends of the new polypeptide fragments resulted can be recognized by specific antibodies. Following separate injections of Botulinum toxin A, B, E or F into the presynaptic element, the cytoplasm is extracted and placed in the presence of antibodies specific for the polypeptide fragments corresponding to P1, P2 and P3. The results are shown in document 5:

**Document 1****Document 2****Document 3****Document 4**

5. Interpret the obtained results.
Botox is composed of Botulinum toxin type A. It is used at low doses in several treatments such as expression of wrinkles due to continuous contractions of the facial muscles.

6. Explain the mode of action and the effects of Botox on reducing facial wrinkles.

Toxins	Antibodies specific for fragments of P1	Antibodies specific for fragments of P2	Antibodies specific for fragments of P3
Toxin A	-	-	+
Toxin B	+	-	-
Toxin E	-	-	+
Toxin F	+	-	-

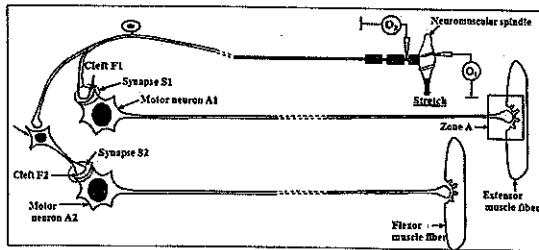
- Absence of immune complex + Presence of immune complex

Document 5

EXERCISE 2 Effects of drugs on genesis and transmission of impulses

In order to study the aspects of the genesis and transmission of the nerve message, series of experiments performed on the nervous circuits involved in the myotatic reflex and the experimental setup is represented in document 1.

Experiment 1: Stretching of increasing intensities E₁, E₂, E₃ and E₄ were applied on the neuromuscular spindle, the amplitude of the receptor potential at oscilloscope O₁ and the amplitude of the action potentials and their frequencies recorded at oscilloscope O₂, are represented in document 2.



Document 1

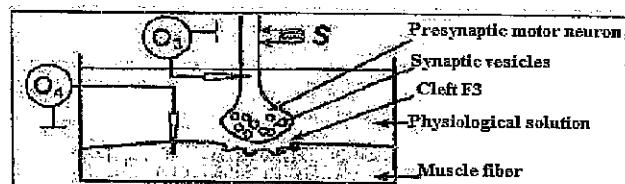
1. Indicate the role of the neuromuscular spindle.
2. Specify the properties for the receptor potential, for the action potential and for the nervous message.

Experiment 2: The concentration of Na⁺ and K⁺ ions in the cell body of motor neuron A₁ and A₂ is measured before and after the injection of neurotransmitter X and Y. Document 3 shows the obtained results.

	In the cell body of A ₁ and A ₂ before the injection of X and Y		In the cell body of A ₁ after the injection in F ₁		In the cell body of A ₂ after the injection in F ₂	
	X	Y	X	Y	X	Y
Concentration of Na ⁺ (U/l)	15	30	15	15	15	15
Concentration of K ⁺ (U/l)	150	150	150	150	150	110

Document 3

- 3.1. Explain the mechanism by which the concentration of Na⁺ and K⁺ ions changes in A₁ and A₂ cell bodies after the injection of neurotransmitters X and Y.
- 3.2. Draw out the consequences of the obtained modifications on the membrane potential of the postsynaptic membrane of A₁ and A₂ cell bodies.
- 3.3. Specify the nature of synapse S₁ and S₂.
- 3.4. Explain, based on what preceded and your acquired knowledge, the coordination of the extensor and flexor muscle activity after stretching the neuromuscular spindle with intensity E₃.



Document 4

In the frame work of studying the neuromuscular transmission, series of experiments were performed. Zone A in document 1 was placed in physiological liquid rich with Ca²⁺ ions. The experimental setup is represented in document 4 and its results in document 5.

4. Determine the effect of each toxin on the neuromuscular transmission.
5. Draw out the role of Ca²⁺ and the mode of action acetylcholine in the neuromuscular transmission.
6. Explain, based in what preceded and your knowledge, the mechanism of the neuromuscular transmission.

Experiment		Results			
		Recording in O ₃	Calcium ions in presynaptic neuron	Acetylcholine rate in F ₃	Recording in O ₄
3	Stimulation S		+	100 nmoles/L	
4	Injection of botulinum toxin in the presynaptic neuron, then stimulation S		+	nul	
5	Addition of conotoxin in the physiological liquid, then stimulation S		-	nul	
6	Injection of curare, toxin having structure similar to acetylcholine, in cleft F ₃ , then stimulation S		+	100 nmoles/L	

+ : presence - : absence

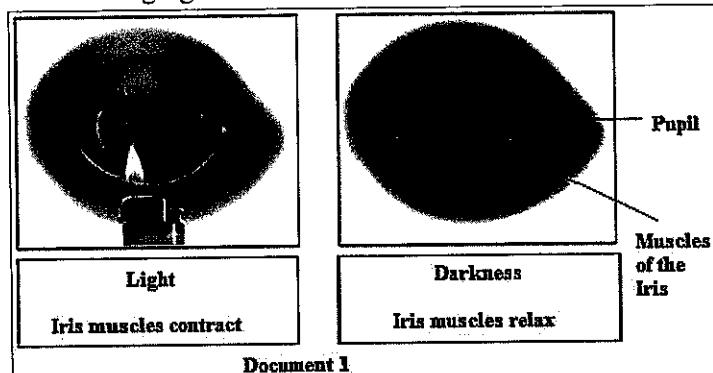
Document 5

EXERCISE 3 Mode of action of Atropine

During routine eye examination, ophthalmologists use eye drops such as "atropine eye drops" to allow examination the part of the eyeball opposite the pupil, the part of the eye through which you see, with complete pupil opening despite the presence of strong light.

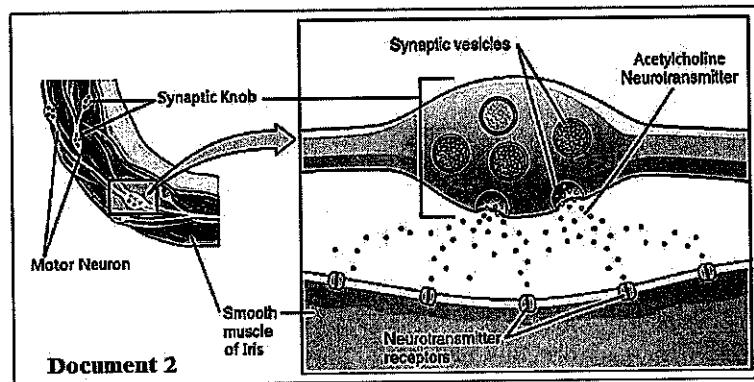
A study was performed to determine the mode of action of atropine.

Pupil reflex, that controls the diameter of the pupil in response to the intensity of light for the adaptation to various light intensity entering the eye, is initiated and the experimental results are illustrated in document 1.



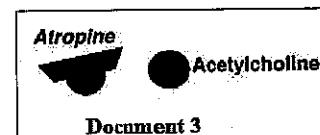
1. Pick out the importance of this reflex.
2. Compare the obtained results.
3. Draw out the effect of light on the Iris muscles.

In the presence of light, the neuromuscular synapse innervating the Iris muscle fibers is schematized in document 2.



Document 3 represents the spatial illustration of the acetylcholine and atropine.

4. Formulate two hypotheses that may explain the mode of action of atropine.



Isolated Iris muscle fibers were perfused in a physiological solution where acetylcholine and atropine effects were studied on these fibers. The experimental results are represented in document 4.

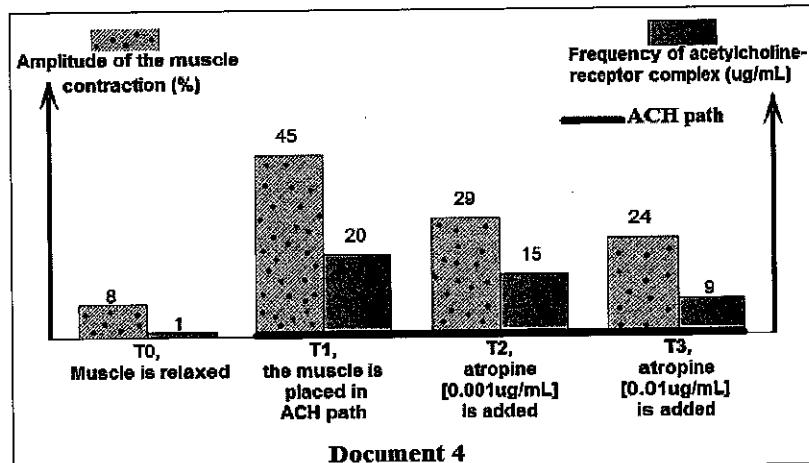
At T0, the muscle is relaxed;

At T1, the muscle is placed in an acetylcholine bath;

At T2, the atropine with concentration 0.001ug/ml, is added to the bath.

At T3, the atropine with concentration 0.01ug/ml, is added to the bath.

5. Determine the effect acetylcholine and atropine at the level if neuromuscular synapse.
6. Explain, from what preceded, the mode of action of atropine.

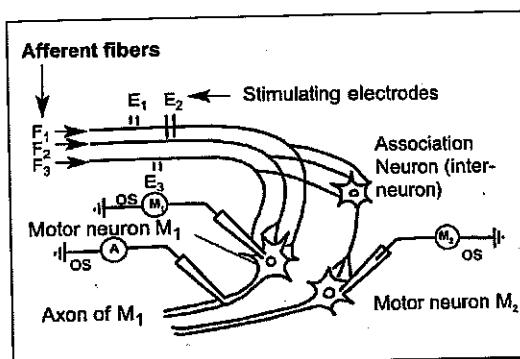
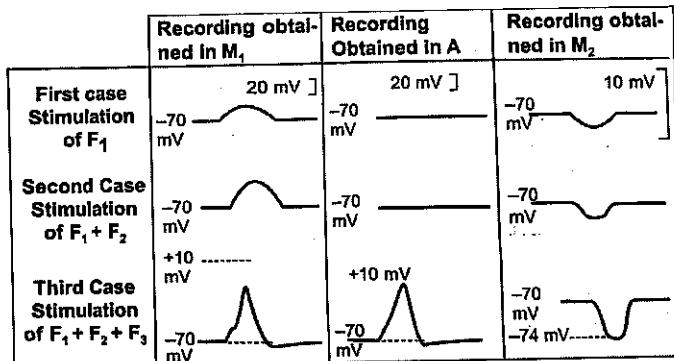


Nervous system

Official exercises

Exercise 1 (4.5 pts) Transmission of the nervous message**Session 2001-1**

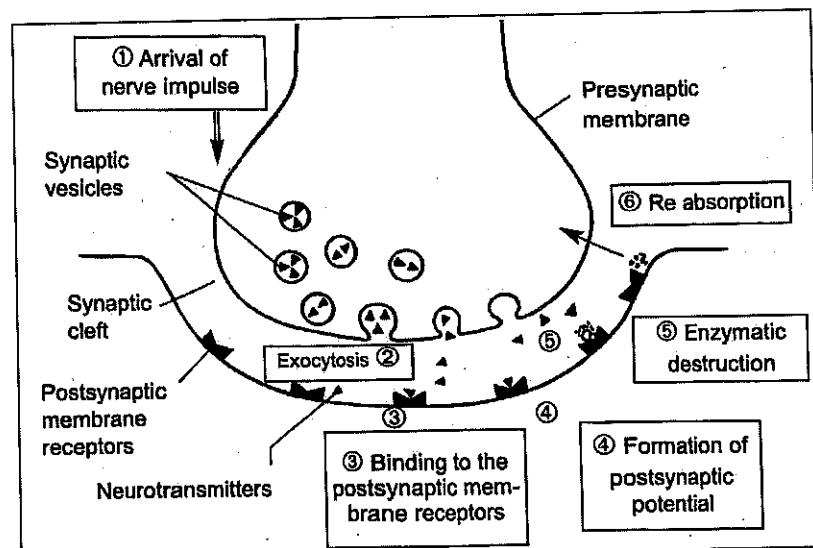
In order to study the transmission of the nervous impulses that originate in afferent sensory fibers leading to the spinal cord, we performed the following experiment. We inserted a microelectrode in a motor neuron M₁ and another microelectrode in a motor neuron M₂. Both present in the spinal cord, in a way that allows recording the activity of these neurons. A third microelectrode inserted in the axon of M₁ permits recording the activity of this axon. We applied effective stimuli having the same intensity to fibers F₁, F₂ and F₃ as indicated in document 2.

**Document 1****Document 2**

1. Explain, using acquired knowledge, the recordings obtained in M₁ and A.
2. What is the role of the interneuron in document 1? Justify the answer with reference to the recording obtained in M₂.
3. Indicate the muscles that are innervated by the motor neurons M₁ and M₂ in a myotatic reflex.

The figure in document 3 represents the steps of the synaptic transmission.

4. Write a short text summarizing the different steps of this synaptic functioning.

**Document 3**

Exercise 2 (3 pts) Number of synapses in a circuit

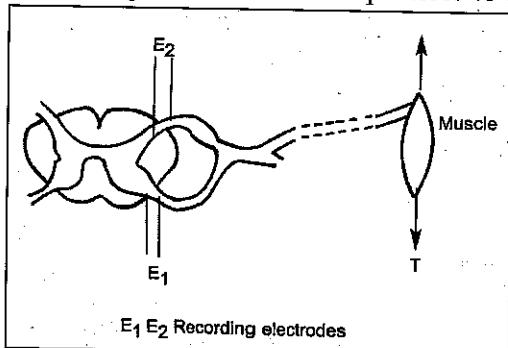
Session 2001-2

The response of a muscle to a stretch is a contraction that takes place in a few hundredths of a second after the beginning of stretching; this is a myotatic reflex.

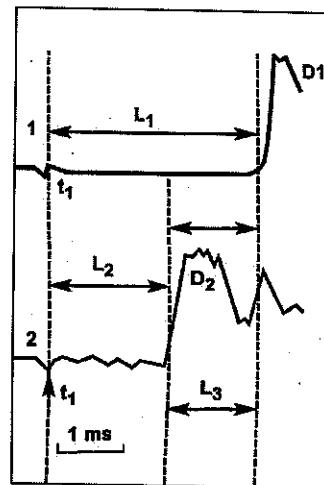
In order to know if the myotatic reflex is a mono or a polysynaptic reflex, we perform the following experiment.

We exert a slight pull T on a muscle at time t_1 , we record the electric response of the ventral root (D1) and that of the dorsal root (D2) of a spinal nerve. Document 1 represents the experimental set up, whereas document 2 represents the obtained results.

N.B. The recording is done as near as possible to the spinal cord.

**Document 1**

1. Explain the form of the response D₂.
2. What do L₁, L₂ and L₃ represent in document 2?
3. Calculate L₃.

**Document 2**

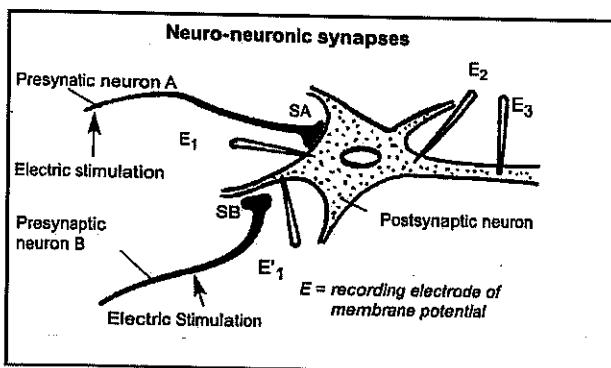
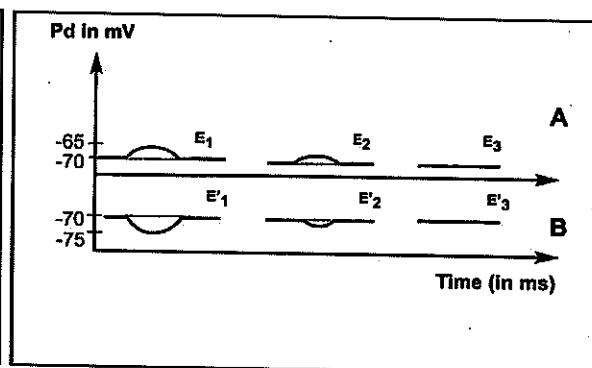
Knowing that the time taken by an impulse to cross a synapse ranges between 1 and 1.5 ms,

4. you deduce from the recordings presented in document 2?

Exercise 3 (2½ pts) Types of synapses

Session 2001-2

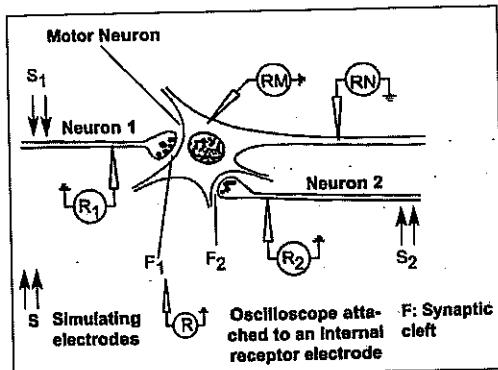
Within the framework of studying synaptic transmission, we perform the following experiment: what can We stimulate separately the presynaptic neurons A and B (document 1) in order to generate an action potential in them. We record the postsynaptic potential with the help of receptor electrodes

**Document 1****Document 2**

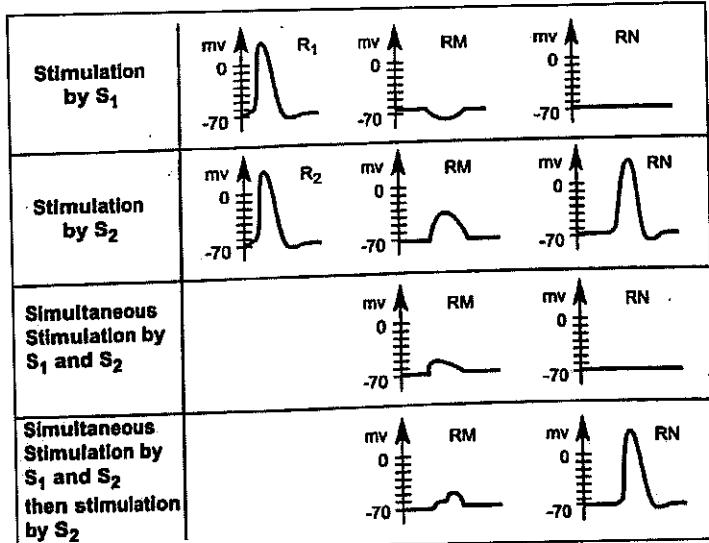
1. Analyze the obtained results.
2. Specify the functional nature of each of the synapses SA and SB.
3. Under what conditions does E3 record the response?

Exercise 4 (4 ½ pts) Synapses and integration**Session 2002-1**

In the spinal cord, the two neurons 1 and 2 are linked to a motor neuron by means of synapses. Documents 1, 2 and 3 represent the experimental set-up and the recordings obtained.



1. Explain, starting from the document 2, and by referring to the acquired knowledge, the integrative role of the motor neuron.

**Document 2**

Document 3 represents the recordings obtained after injection of some chemical substances in the synaptic clefts F1 and F2.

2. Specify from document 3, the neurotransmitter acting in each of the two synapses: the synapse between neuron 1 and the motor neuron; and the synapse between neuron 2 and the motor neuron.

Performed operations	Injection of GABA in F ₁	Injection of GABA in F ₂	Injection of acetylcholine in F ₁	Injection of acetylcholine in F ₂
Recordings in RM	mv -70	mv -70	mv -70	mv -70

Document 3

Exercise 5 (3 ½ pt) Synaptic transmission

Session 2002-2

To specify the pattern of neurotransmission, we performed the following experiments.

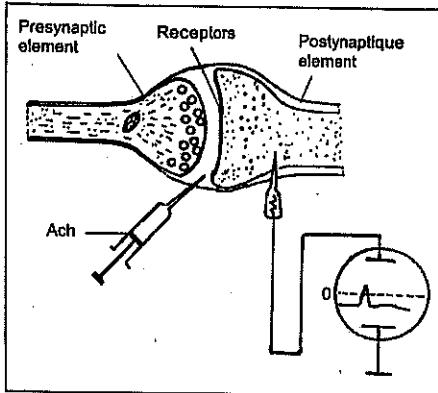
First experiment: We injected into a synapse (document 1) increasing amounts C₁, C₂, C₃ and C_A of acetylcholine (Ach), and recorded the response of the postsynaptic element, document 2.

Second experiment: We injected labeled acetylcholine into an Ach-synapse. By means of autoradiography, the plasma membrane of the postsynaptic element appeared radioactive.

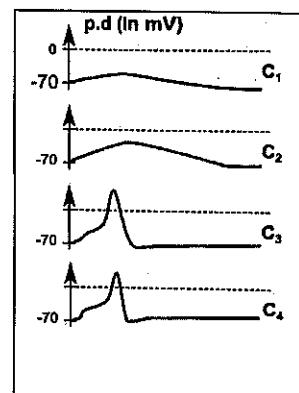
Third experiment:

We injected curare into another Ach-synapse; then we injected increasing amounts of Ach. Neither EPSP nor action potential was obtained.

When we injected labeled curare into the same Ach-synapse, the postsynaptic membrane appeared radioactive.

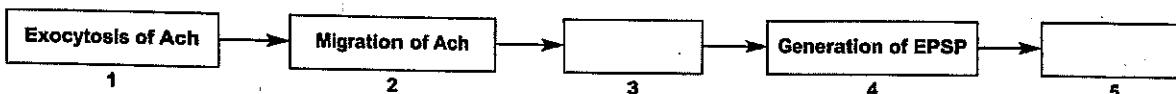


Document 1



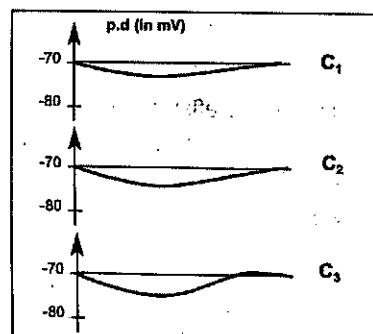
Document 2

1. Interpret the experimental results.
2. Complete the functional diagram of the neurotransmission mechanism represented below by filling the empty boxes 3 and 5 with the appropriate expressions.



Fourth experiment: We injected increasing amounts of GABA (Gamma amino butyric acid) in another synapse and recorded the response of the postsynaptic element (document 3)

3. Interpret the experimental results in document 3.



Document 3

Exercise 6 (4 pts) Action of morphine

Session 2003-1

Pain sensation, which is of a cutaneous origin, necessitates the intervention of peripheral receptors, conductors, and an integration system.

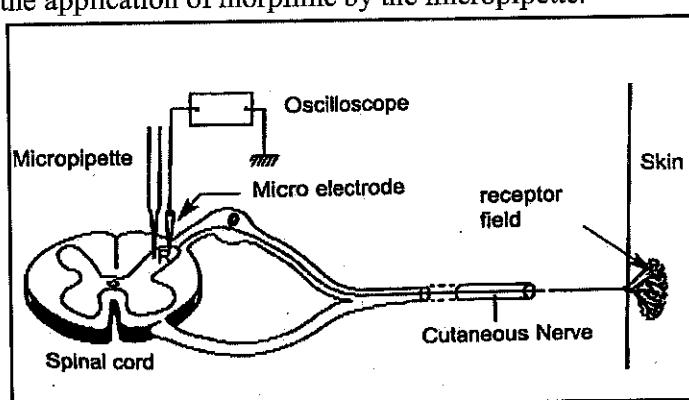
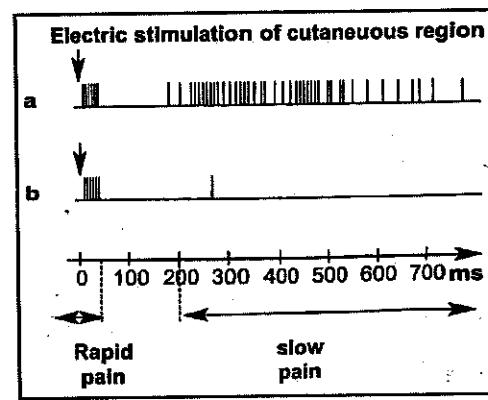
It is proposed to study the way by which the spinal cord intervenes in the transmission of nervous messages.

Document 1 presents the neural links between the skin and the spinal cord.

We insert, at the level of the neurons of the dorsal horn of the spinal cord (zone R), a microelectrode, which is connected to an oscilloscope for recording the response of these neurons. A micropipette is used for eventual injection of an active substance.

A strong electric stimulation of the corresponding cutaneous region provokes a short and rapid message of pain sensation called "rapid pain" followed by a delayed pain sensation, longer than the first one called "slow pain".

The obtained recording is presented in document 2a. The recording in document 2b, is obtained after the application of morphine by the micropipette.

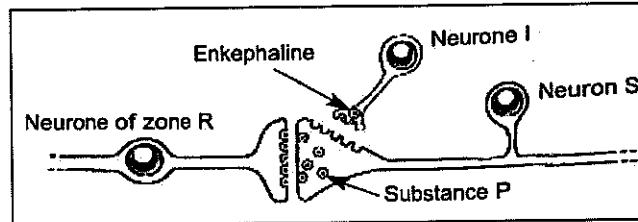
**Document 1****Document 2**

- 1.1. Analyze document 2.
- 1.2. Conclude the action of morphine.

Using the immunofluorescence technique, Hokfelt and his collaborators have localized, at the level of zone R of the dorsal horn of the spinal cord (doc. 1), two chemical substances: substance P and enkephalin.

Document 3 shows the details of zone R.

2. Formulate a hypothesis concerning the mode of action of morphine at the level of zone R.

**Document 3**

Hokfelt and his collaborators performed a series of experiments:

First experiment: A strong stimulation of neuron S provokes a pain sensation associated to a decrease in the number of substance P vesicles, and the appearance of a nervous message passing towards the brain. Once liberated, substance P is rapidly inactivated.

Second experiment: The stimulations applied on neuron I then on neuron S show a decrease in the number of enkephalin vesicles and a slight decrease in the number of substance P vesicles.

Third experiment: Before the stimulation of neuron S, we apply a micro-injection of morphine in synapse I - S the number of substance P vesicles does not decrease, it remains constant.

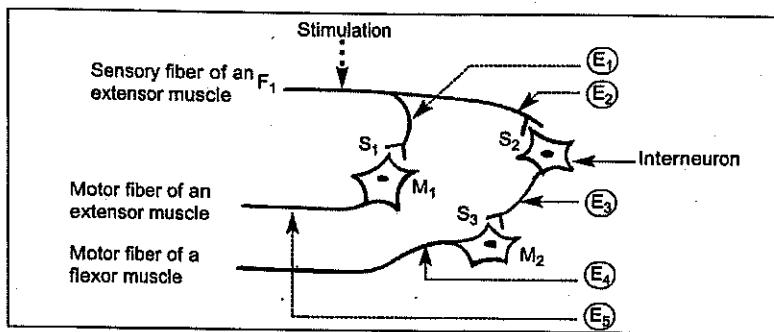
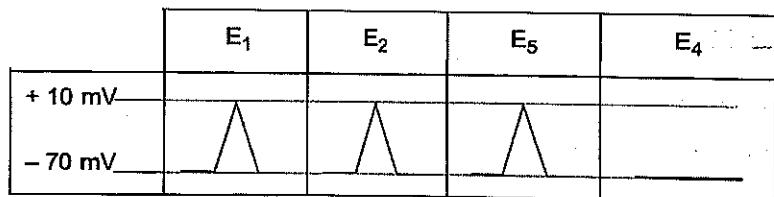
3. Determine, based on the first two experiments, the role of substance P and that of enkephalin.
4. Does the result of the third experiment validate the hypothesis formulated in (b)? Justify the answer.

Exercise 7 (4 ½ pts) Synapses of myotatic reflex network

Session 2003-2

In order to specify the type of synaptic transmission involved in a myotatic reflex (document 1), the following experiments were done.

First experiment: We stimulated the sensory fiber F1 of an extensor muscle. The recordings are presented in document 2.

**Document 1****Document 2**

Second experiment: We injected two types of neurotransmitters (acetylcholine and GABA) into the synaptic clefts S1, S2 and S3. The obtained recordings are shown in document 3.

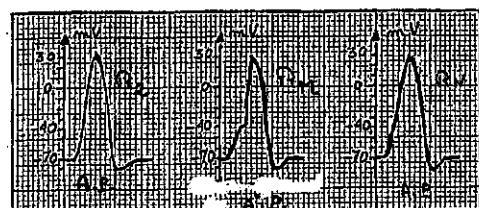
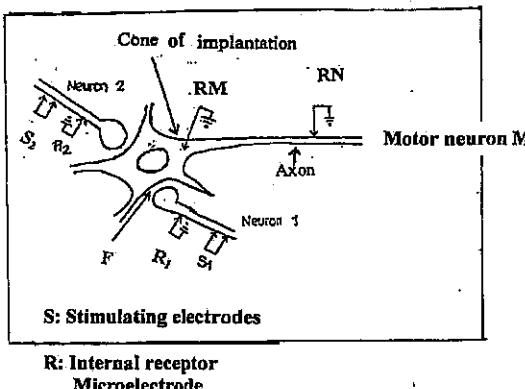
Synaptic Clefts	Neuro transmitters	E ₃	E ₄	E ₅
S ₁	Ach	- 70 mV	- 70 mV	+ 10 mV - 70 mV
	GABA	- 70 mV	- 70 mV	- 70 mV
S ₂	Ach	+ 10 mV - 70 mV	- 70 mV	- 70 mV
	GABA	- 70 mV	- 70 mV	- 70 mV
S ₃	Ach	- 70 mV	+ 10 mV - 70 mV	- 70 mV
	GABA	- 70 mV	- 70 mV	- 70 mV

Document 3

- Interpret the experimental results in document 2 and 3.
- What movement is expected following the stimulation of F1? Justify the answer.
- How do you qualify the two muscles participating in this movement?

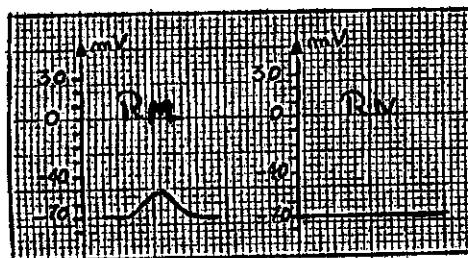
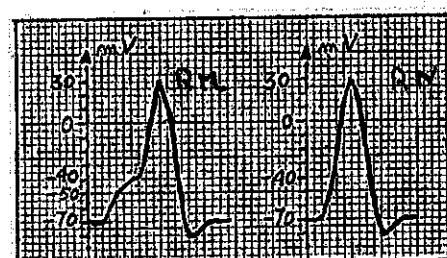
Exercise 8 (5 ½ pts) Properties of the motor neuron

In the framework of studying the transmission of a nervous message, we perform the experimental set-up represented in document 1. The axon terminal of neuron 2 has vesicles that contain acetylcholine. The axon terminal of neuron 1 has vesicles that contain GABA. If we effectively stimulate neuron 2 at S_2 , we obtain the recordings presented in document 2.

**Document 1****2****1.1.** Analyze these recordings.**1.2.** What can you conclude?

We stimulate at S_1 and S_2 simultaneously; we obtain the recordings in document 3.

When we stimulate simultaneously at S_1 and S_2 , followed immediately by the stimulation at S_2 , we obtain the recordings in document 4.

**Document 3****Document 4**

2. Explain the property of the motor neuron revealed by these results.

With the interest of specifying the effect of the drug, barbiturate, on the chemical synapse involving GABA, we perform the following experiments:

1st experiment: we inject into F, at the same time, GABA and barbiturates. The recorded activity at RM reveals a hyperpolarization, which is more significant than when GABA was injected alone.

2nd experiment: if radioactive barbiturates were used in experiment 1, we notice, after the injection, that radioactivity is found to be localized at the level of the postsynaptic membrane.

Based on the results of the above two experiments,

3. Specify the role of barbiturates in this synapse and at what level of the synapse they act.

There exists, in the central nervous system, numerous neuro-neuronal synapses where GABA is involved (document 1).

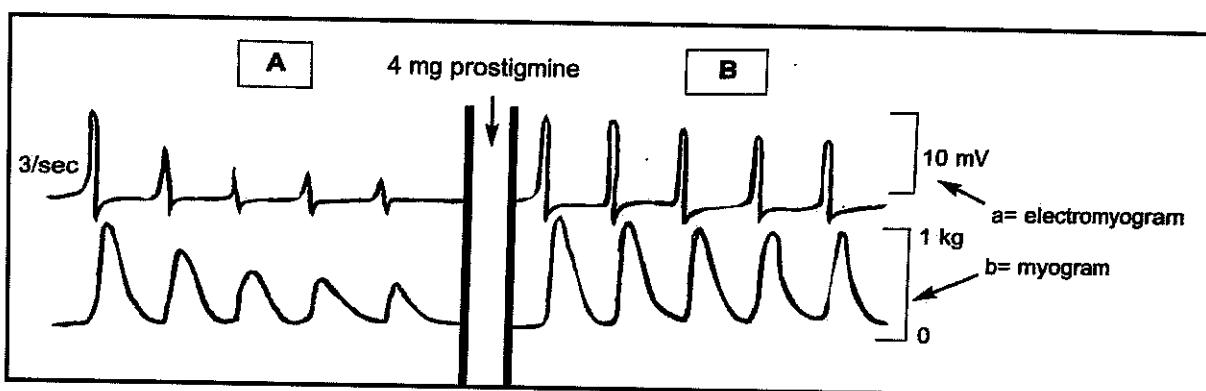
4. Explain, by referring to the acquired knowledge, the effect of barbiturates on the activity of the muscles of an organism.

Exercise 9 (4½ pts) Muscular dysfunction**Session 2004-2**

Myasthenia is a muscular disease characterized by great fatigue when doing an effort. The motor deficit appears, particularly during a sustained or repeated skeletal muscular activity. When fatigue is at maximum, the muscle remains excitable directly by an electric stimulation but it cannot be excited by means of its nerve.

Document 1-A represents the simultaneous recordings of the electric response (a) and the mechanical response (b) of the adductor muscle of the thumb by the stimulation of the cubital nerve at a frequency of 3 per second in an individual having myasthenia.

Document 1-B shows the recordings in the same individual and by the same stimulation done 15 minutes after the injection of 4mg of prostigmine. Prostigmine is an inhibitor of acetylcholinesterase, an enzyme that destroys acetylcholine.



Document 1

1. Compare the recordings before and after the injection of prostigmine.
2. How can you explain, by referring to the given and to the acquired knowledge, the results obtained in B?
3. Are the muscle fibers affected in this individual? Justify the answer.
4. The nerves, in individuals having myasthenia, are healthy. Formulate two hypotheses concerning the cause of this disease.

Research has shown that 95% of the individuals having myasthenia have antibodies, which block or destroy the membrane receptors of the acetylcholine.

5. To which type of disease does myasthenia belong?
6. Which of the two hypotheses is valid? Justify the answer.

A pregnant woman having myasthenia, gives birth to an infant who presents, at birth, muscular paralysis, that disappears after a few weeks or a few months.

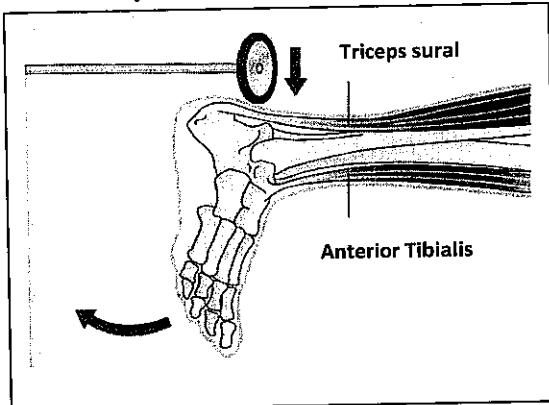
7. How can you explain this particularity?

Exercise 10 (4 ½ pts) Myotatic reflex

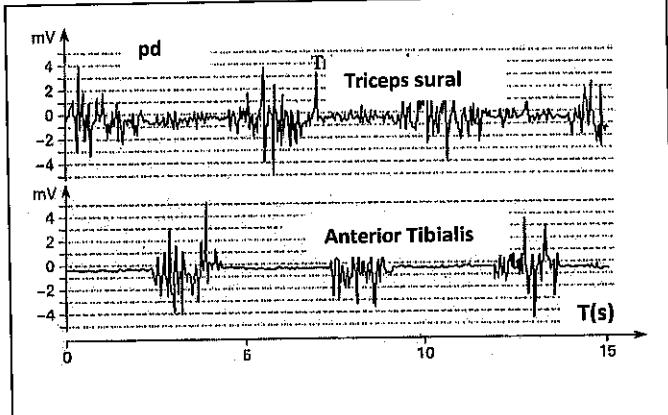
To understand the activity of the two muscles of the lower leg, the anterior tibialis and the triceps sural, during a reflex act and during their voluntary movement, the following experiments are done and the results are presented in following documents.

1st experiment: Stretching of the triceps sural by hitting the Achillean tendon, connected to the muscle, immediate extension of the foot and the contraction of the mentioned muscle is provoked, document 1.

2nd experiment: We place electrodes on the skin of a person at the level of the triceps sural and the anterior tibialis, and we ask this person to perform alternating movements of his foot: extension followed by flexion. The obtained recordings are presented in document 2.



Document 1



Document 2

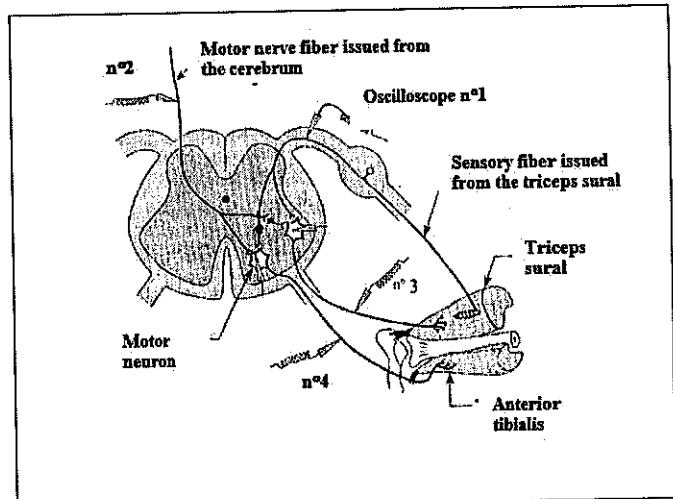
- What type of reflex is revealed in the first experiment? Justify the answer.
- Interpret the results of the second experiment.

In order to know if a person is capable to control an Achillean reflex we perform the experimental set-up shown in document 3. We record the electric activity at the level of the triceps sural, the anterior tibialis, and the corresponding network of neurons, in the following two cases:

Case A: Hitting the Achillean tendon.

Case B: Hitting the Achillean tendon during a strong voluntary contraction of the anterior tibialis.

The results are presented in document 4.



Document 3

	Obtained recordings at the level of the oscilloscopes				Muscular activity	
	n° 1	n° 2	n° 3	n° 4	Triceps sural	Anterior tibialis
Case A	+	-	+	-	Contraction	Relaxation
Case B	+	+	-	+	Relaxation	Contraction

(+) presence of action potential

(-) absence of action potential

Document 4

3.1. Compare the obtained results.

3.2. Conclude the role of the cerebrum in this activity.

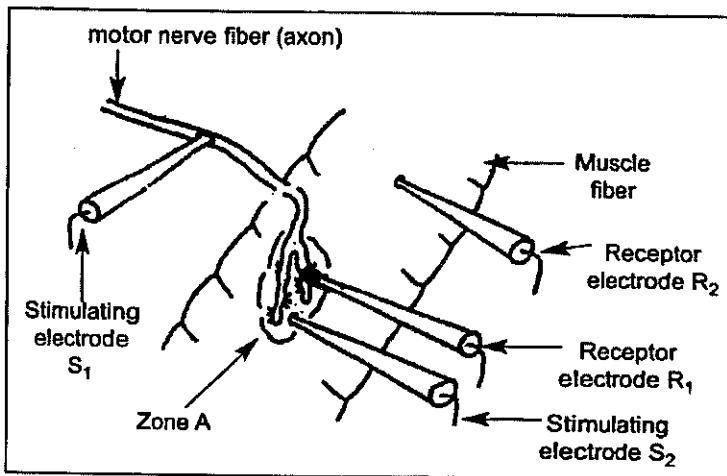
Exercise 11 (5 pts) Motor end plate

Session 2005-2

We propose to study how a nerve fiber gives order to a muscle fiber to contract. For this sake, we do an experimental study on an isolated muscle fiber connected to its motor nerve fiber, document 1.

Experiment 1

We stimulate the motor nerve fiber with electrode S₁. The obtained recordings at the levels of electrodes R₁ on the presynaptic membrane and R₂ on the muscle fiber are shown in document 2.

**Document 1****Experiment 2**

We stimulate the muscle fiber with electrode S₂ placed at the level of postsynaptic membrane.

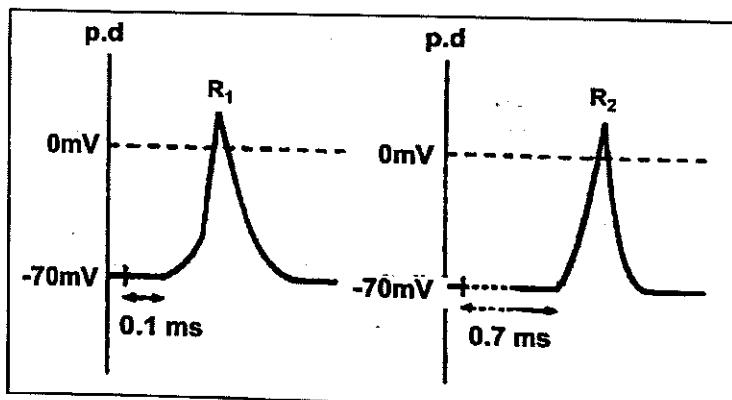
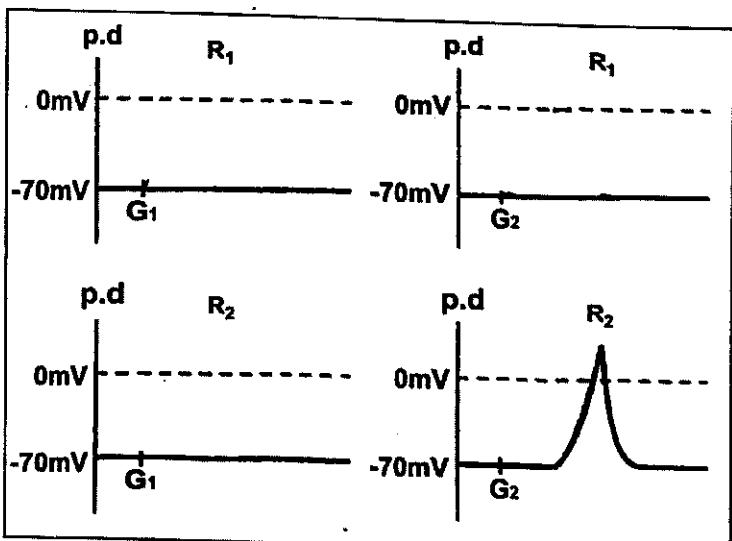
The muscular action potential takes 0.1 ms to reach R₂.

1. Analyze the first experiment.
2. Determine from the two experiments the time needed by a nerve impulse to traverse the synapse.

Experiment 3

With a micropipette, we put on the plasma membrane of the muscle fiber at the level of zone A, a small drop of acetylcholine G₁, then we put another small drop of acetylcholine G₂, which is more concentrated than G₁. The obtained recordings at the level of R₁ and R₂ are shown in document 3.

3. Interpret the obtained recordings.
4. Explain how the nerve fiber gives order to the muscle fiber to contract.

**Document 2****Document 3**

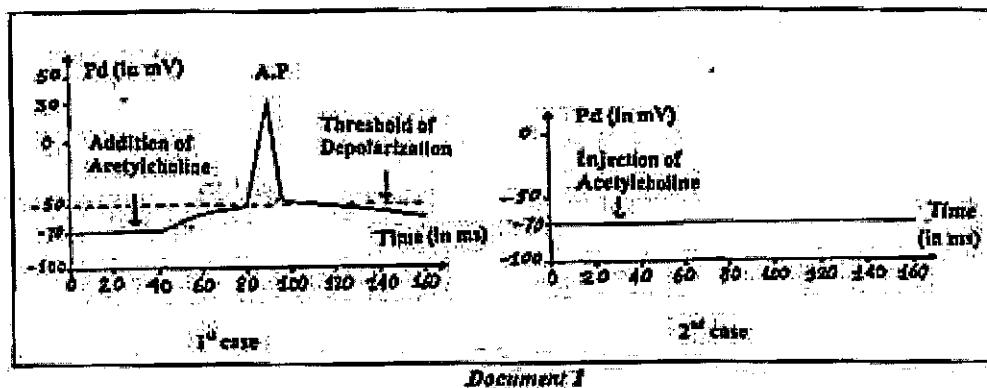
Exercise 12 (8 pts) Site of action of neurotransmitters

In order to understand the intervention of acetylcholine in the functioning of a neuro-muscular synapse, we depend on the results of the following experiments;

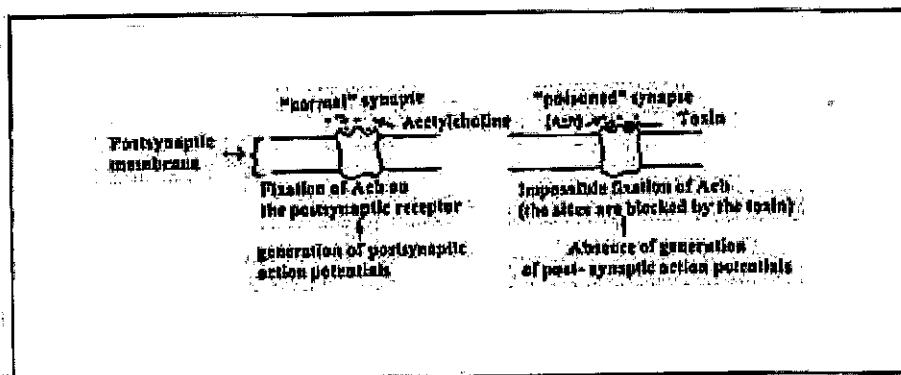
1st experiment: we isolate some muscle fibers and we record the variations of membrane potentials of these fibers under the action of acetylcholine in two different cases:

- Case 1: we add a sufficient quantity of acetylcholine into the synaptic cleft.
- Case 2: we inject the same quantity of acetylcholine inside the muscle fibers.

The results of the recordings are shown in document 1.



2nd experiment: we add into this synapse α -bungarotoxin, a poison found in the venom of the snake, then we add acetylcholine into the synapse, document 2. No muscular contraction is recorded.



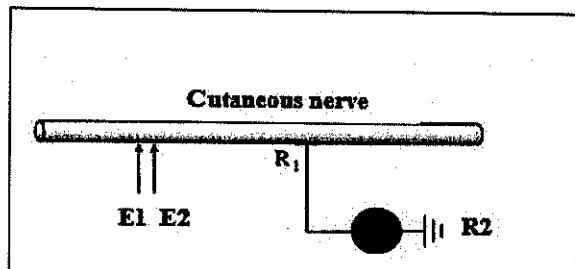
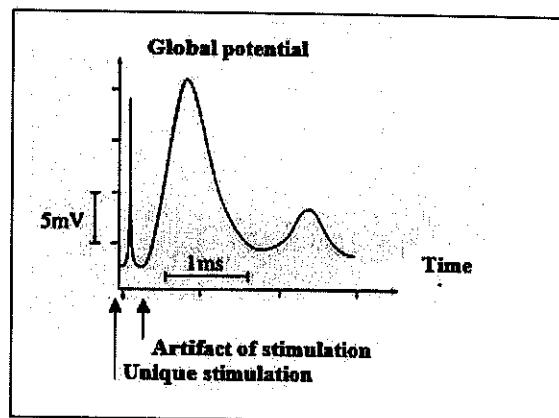
1. Interpret the experimental results of each of the two documents 1 and 2.
2. Explain, by referring to the acquired knowledge, the steps of the transmission of the nervous message at the level of a neuro-muscular synapse.

Exercise 13 (4 pts) Global potential

Session 2006-2

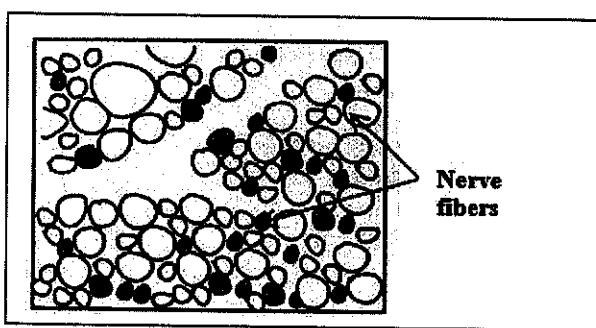
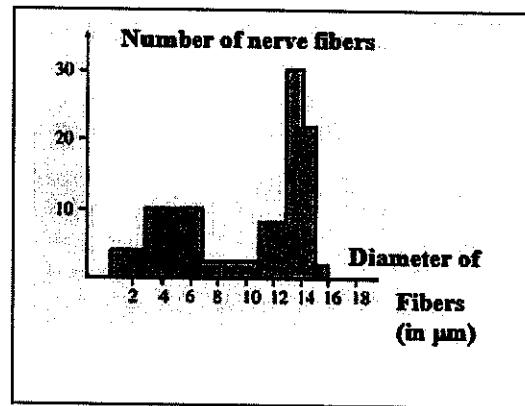
In the framework of studying the electric activity of a mammalian nerve, we establish the experimental set-up shown in document 1. E1 and E2 are stimulating electrodes while R1 and R2, which are placed far from E1 and E2, are recording electrodes. R1 is placed on the surface of the nerve and R2 is connected to a fixed potential.

By the help of E1 and E2, we apply a unique stimulus on the nerve, of intensity above threshold. The response of the nerve to this stimulus is shown in document 2, where the curve shows two successive global potentials instead of one global potential.

**Document 1****Document 2**

1. Draw out the problem, which arises in this study.
2. Formulate a hypothesis that explains the obtained recording.

In order to verify the formulated hypothesis, studies are done on this nerve whose results are shown in documents 3, 4, and 5.

**Document 3: Transverse section done at the level of the nerve****Document 4: Distribution of the nerve fibers according to their diameters**

The speed of propagation of the action potential is 50 meters per second in the nerve fibers having a diameter around 14 μm ; and is 10 meters per second in the nerve fibers having a diameter around 4 μm .

Document 5

3. Is the hypothesis that you have formulated validated? Justify the answer in reference to documents 3, 4, and 5.
4. Explain the difference in the amplitude between the two global potentials obtained?

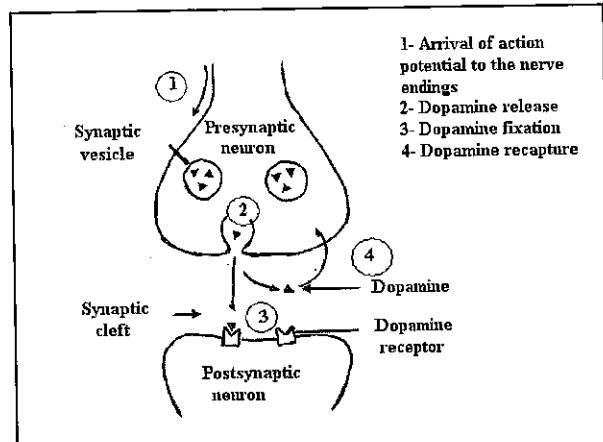
Exercise 14 (4½ pts) Action of cocaine

Session 2007-1

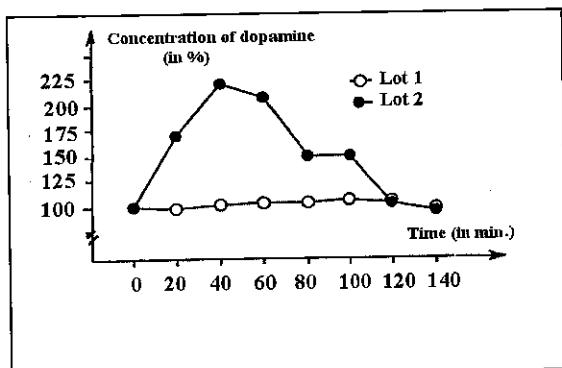
Studies are done to show the mode of action of cocaine at the level of the dopamine synapse. Dopamine is a cerebral neurotransmitter. Document 1 reveals the functional diagram of the dopamine synapse.

1. Write a short text describing the mode of action of this synapse.

We measure the concentration of dopamine in the synaptic cleft in two lots of rats. The rats of lot 1 are kept as a control and those of lot 2 received an injection of cocaine at time $t = 0$ minute. The results are shown in document 2.



Document 1



Document 2

2. Interpret the obtained results.

Based on documents 1 and 2:

3. Propose two hypotheses that explain the mode of action of cocaine at the level of this synapse.

Document 3 indicates the effects of cocaine on the nervous system.

Cocaine disrupts the fragile balance that allows the few billions of neurons of our brain to function... In the brain, the privileged target of cocaine, are the neurons that secrete dopamine. Normally, the neurotransmitter substances are liberated by a neuron and passes into the synaptic cleft to fix on receptors of the next neuron. Some are recaptured by a specific pump to be liberated later when needed. Cocaine blocks this pump of dopamine recapture. As a consequence: the neurotransmitter stimulates the neighboring neurons permanently. Under the repeated effects of cocaine, the neurons adapt to the abnormally elevated concentration of this substance. The brain is thus, forced to maintain an increased production of this neurotransmitter. This production can only be maintained by the frequent consumption of the drug. This leads to the anxious behavior of cocaine addict in constantly searching for the drug.

Document 3

4. Indicate which of the two proposed hypotheses is validated in document 3? Justify the answer.
5. Pick out from the text the statements that indicate that cocaine consumption leads to tolerance.

Exercise 15 (5 ½ pts) Characteristics of the synaptic transmission

Session 2007-2

To understand the mechanism of transmission of the nervous message at the level of a synapse, experiments were carried out on two neurons N_1 and N_2 of a squid, using the setup that appears in document 1.

1st experiment: The nerve fiber of N_1 was stimulated by S_1 . An action potential was recorded in R_1 then in R_2 .

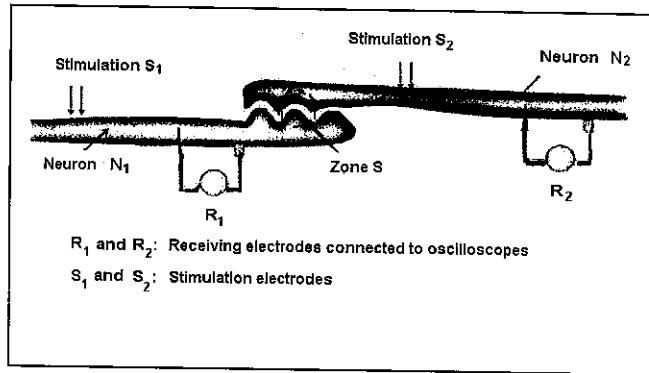
2nd experiment: The nerve fiber of N_2 was stimulated by S_2 . An AP was recorded only in R_2 .

3rd experiment: A micro-drop of acetylcholine was deposited at the level of zone S between N_1 and N_2 . An AP was recorded only in R_2 .

4th experiment: A micro-drop of acetylcholine was injected in neuron N_1 and another drop in N_2 . No AP was recorded in R_1 or R_2 .

1. Interpret the results of the 1st and 2nd experiments.

2. Deduce from the 3rd and 4th experiments the level of the nerve cell at which acetylcholine acts. Documents 2a and 2b present electronic micrographs of the synapse at the level of zone S at two different moments.

**Document 1****Document 2a****Document 2b**

3. Specify the state in which this zone was found when each of the micrographs was taken.

The study of a synapse made it possible to establish the relation between the frequency of the presynaptic action potentials, the number of vesicles that release their neurotransmitter, and the quantity of acetylcholine liberated into the synaptic cleft. Document 3 shows the results.

4. Determine, based on the analysis in document 3, how the nervous message is coded during synaptic transmission.
5. Explain, based on what preceded, and with reference to acquired knowledge, how the nervous message is transmitted at the level of the synapse.

Frequency of presynaptic AP (a.u)	1	2	4	6
Number of vesicles (in thousands)	1	2	4	6
Quantity of acetylcholine (in a.u)	100	200	400	600

Document 3

Exercise 16 (5 pts) Muscles involved in myotatic reflex

In the framework of studying some aspects of the control mechanism of muscle activity during dancing, studies were carried out and summarized in document 1.

The movements of a dancer are performed in sequences, which are not always predictable, since each of these movements is triggered by an intention: the body is then used as a means of expression.

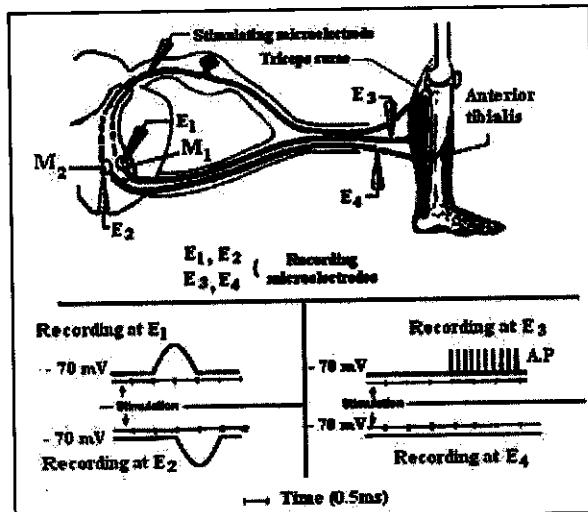
However, any body's movement is hindered by a force – gravity – which attracts it towards the ground. To control body movement and to reach equilibrium, the dancer uses muscles that block some joint movements and prevent falling down. The posture is thus maintained thanks to a constant adjustment of the muscle: for instance, every time a muscle is stretched, it contracts.

Document 1

- 1- Pick out from document 1 a statement that justifies the presence of a myotatic reflex, and another one that justifies the presence of a voluntary motor activity.
- 2- Indicate the nerve center responsible for each of these activities.

In order to understand the functioning of the neurons' circuits implied in maintaining posture during dancing, and to know how muscles interfere in maintaining the body's equilibrium the following experiments are performed.

1st experiment: A nerve fiber issued from a neuromuscular spindle of an extensor, the triceps surae is stimulated. This stimulation leads to modifications of the electric status of two motor neurons, M₁ and M₂, located at the level of the grey substance of the spinal cord. One of these motor neurons innervates the extensor while the other innervates the flexor: the anterior tibialis. Document 2 reveals the experimental set up and the results of the recordings.

**Document 2**

- 3- Analyze the obtained recordings.
- 4- Draw out the effect of the activity of the motor neurons on the concerned muscles.
- 5- Determine, by referring to the recordings E₁ and E₂, the number of synapses implied in each of the concerned neurons' circuits knowing that the transmission of a nerve message at the level of a synapse needs 0.5 ms.

2nd experiment: Experiment 1 is repeated and at the same time we stimulate a nerve fiber issued from the superior nerve centers, related to motor neuron M₂ that is linked to anterior tibialis. Many action potentials were recorded at E₄ and no recording was obtained at E₃.

- 6- Specify, based on the obtained results, the effect of this stimulation on both muscles.

Myotatic reflex is a muscle response triggered by a stimulus whose receptor is the neuromuscular spindle.

Tapping the Achillean tendon provokes the stretching of the foot's extensor muscle into variable lengths. Simultaneously, we record the nerve message transmitted all along a nerve fiber issued from the neuromuscular spindle of this muscle. The results are shown in document 1.

- Interpret the recordings obtained.

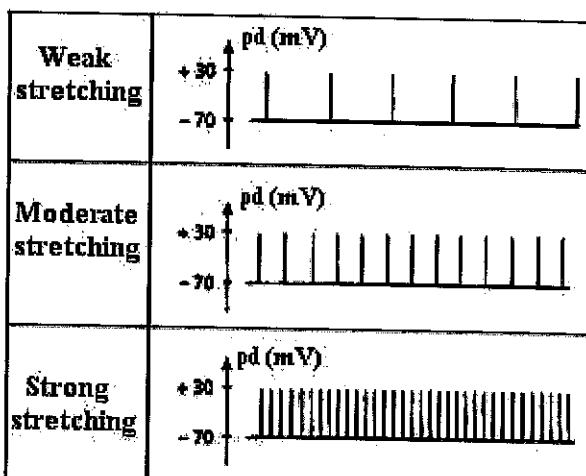
The nerve fibers issued from the neuromuscular spindles are connected, inside the spinal cord either directly or by means of interneurons, to the motor neurons of two muscles: one is an extensor, and the other is a flexor.

The activity of these motor neurons is recorded in response to an afferent message. The results are shown in document 2. For each recording obtained, arrow "1" corresponds to the beginning of the stimulation and arrow "2" corresponds to the end of the stimulation.

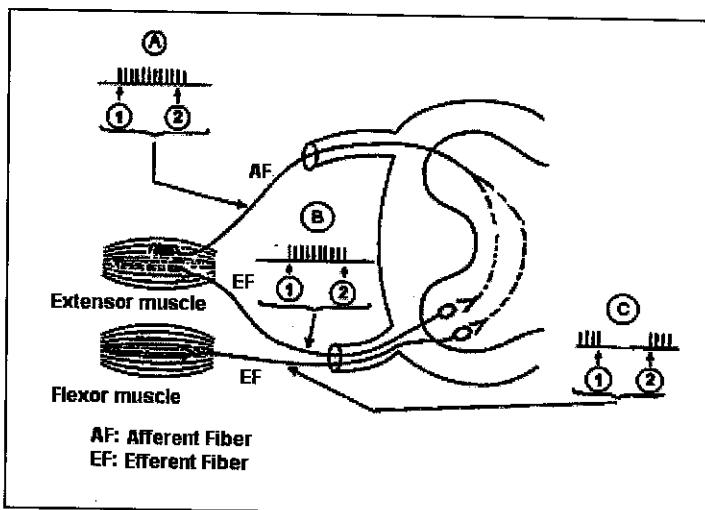
- Compare the obtained recordings.
- Explain the role of the spinal cord in the establishment of this reflex and specify the neuronic circuit implied.

The tensions of the extensor and flexor foot's muscles during this reflex were recorded as shown in document 3.

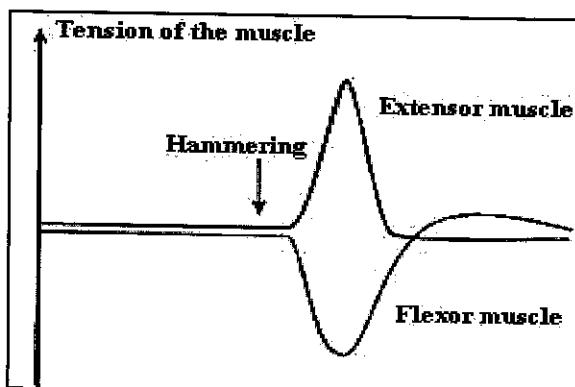
- Indicate the movement that was done. Justify your answer based on documents 2 and 3.
- What can these muscles be qualified as? Justify the answer.



Document 1



Document 2



Document 3

Exercise 18 (5 pts) Action of benzodiazepine

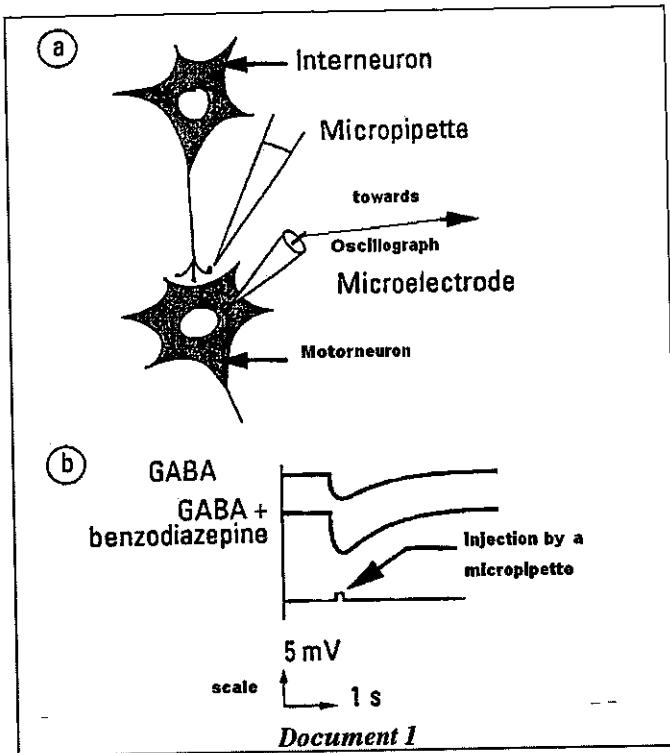
Nowadays, certain molecules that belong to the benzodiazepines family are used in treating anxiety.

In order to study the action of these molecules on the muscular activity, we record by means of a microelectrode the electric activity of the postsynaptic motor neuron following the injection of GABA and/or benzodiazepine into the synaptic cleft using a micropipette. The experimental set up (a) and the results (b) are represented in document 1.

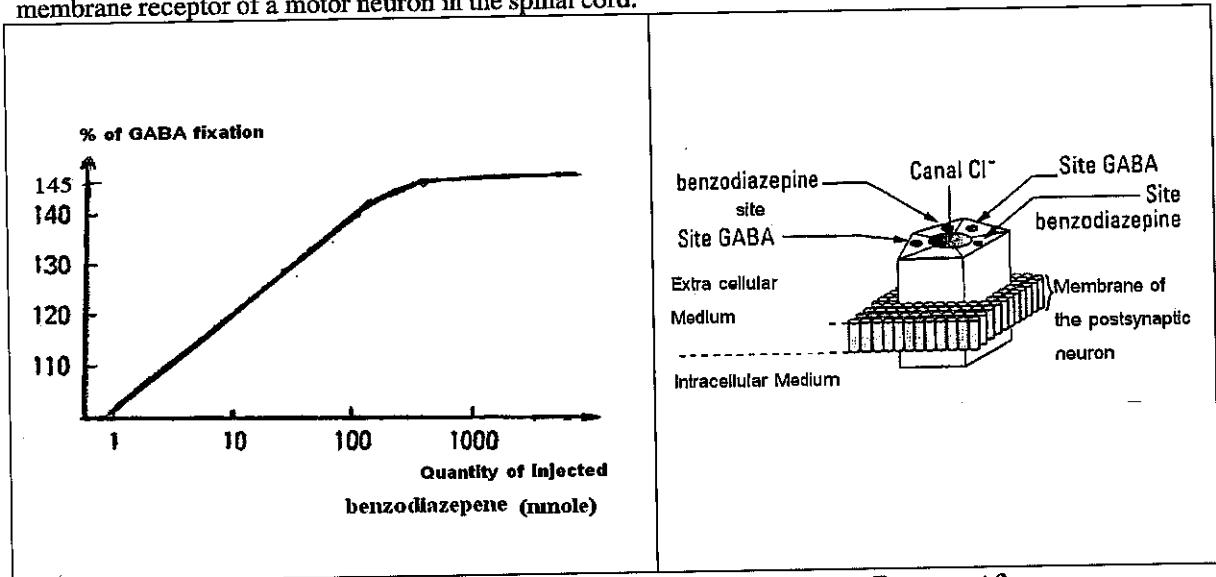
- Determine, in reference to document 1, the nature of the studied synapse.

Researchers found that rats administered a chemical substance, called picrotoxin, exhibit involuntary muscular contractions accompanied by signs of anxiety.

- Specify the effect of picrotoxin and benzodiazepine at the level of this synapse.



In order to understand thoroughly the mode of action of benzodiazepene, we measure the percentage of fixation of GABA on its receptors in function of the quantity of benzodiazepene injected into the synaptic cleft. The results are shown in document 2. Document 3 reveals the organization of the membrane receptor of a motor neuron in the spinal cord.



- Represent, in a table, the data shown in document 2.
- What can you deduce starting from document 2?
- Explain, based on the information derived from all documents, the mode of action of benzodiazepene on the muscular activity.

Exercise 19 (5 pts) Role of morphine

Session 2009-2

Pain sensation necessitates the intervention of several neuronic circuits. Document 1 represents the structures implicated in pain sensation and in its modulation.

We study certain mechanisms which control the transmission of nociceptive message or pain message, in an attempt to show the mode of action of enkephalin and morphine.

In two different experiments 1 and 2, we stimulate at S1 the nociceptors of the skin using the same effective intensity and we record the electric activity of three nerve fibers:

- Sensory nerve fiber by an electrode E1 connected to oscilloscope O1;
- Nociceptive medullary nerve fiber by an electrode E2 connected to oscilloscope O2; and
- Nerve fiber of enkephalin interneuron by an electrode E3 connected to oscilloscope O3.

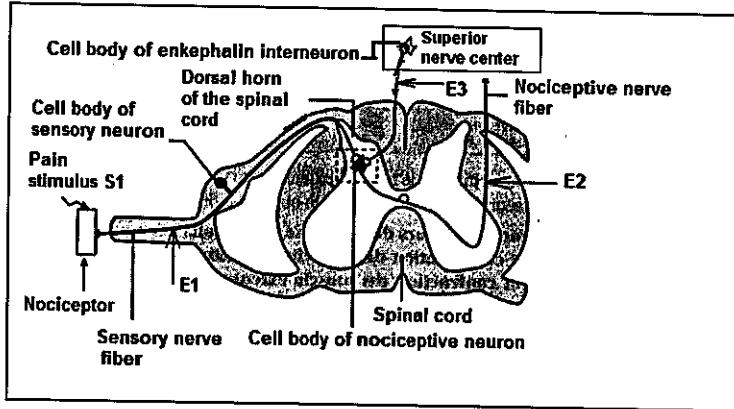
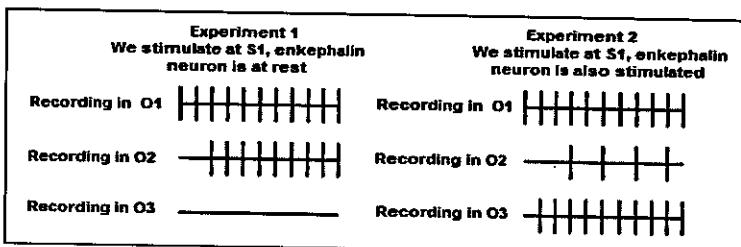
The conditions and the obtained results are shown in document 2.

1- Draw out, in reference to document 2, the role of enkephalin. Justify the answer.

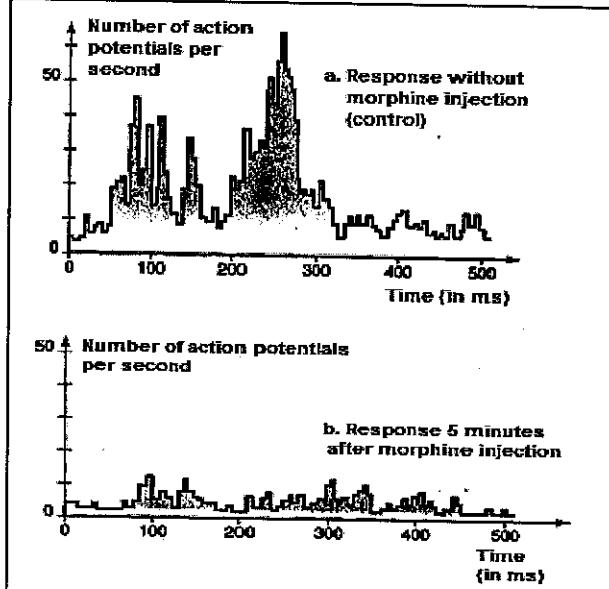
2- Explain how the results recorded by O2 in experiment 2 put in evidence the integrative role of the nociceptive neuron.

In the framework of studying the action of morphine on the medullary nociceptive neuron, we perform experiments 3 and 4.

Experiment 3: By the help of a microelectrode, we record the activity of the medullary nociceptive neuron at the level of the dorsal horn after an intense electric stimulation of the sensory fibers.

**Document 1****Document 2**

N.B. Each vertical line corresponds to an action potential (A.P.)

**Document 3**

Experiment 4: Under the same conditions of experiment 3, we also record the activity of the medullary interneuron after the injection of a morphine dose by a micropipette at the level of the dorsal horn. Document 3 shows the obtained results.

3- Determine, from document 3, the role of morphine. Morphine and enkephaline are agonist substances.

4- Justify this statement.

Exercise 20 (5 pts) Properties of motor neuron

In order to study the characteristics of a nerve message in an achillian reflex before and after it passes through the spinal cord, we use the experimental set up presented in document 1 and we realize the experiments described below.

The experimental set up in document 1 shows the location of the stimulating electrodes on the afferent fibers and that of recording electrodes on different oscilloscopes. Oscilloscope O1 permits recording the effect of stimulations of one or more afferent fibers; Oscilloscopes O2 and O3 permit recording the electric responses of motor neurons M1 and M2 respectively at the level of the implantation cone. Oscilloscope O4 permits recording the electric activity at the level of the axon of motor neuron M1.

Experiment 1 : We apply two successive effective stimulations on one of the afferent fibers Fa1, and we vary the time between these two stimulations. The results, recorded by O2, are shown in document 2.

1- Interpret the results obtained in document 2.

Experiment 2 : We apply stimulations of increasing intensities on the afferent fibers and we record the results on the four oscilloscopes (document 3).

2- Explain the recordings obtained by O1.

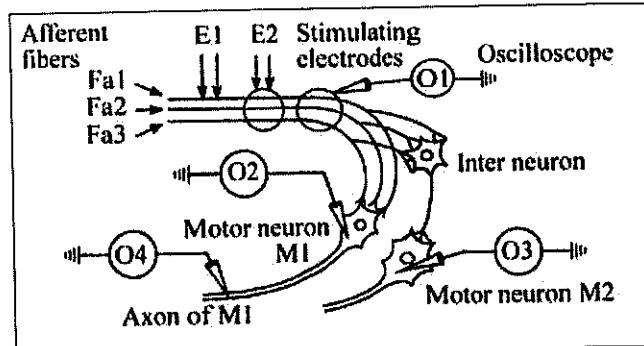
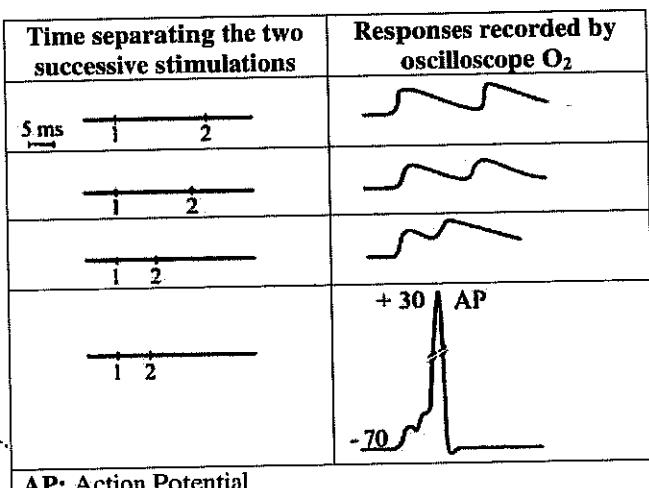
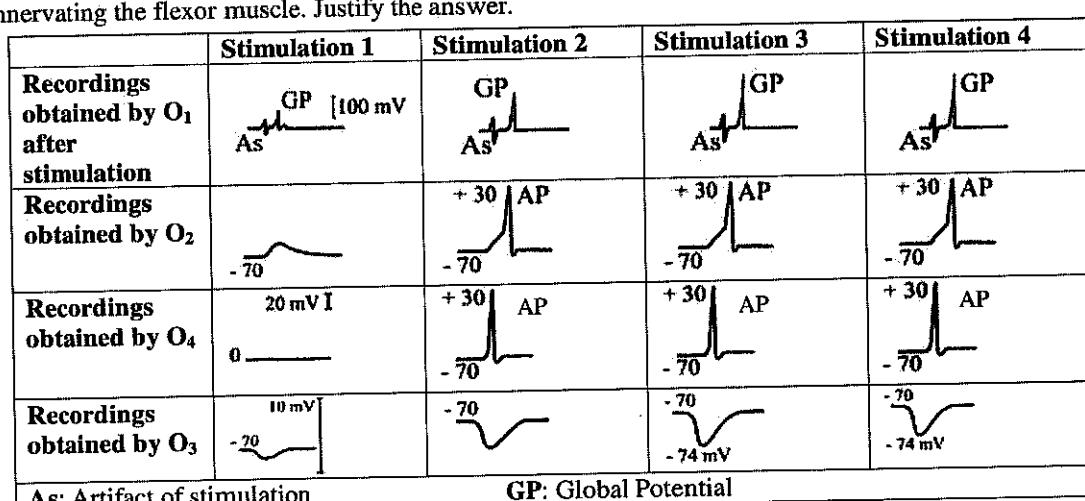
3-1-Compare the recordings obtained by O2 and O4.

3-2- Draw out the corresponding characteristics of the nerve message.

4- Indicate the type of summation which is revealed by experiment 2 at the level of the motor neuron M1. Justify the answer.

Each of the motor neurons M1 and M2, innervates a muscle intervening in the achillian reflex that provokes the extension of the foot.

5- Specify, by referring to documents 1 and 3, and by using the acquired knowledge, the motor neuron innervating the flexor muscle. Justify the answer.

**Document 1****Document 2****Document 3**

Exercise 21 (5 pts) Neuromuscular disease

Session 2010-2

Myasthenia is a neuromuscular disease characterized by a difficulty in performing efficient muscular contractions. In order to determine the cause of this difficulty we performed experiment 1.

Experiment 1

A microelectrode introduced into a muscle fiber of the leg muscle permits the recording of the electrical activity, obtained in the case of a healthy individual (A) and a myasthenic individual (B), following the stimulation of the motor neuron.

Document 1 shows the experimental set-up and the results.

1. Analyze the obtained recordings.
2. Specify, by referring to document 1 and to the acquired knowledge, the physiological consequences that can be observed at the level of the muscles of these two individuals.

Physicians thought that the abnormal functioning of the neuromuscular junction might be at the origin of myasthenia.

Document 2 presents the organization of the neuromuscular junction or motor end plate.

3. Label each of the structures 1, 2, 3 and 4 of document 2.

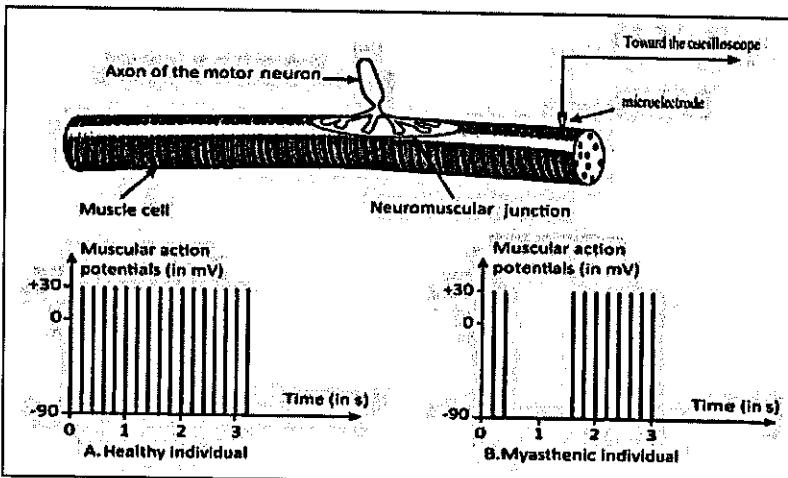
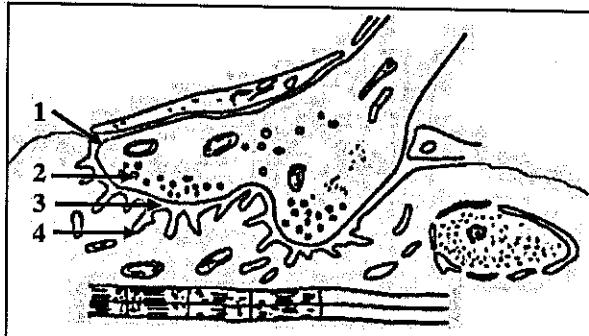
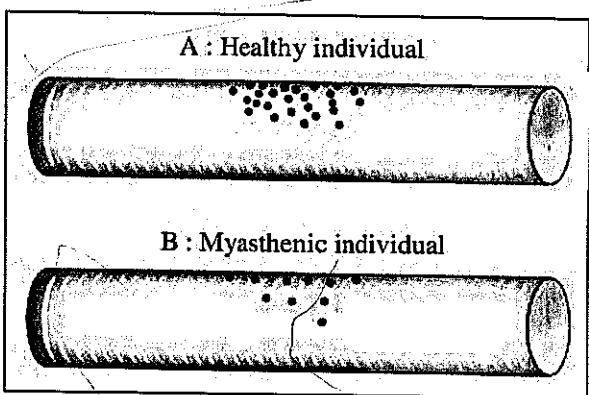
In order to determine the origin of this disease we performed experiment 2.

Experiment 2

- α -bungarotoxin, a toxic molecule extracted from some snakes' venom, has a spatial configuration that is similar to that of acetylcholine which is the neurotransmitter of the neuromuscular synapse. α -bungarotoxin has the capacity to bind acetylcholine receptors. Its injection to a healthy mouse induces immediately symptoms that are similar to those of myasthenia.
- Biopsies of muscular tissues are performed to a healthy individual (A) and to a myasthenic individual (B).

Removed cells are placed in presence of radioactive α -bungarotoxin. This toxin is then localized, by autoradiography, on the membrane of a muscle cell in the form of black grains. Document 3 shows the obtained results.

4. Compare the two autoradiographies A and B of document 3.
5. Determine, referring to experiment 2, the origin of myasthenia.
6. Referring to the information drawn out from documents 1 and 3, write a text that explains the symptoms of this disease.

**Document 1****Document 2****Document 3**

Exercise 22 (5 pts) Achillean reflex and voluntary movement**Session 2011-1**

An individual can control or inhibit an achillian myotatic reflex by voluntary muscle activity. Several experiments were performed in order to explain the interaction between voluntary activities and reflexes. The experimental set ups and results are presented in documents 1, 2 and 3.

Document 1 presents the structures involved in the achillian reflex.

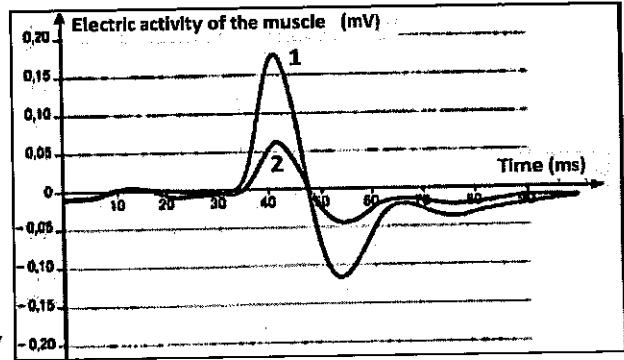
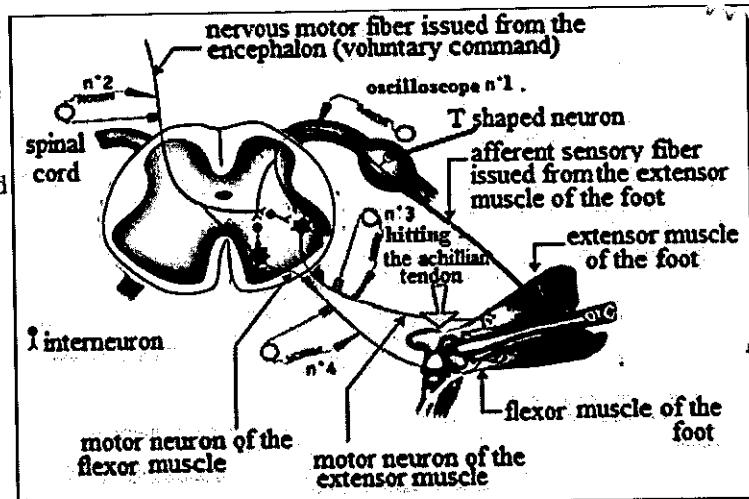
Document 2 shows the electromyogram of the extensor muscle of the foot upon hitting the Achillian tendon in the absence of voluntary flexion of the foot (curve 1) and during slight voluntary flexion of the foot (curve 2).

- 1- Interpret the results of document 2.

Document 3 presents the recordings of the electric activity of the neuronal network involved in the achillian reflex obtained under the same experimental conditions as those of document 2.

- 2- Match each of the cases A and B in document 3 to its corresponding curve 1 or 2 in document 2. Justify the answer.
- 3- Explain the results obtained at the level of oscilloscope n° 3 in document 3 in the cases A and B.

We ask this individual to perform a strong voluntary flexion of his foot before hitting the achillian tendon.

Document 1**Document 2**

- 4- Based on document 3, draw in this case, the recordings obtained at the level of the oscilloscopes n° 1, 2, 3 and 4. Justify the answer for each recording.

Recordings of the activity of the neuronal network	Oscilloscope			
	n° 1	n° 2	n° 3	n° 4
Case A		—		—
Case B				

Document 3

N.B: Each vertical line corresponds to an action potential

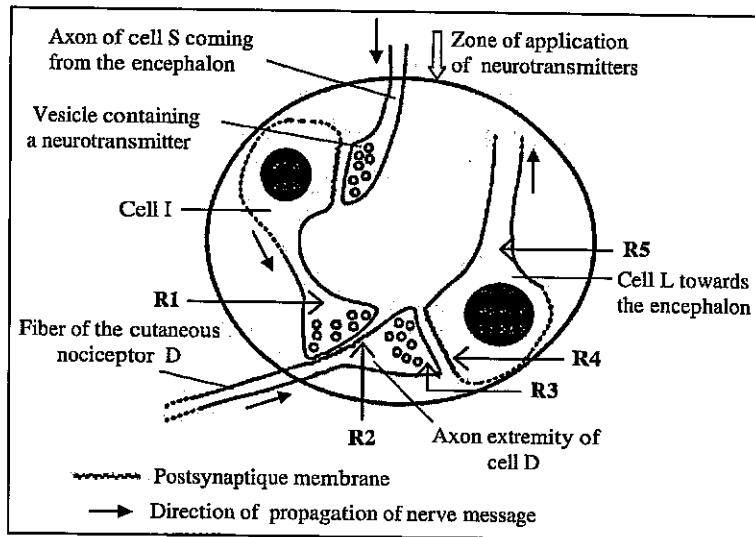
Exercise 23 (5 pts) Neurotransmitters and pain

Session 2011-2

In the posterior horn of the spinal cord, we observe cells I in addition to extremities of fibers of cells D and S as well as cell bodies of cells L (doc.1).

In the framework of studying the transmission of the pain message, we apply the same molar concentration of neurotransmitters, enkephalin or substance P, in the defined zone of document 1.

We record, using the microelectrodes R1, R2, R3 and R4, the membrane potentials of cells I, D, and L with respect to a reference potential. The results are presented in document 2.

**Document 1**

	Evolution of the membrane potentials at the level of the recording electrodes			
	R1	R2	R3	R4
Application of enkephaline	-70 —	-70 ~	-70 —	-70 —
Application of substance P	-70 —	-70 —	-70 —	-70 ~

Document 2

- 1- Specify the role and site of action of each of the used neurotransmitters.

We stimulate a cutaneous nociceptor D whose fibres are responsible for the slow transmission of intense and prolonged pain. We stimulate again the same cutaneous nociceptor D with the application of serotonin neurotransmitter.

The obtained recordings of R1, R2, R3 and R5 of these experiments are shown in document 3.

	Evolution of the membrane potentials at the level of the recording electrodes			
	R1	R2	R3	R5
Case A: Stimulation of the cutaneous nociceptor D without application of any substance	-70 —	AP 0 -70	0 -70	0 -70
Case B : Stimulation of the cutaneous nociceptor D with the application of serotonin	0 -70	-70 ~	-70 —	-70 —

Document 3

- 2- Interpret the obtained results in case A.
 3-2- Compare the recordings obtained in case B to those obtained in case A.
 3-2- Draw out the role and the site of action of serotonin.
 4- Explain, from what precedes, how the encephalon interferes in blocking the transmission of the pain message.

Exercise 24 (5 pts) Activity of antagonistic muscles

Session 2012-1

In order to study the coordinated behavior of the flexor and the extensor muscles of the leg during a reflex action in mammals, we record in a spinal animal (an animal whose spinal cord was sectioned), the variations in the intensity of the contraction of the quadriceps, the extensor muscle of the leg, under the effect of stretching starting from time 0. Keeping the extensor muscle stretched, at moment S, we stretch muscle X and at moment B we stretch simultaneously both muscles X and Y of the thigh. Document 1 shows the obtained recordings.

- 1- Name the type of reflex occurring between 0s and 1s. Justify the answer.
- 2- Interpret the obtained results.

To better understand the role of the spinal cord in this reflex, we performed effective stimulations S1

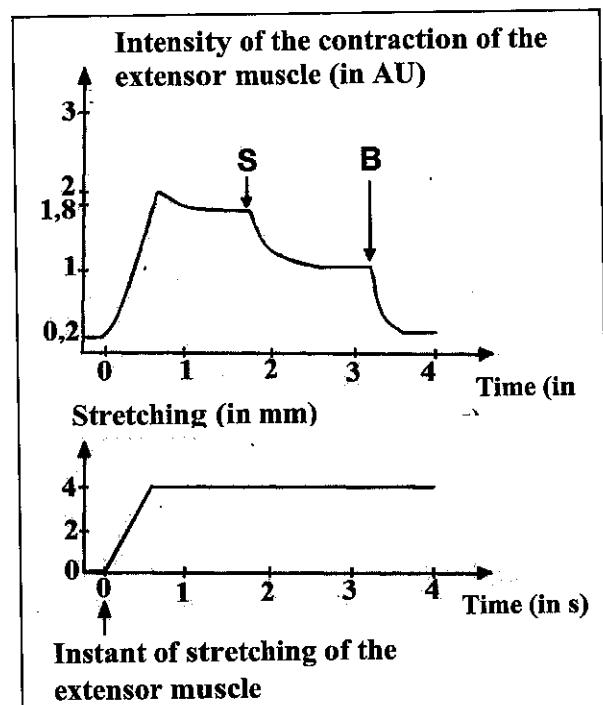
and S2 respectively on each of the afferent fibers 1 and 2 issued from the muscles mentioned above. Document 2 represents the neural circuits in the spinal cord and the experimental set up.

Document 3 shows the recording obtained at the motor neuron M of the extensor muscle for each of the performed stimulations.

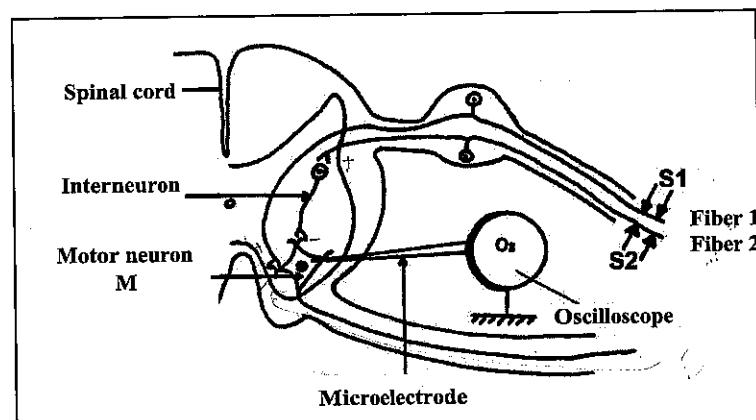
- 3- Match each of the two afferent fibers 1 and 2 to its corresponding muscle involved in this reflex (extensor, muscle X or muscle Y).

Justify the answer by specifying the type of each involved synapse (documents 2 and 3).

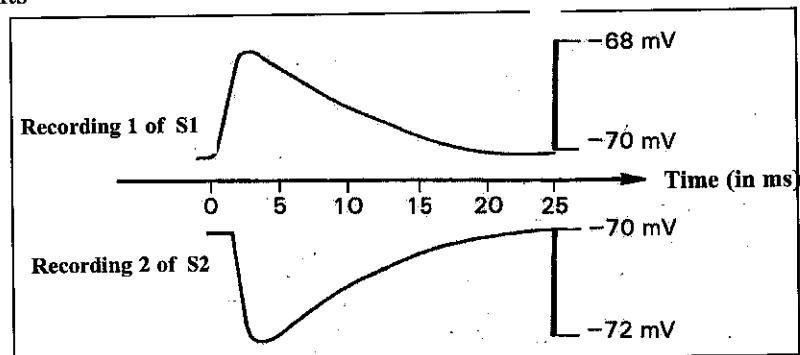
- 4- Explain, on what was preceded, the role of the motor neuron M at moment S.



Document 1



Document 2

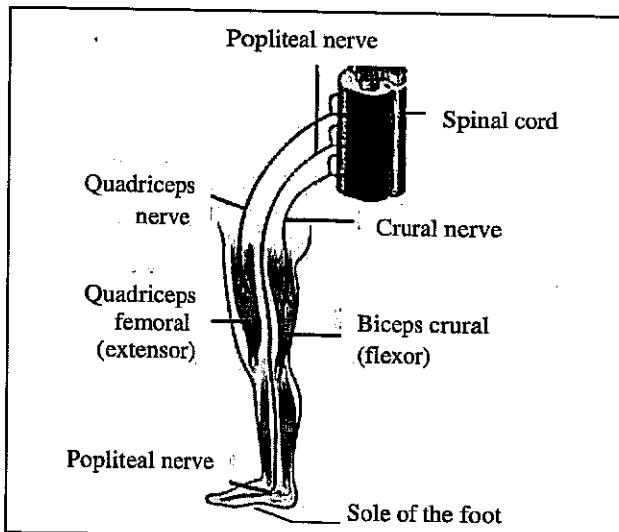


Document 3

Exercise 25 (5 pts) Protection reflex

In a man who has been accidentally subjected to a section in the upper level of his spinal cord, the contact of a hot object with the skin of the sole of the foot causes systematically a protection reflex that is manifested by the flexion of the corresponding lower limb. We aim to study the mechanisms implicated in such a response.

Document 1 shows the muscles and the nerves involved in such a protection reflex. Document 2 represents the results of an experimental study performed on a spinal animal (cat) having only the spinal cord as a nervous center. The muscle structure and the innervation of this animal are similar to those of humans.

**Document 1**

Experiments	Popliteal nerve	Crural nerve	Nerve of the quadriceps
Sectioning of the nerve	disappearance of the flexion of the lower limb	disappearance of the contraction of the biceps crural	disappearance of the contraction of the quadriceps femoral
Excitation of the central end*	flexion of the lower limb	No reaction	No reaction
Excitation of the peripheral end *	No reaction	contraction of the biceps crural	contraction of the quadriceps femoral

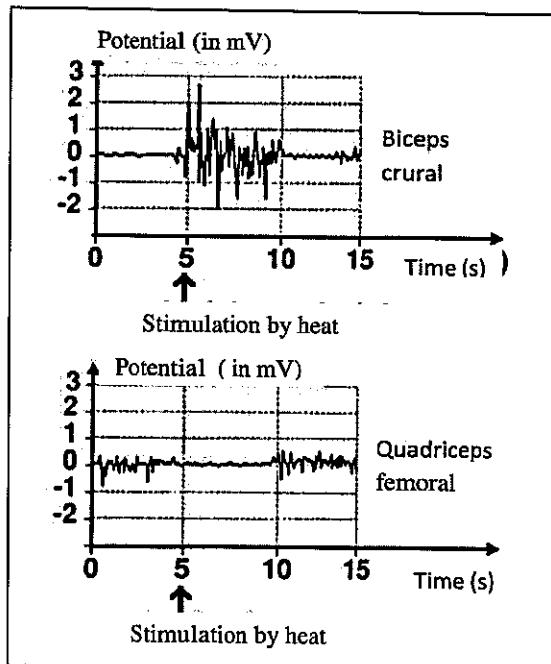
* At the level of the sectioning of a nerve, the end that is still attached to the nervous center is called the central end whereas the end that is still attached to the peripheral organs (muscle or skin) is called the peripheral end.

Document 2

- Specify, based on the experimental results, and for each nerve, whether it plays an afferent / sensory role or an efferent / motor role in this reflex. Justify the answer.

Document 3 represents the electromyograms recorded at the level of the biceps crural and the quadriceps femoral before and after stimulation by heat at time 5 seconds.

- Compare these electromyograms. What can you draw out?
- Draw a functional diagram relating the structures involved in this protection reflex.
- Give one difference between the protection reflex and the myotatic reflex.

Document 3

Exercise 26 (5 pts) Ecstasy, euphoria or depression?

Ecstasy is a synthetic drug derived from amphetamine. Its effects are described in the text below:

« ...if the quantity of the consumed ecstasy is limited, the consumer becomes euphoric, very talkative, and feels extreme happiness. This phase can last 2 to 4 hours depending on the dose and the individual's sensibility. It is followed by a "descent" period marked by exhaustion and even a strong depressive syndrome... »

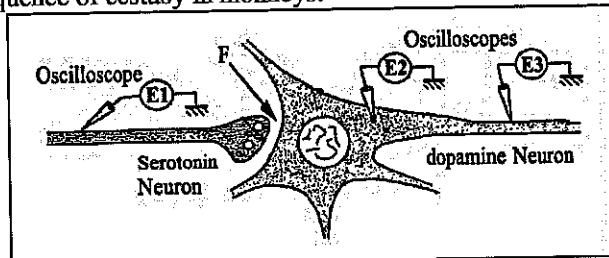
In monkeys, on the long term, ecstasy provokes irreversible destruction of neurons. In humans, we could assume that there is neuronal destruction that can be permanent ... »

1. Pick out from the text :

1.1- The effect of ecstasy 1 hour and 5 hours after consuming a limited dose of ecstasy.

1.2- The statement that shows the long term consequence of ecstasy in monkeys.

To better understand the effects of ecstasy on the nervous system, the activity of a dopamine-releasing neuron connected to a serotonin-releasing neuron (document 1) is studied. For this reason, two successive stimulations separated by different intervals of time are applied on the serotonin-releasing-neuron.



The obtained results are shown in document 2.

Document 1

Conditions	Recordings of E1	Recordings of E2	Recordings of E3
2 stimulations separated by a long time interval			
2 stimulations separated by a short time interval			

Document 2

- Determine if the synapse F is excitatory or inhibitory.
- Indicate, at the level of dopamine-releasing neuron, the type of summation revealed by this experiment. Justify the answer.
- Justify, by referring to document 2, the following expression: "Only the action potential propagates at the level of a neuron".

Pleasure sensation is related to the activity of certain dopamine-releasing neurons situated in the encephalon. Document 3 summarizes the effects of consuming ecstasy on the serotonin-releasing neurons and dopamine-releasing neurons.

Measured parameters at the level of neurons	Serotonin-releasing neurons				Dopamine-releasing neurons
	Frequency of action potentials at the level of serotonin-releasing neurons	Activity of serotonin synthesis	Amount liberated serotonin	Activity of the pump that recaptures serotonin	Frequency of action potentials at the level of dopamine-releasing neurons
Without ecstasy	++	++	++	++	++
0 to 4 hours after the consumption of ecstasy	++	++	++++	+	++++
Beyond 4h from ecstasy consumption	++	0	0	Not measured	+

Document 3 N.B : the number of + indicates the importance of the phenomenon

- Explain the intervention of the serotonin-releasing neurons and the dopamine-releasing neurons after ecstasy intake in the cases:
 - sensation of euphoria.
 - state of depression.

Exercise 27 (5 pts) LSD and hallucinations

Session 2013-2

Albert Hofmann is best known for discovering a powerful synthetic drug, the LSD. In one of his books, he described his sensations after he voluntarily took this drug in the frame work of experimental automedication.

« Everything in my field of vision was oscillating and distorted as if seen in a curved mirror. I also had the sensation that the bike was not moving even though my assistant told me later that we have been moving fast. When I arrived home, dizziness and weakness sensation were more serious in a way that I couldn't stand up and was obliged to lie down on a sofa-bed.
Later, I noticed that the way all acoustic perceptions, such as the sound of a door handle or that of a car passing by the house, were transformed into visual perceptions. Every sound generated a corresponding animated image with a particular form and color. »

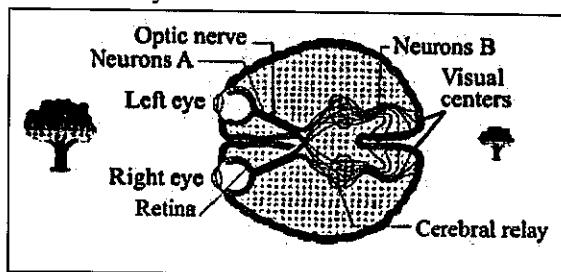
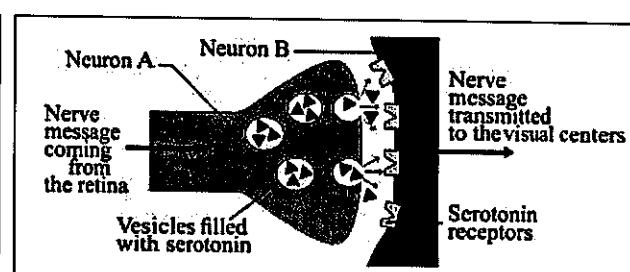
Document 1

- 1- Knowing that hallucination is defined as «perception without any object to perceive», show that the LSD is a powerful hallucinogen.
- 2- Justify that the LSD doesn't modify only the visual perceptions of the individual.

To better understand the action of LSD and its effects, the following studies are performed.

Stimulations applied on neurons A produce visual perceptions. Document 2 shows the encephalic visual pathways involved in these types of perceptions.

Document 3 represents the scheme of the synapse between the two types of neurons A and B at the level of the cerebral relay.

**Document 2****Document 3**

Effective stimulations of increasing intensities ($I_1 < I_2 < I_3$) are applied on neuron A. The amount of serotonin in the synaptic cleft is measured and the nervous message at the level of neurons A and B are recorded. The results are shown in document 4

Intensity	Frequency of AP at the level of the neuron A	Amount of serotonin (in AU)	Frequency of AP at the level of the neuron B
I_1	5	1.5	8
I_2	9	2.5	13
I_3	12	3	18

Document 4

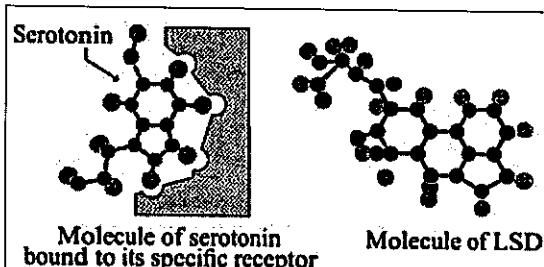
- 3- Explain the steps of synaptic transmission of the nervous message coming from the retina via neurons of type A before reaching the visual centers.

- 4- Draw a histogram showing the variation of the amount of serotonin as a function of the intensity of stimulation.

5-1- Analyze the obtained results.

5-2-Draw out the form in which the nervous message is coded at the level of the neuron as well as that at the level of the synapse.

Document 5 shows the molecular structure of serotonin and that of LSD.

**Document 5**

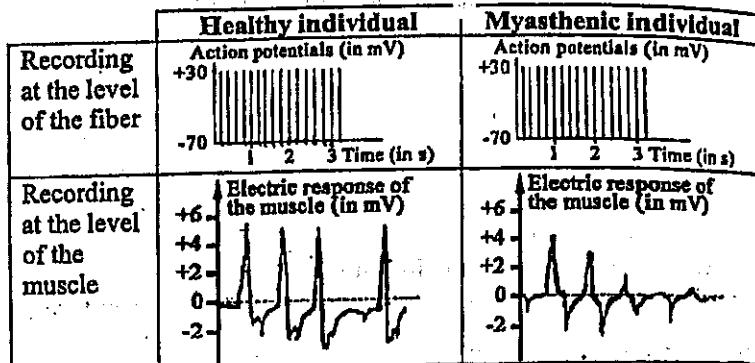
- 6- Suggest, referring to all what precedes, an explanation of the mode of action of LSD in the genesis of visual hallucinations.

Exercise 28 (5 pts) Myasthenia

Myasthenia is a neuromuscular disease characterized by weakness of skeletal muscles. It can affect any muscle; patients can hardly keep their eyelids open, have respiratory difficulties... To better understand the biological mechanisms leading to the previously indicated symptoms, researchers performed the following studies.

- In two persons, one being healthy and the other myasthenic, an effective stimulation is performed directly on a muscle. Identical muscular contractions are observed for the two persons.

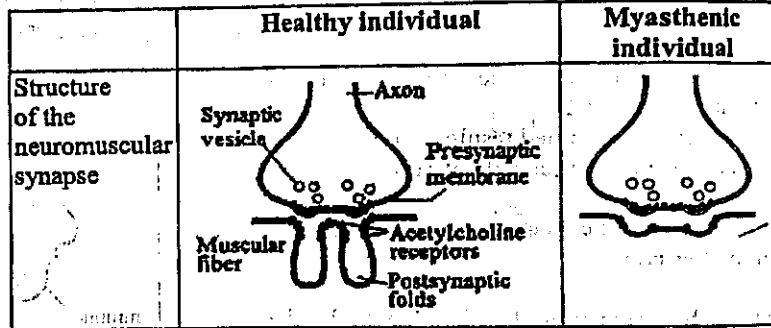
- A motor neuron is effectively stimulated and the electrical activity of its fiber and that of its corresponding muscle are recorded using receptor microelectrodes. The results are presented in document 1.



Document 1

- Show that myasthenia is due to a synaptic malfunction.

Antibodies X that are absent in healthy persons are detected in the blood of myasthenic individuals. The injection of these antibodies to a healthy animal induces temporary myasthenia.



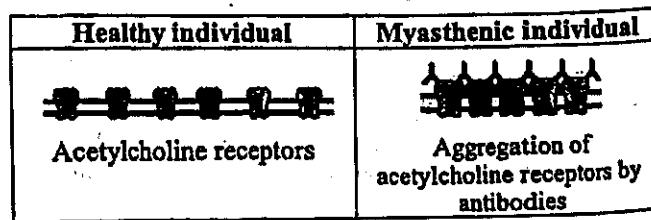
Document 2

- Determine the origin of this disease.
- Formulate two hypotheses concerning the mode of action of antibodies X.

- An electrography of the neuromuscular synapse is performed in two individuals; a healthy one and a myasthenic one. Document 2 shows the schemes of the structure of the observed synapses.
- Compare the synaptic structures (doc.2) in these two individuals.

In order to determine the origin of the observed differences, a more advanced observation of the neuromuscular synapse is performed.

Document 3 shows the postsynaptic membrane in a healthy individual and in a myasthenic one.



Document 3

- Draw, by referring to document 3, the post synaptic potential recorded following an effective stimulation of the motor neuron for each of the healthy and the myasthenic individual.
- Explain, based on what precedes, the mechanisms that occur in the body and that are at the origin of the symptoms observed in a myasthenic individual.

Exercise 29 (5 pts) Mode of action of Botox

Session 2015-1

Botulinum toxins are at the origin of a serious disease called Botulism. This disease affects all the muscles and may lead to paralysis of the respiratory muscles thus causing death. However, these toxins are frequently used by all men and women who want to eliminate the signs of aging (anti-wrinkles treatment). This is realized by injecting these toxins "Botox" every 6 months.

In order to determine the mode of action of Botox, the following experiments are performed.

Experiment 1: In a physiological culture medium, using an appropriate experimental set up, four effective stimulations of increasing intensities are applied on a motor neuron that innervates a skeletal muscle.

For each of the applied stimulations, a muscular contraction is observed. The frequency of action potentials at the level of the presynaptic motor neuron (doc.1), the concentration of calcium in the presynaptic terminal bud (doc.2), and the quantity of acetylcholine released in the synaptic cleft (doc.3) are measured for each of the applied stimulations.

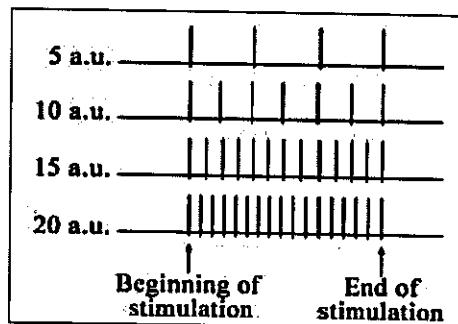
- 1- Interpret the obtained results in document 1.
- 2- Draw the curve that shows the variation of the quantity of acetylcholine as a function of the intensity of stimulation.
- 3- Specify the type of coding of the nervous message that is revealed by each of the documents 2 and 3.

Experiment 2: Botox is added to the culture medium of the experimental set up of experiment 1. The same stimulations as well as the same measurements are repeated. Same results as those of experiment 1 are obtained except for the quantity of the released acetylcholine. In addition, no muscular contraction is observed for the 4 intensities of stimulation.

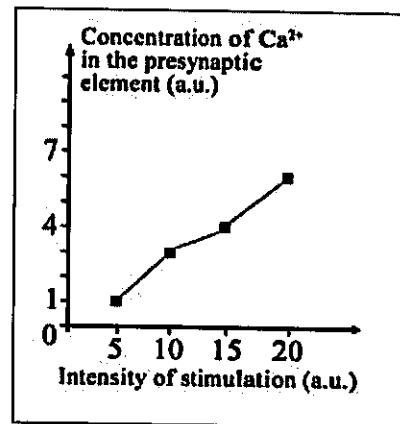
- 4- Formulate a hypothesis explaining the mode of action of Botox on the transmission of the nervous message.

Experiment 3: The presynaptic vesicles of the motor neuron of a frog are labeled by a fluorescent dye. This neuron is placed in a medium with or without botulinum toxin. The intensity of fluorescence inside the presynaptic bud is measured before and after stimulating this neuron. The results are presented in document 4.

- 5- Determine, referring to experiment 3, the quantity of acetylcholine that should be released in experiment 2.
- 6- Explain how Botox eliminates the signs of aging without causing death by intoxication.



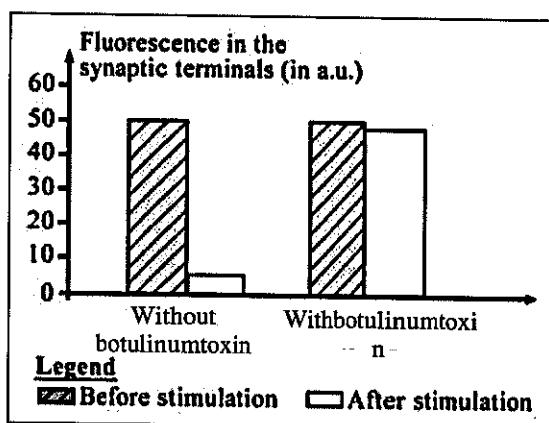
Document 1



Document 2

Intensity of stimulation (a.u.)	Quantity of released acetylcholine (a.u.)
5	30
10	40
15	50
20	60

Document 3

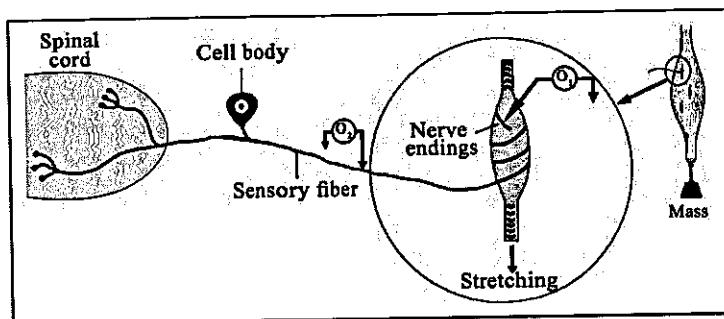


Document 4

Exercise 30 (5 pts) Nervous coding of sensory information

When a dog is kept on a leash, the muscles of the arms react immediately to all the traction variations they undergo. This is a reflex.

In order to study the coding of the message involved in this reflex, the following experiments are performed using the experimental set up presented in document 1.



Document 1

Experiment 1: The arm muscle is stretched five times using increasing masses. The obtained responses are recorded by oscilloscope O1 at the level of the neuromuscular spindle (document 2) and by oscilloscope O2 at the level of the sensory nerve fiber (document 3). Meanwhile more and more important contractions are observed at the level of the stretched muscle.

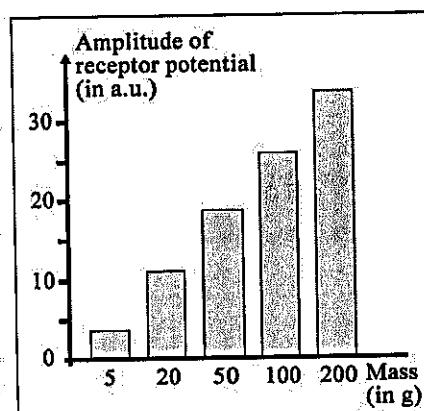
- 1- Show that this is a "myotatic reflex".
- 2- 2-1- Analyze the results of each of the documents 2 and 3.
2-2 Conclude the type of coding of the nerve message at the level of the neuromuscular spindle and at the level of the sensory fiber.

Experiment 2: This muscle is subjected twice to the same effective stretching of 750 µm at different velocities. The response for each stretching is recorded at the level of the sensory fiber. The obtained results are presented in document 4.

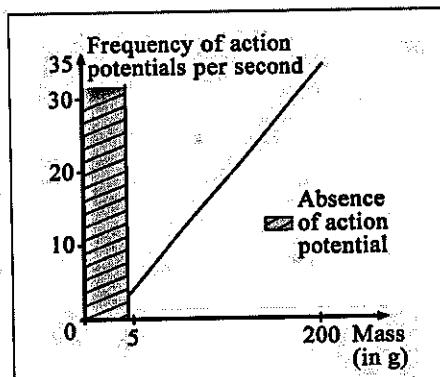
- 3- What can you draw out from document 4?

Experiment 3: The arm muscle, like in experiment 1, is stretched five times using increasing masses. The amount of the neurotransmitter (acetylcholine) that is released at the level of the synapse involved in the neural circuit of this reflex is measured. The obtained results are shown in document 5.

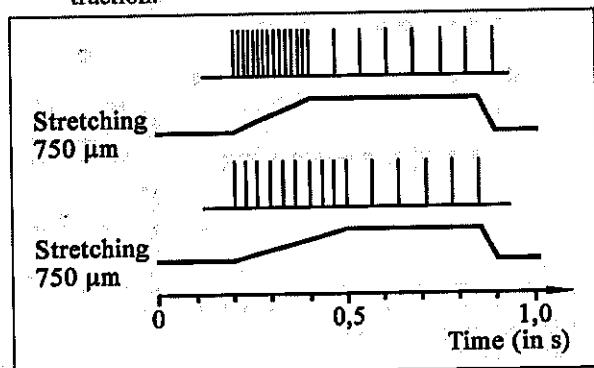
- 4- Draw the curve showing the variation of the amount of released acetylcholine as a function of the intensity of stretching.
- 5- Deduce the type of coding of the nervous message at the level of a synapse.
- 6- Show how the arm muscles react in an adapted way to each traction.



Document 2



Document 3



Document 4

- 7- Schematize the neuronal circuit and the structures involved in this reflex.

Intensity of stretching (in a.u.)	5	10	15	20	25
Amount of released acetylcholine (in a.u.)	20	30	40	50	60

Document 5

Exercise 31 (5 pts) Analgesia without morphine

Session 2016-1

Morphine is an analgesic substance (pain-killer) that acts at the level of enkephalin and endorphin synapses. The latter substances are neurotransmitters that are naturally produced in the brain and in the spinal cord, while morphine is exogenous. Its excessive usage causes physical and psychological dependence as well as respiratory and digestive troubles.

- 1- Explain how morphine acts at the level of enkephalin synapses.

In order to avoid the secondary effects of the use of morphine, researchers have tried to find other endogenous analgesic substances. Some of their studies are represented in the following experiments.

Experiment 1: Researchers have injected serum to rats without or with analgesic, morphine or endorphin.

Then, they put each rat in zone P of a box whose surface is divided into two zones: zone P (periphery) that is covered by sharp ends causing intense pain, and zone S (center) without sharp ends. Then, during three minutes, they measured the average duration during which the rats stayed in zone P. This duration indicates the analgesic effect of the studied substance. Document 1 shows the conditions as well as the results of the experiment.

Animals	Injections	Duration of staying in zone P (sec)
A	-	5
B	Morphine (6 mg/kg)	72
C	Endorphin (6 mg/kg)	5

Document 1

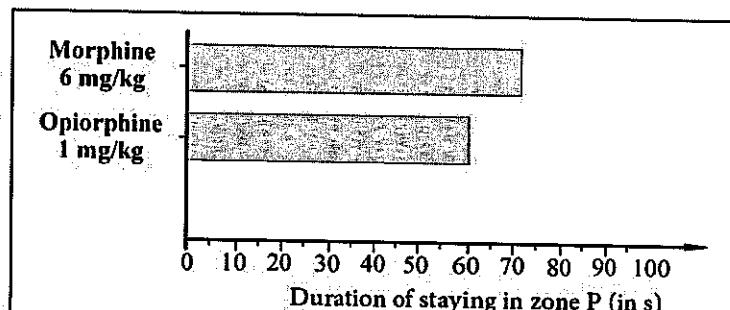
- 2- What can you deduce from experiment 1?
- 3- Formulate a hypothesis explaining the result obtained upon the injection of endorphin.

Experiment 2: Researchers have injected endorphin marked by radioactive tritium in the blood of a group of animals. The performed tests reveal the absence of radioactivity in the brain and in the spinal cord. Moreover, even in blood, endorphin disappears rapidly, but other radioactive molecules appear.

- 4- Show that experiment 2 explains the result obtained in rats C.

Experiment 3: Other researchers of Pasteur institute have identified a new analgesic substance, secreted naturally in the saliva of humans, the opiorphine. They have tested opiorphine on rats. They repeated experiment 1 but they injected opiorphine instead of endorphin. The experimental conditions as well as the results are represented in document 2.

Document 3 shows information concerning opiorphine.

**Document 2**

- 5- Show, by referring to document 2, that opiorphine is an effective analgesic.
- 6- Explain how opiorphine acts as an analgesic.
- 7- Draw out two reasons why opiorphine seems to be a molecule whose therapeutic value is more important than that of morphine.

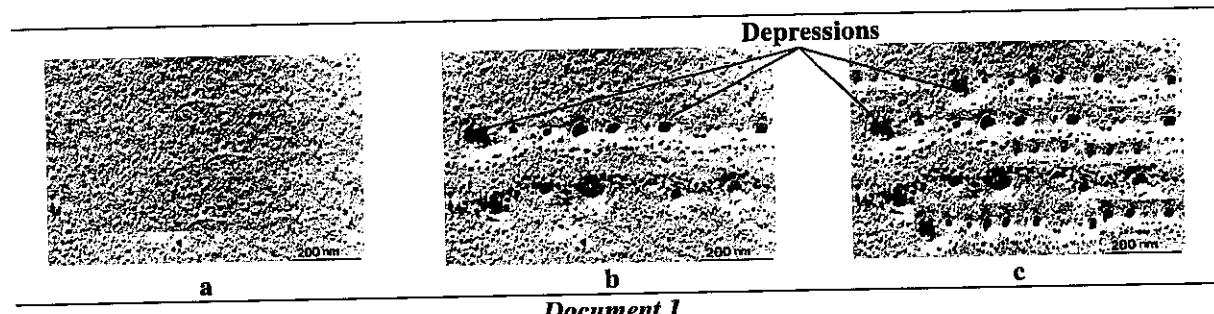
Opiorphine has an analgesic power for thermal and mechanical pain as well as chronic pain. Opiorphine seems to protect enkephalin from the effects of the enzyme NEP present in the cleft of enkephalin synapses. It is not necessary to increase the doses of opiorphine to obtain the same anti-nociceptive effect. It doesn't cause constipation and its addictive effect is much reduced.

Document 3

Exercise 32 (5 pts) Synaptic transmission**Session 2016-2**

Nervous messages are transmitted along the nerve fibers and traverse synapses. In order to study the mechanisms of the synaptic transmission and the effect of certain exogenous substances, Norcuron and TEPP, the following studies were performed.

Study 1: electronographs of the external side of the presynaptic membrane were performed in different cases: case "a" where the presynaptic neuron is not stimulated, and cases "b" and "c" where this neuron is stimulated respectively with increasing intensities I_1 and I_2 which are above the threshold. The results are shown in document 1. The depressions represent the fusion of the vesicles with the presynaptic membrane.

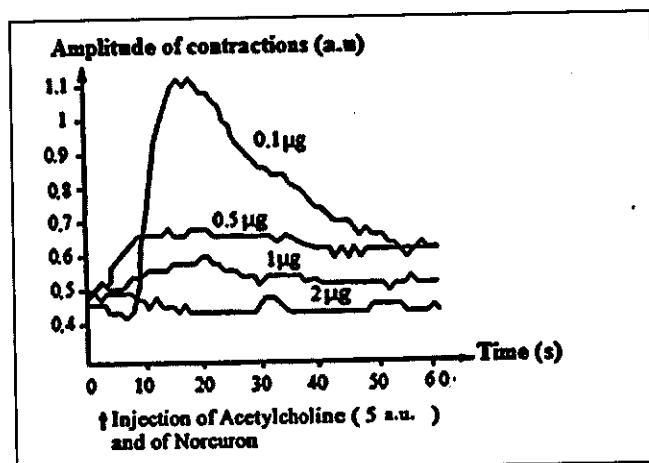
**Document 1**

- Justify, based on document 1, that the exocytosis of neurotransmitters at the level of a synapse is amplified with the increase of the intensity of stimulation.

Study 2: an experimental set up is used to measure the quantity of acetylcholine released in the synaptic cleft of a neuromuscular synapse as well as the amplitudes of the muscular contractions in the three cases "a", "b" and "c" of study 1. The obtained recordings are shown in document 2.

- Draw a histogram showing the variation of the quantity of acetylcholine and that of the amplitude of the muscle contraction in the three cases "a", "b" and "c".
- Indicate the type of coding of the nervous message at the level of a synapse. Justify the answer by referring to document 2.

Case	a	b	c
Quantity of Acetylcholine (a.u.)	1	3	5
Amplitude of the contraction (a.u.)	0.1	0.5	1.5

Document 2**Document 2**

Study 3: in the synaptic cleft, 5 a.u. of acetylcholine are injected simultaneously with increasing amounts (from $0.1\mu\text{g}$ to $2\mu\text{g}$) of Norcuron, a substance that has similar molecular structure to that of acetylcholine. Document 3 shows the recordings of muscular contractions obtained for each amount of Norcuron.

- What can be deduced from the results of document 3?

Study 4: TEPP is injected in insects. Symptoms characterized by a period of convulsions followed by permanent contraction of muscles are observed.

- Determine whether each of the substances TEPP and Norcuron is agonist or antagonist relative to acetylcholine.

Exercise 33 (5 pts) Effect of an insecticide

Session 2017-1

Farmers use organophosphorous insecticides to kill insects. Some of these insecticides such as pyrethrum alter the function of the nervous system thus blocking respiration leading to death by asphyxia. In fact, the respiratory movements are ensured by contractions followed by relaxations of the respiratory muscles. In order to better understand the mode of action of pyrethrum, the following experiments are performed.

Experiment 1: the gastrocnemius muscle of a frog and the nerve connected to it are immersed in a physiological medium. An effective stimulation of intensity I is applied on this nerve in the presence and absence of pyrethrum. For each stimulation, the amplitude and the duration of the muscle contraction are recorded. The results are presented in document 1.

- 1- Represent in a table the results of document 1.
- 2- 2-1- compare the obtained results.
2-2- what can you conclude?
- 3- Formulate two hypotheses explaining the mode of action of pyrethrum.

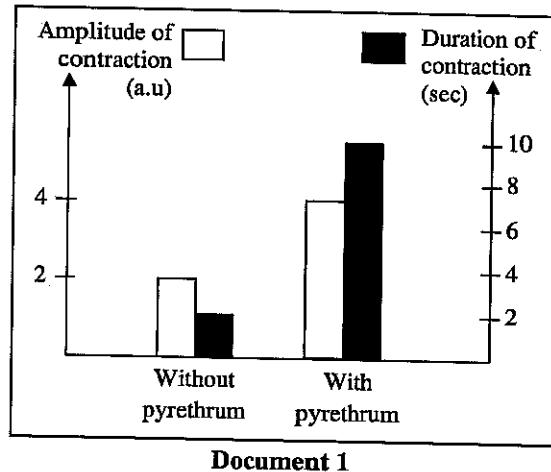
Document 2 shows the ultrastructure of the neuromuscular synapse.

- 4- Identify, which of the structures 1, 2, or 3 corresponds to the presynaptic neuron.

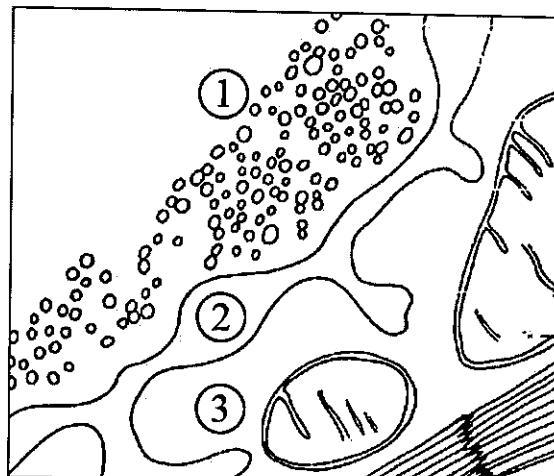
Experiment 2: a micro-drop of pyrethrum marked by radioactive phosphorus is injected at the level of the neuromuscular synapse. Concentrated radioactivity is observed at the level of the synaptic cleft.

Profound analyses show that the pyrethrum molecules are associated with acetylcholinesterase, an enzyme that degrades acetylcholine molecules that are fixed on the receptors of the postsynaptic membrane.

- 5- Explain, referring to what precedes, how can pyrethrum lead to death by asphyxia.



Document 1



Document 2

Exercise 34 (5 pts) Control of reflex

Session 2017-2

In order to understand how the myotatic reflex can be controlled, many studies are carried out on different fibers, the sensory fibers and motor fibers involved in this reflex.

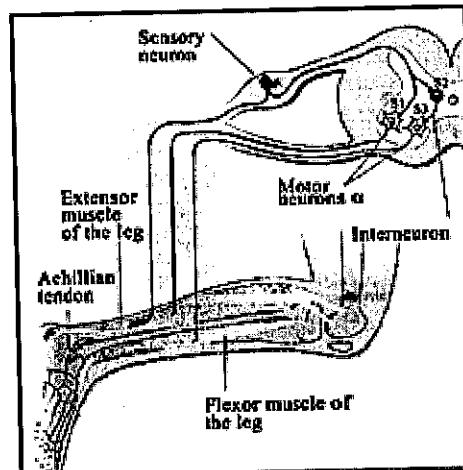
1- Define the myotatic reflex.

Study 1:

The extensor muscle is stretched and the sensory and the motor messages are recorded in two different situations: in the first situation the flexor muscle is at rest, and in the second situation the flexor muscle is strongly stretched.

Document 1 shows the concerned muscles with their nervous connections.

Document 2 shows the experimental conditions as well as the obtained recordings during the same duration in the two situations.



Document 1

2- Compare the neuronic circuits innervating these antagonistic muscles involved in this reflex.

	Situation 1 Flexor muscle at rest	Situation 2 Flexor muscle strongly stretched
Electrical recordings	Fiber issued from the neuromuscular spindle of the extensor muscle	
	Fiber issued from the motor neuron α innervating the extensor muscle	
	Fiber issued from the motor neuron α innervating the flexor muscle	

Document 2

3- Determine, based on the results of the first situation (doc 2), the contracted muscle and the relaxed one.

4- Indicate the role of the interneuron.

5- Explain the role of the motor neuron α of the extensor muscle in the second situation.

Study 2

The extensor muscle is stretched and the activities of the sensory fiber and the motor fiber of this muscle are recorded with or without the voluntary contraction of the flexor muscle. The results are presented in document 3.

Stretching of the extensor muscle		
	Flexor muscle at rest	Voluntarily contracted flexor muscle
Electrical recordings	Fiber issued from the neuromuscular spindle of the extensor muscle	
	Fiber issued from the motor neuron α innervating the extensor muscle	

Document 3

6- Deduce the action of the superior nerve centers on the studied reflex.

Exercise 35 (5 pts) Cause of muscle paralysis

Session 2018-1

In the framework of studying certain cases of muscle paralysis, researchers carried on experiments on animals which exhibit complete paralysis of their muscles. In order to determine the origin of this paralysis, the following experiments are performed on a normal animal another paralyzed one. These experiments are performed on the motor neuron N connected to muscle M by synapse F.

Experiment 1:

Effective stimulations are directly applied on muscle M in each of the two animals. Muscular contraction is observed in both cases.

Experiment 2:

Effective stimulations are applied on motor neuron N innervating muscle M in each animal. The results and the experimental conditions are shown in document 1.

1. Show that the paralysis of this animal is due to dysfunctioning of the synapse.

A group of researchers formulate the following hypotheses concerning the cause of the synaptic dysfunctioning in the animal affected by muscle paralysis.

Results of effective stimulation of motor neuron N		
Normal animal	Nerve Message at the level of motor neuron N	Contraction of muscle M
Paralyzed Animal	Nerve Message at the level of motor neuron N	No contraction of muscle M
Document 1		

H1: Muscle paralysis is due to the blockage of exocytosis of acetylcholine in the synaptic cleft.

H2: Muscle paralysis is due to nonfunctional postsynaptic receptors of acetylcholine.

H3: Muscle paralysis is due to a deficiency in the production of acetylcholine by the presynaptic neuron.

These researchers performed experiments 3, 4, and 5 to verify these hypotheses.

Experiment 3:

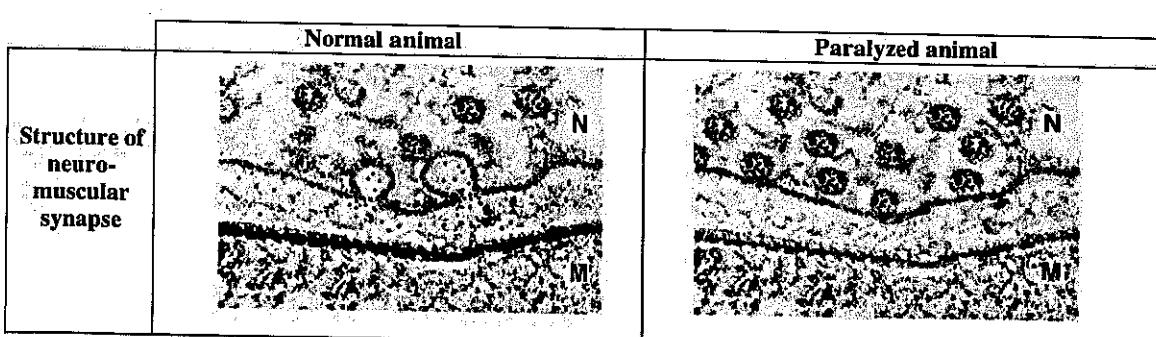
The analysis of the content of the synaptic vesicles of the neuromuscular synapse in the paralyzed animal reveals the presence of acetylcholine, similar to that in the normal animal.

Experiment 4:

Acetylcholine in the neuromuscular synapse of the paralyzed animal is extracted and injected into the synaptic cleft between N and M, in both the paralyzed animal and the normal animal. Contraction of muscle M is observed in both animals.

2. Determine, after studying the results of each of the experiments 3 and 4, the two rejected hypotheses.

Experiment 5: Radioactive choline, a substance transformed by the neuron into acetylcholine, is injected into neuron N of the normal and paralyzed animals. Then, neuron N in both animals is stimulated. Document 2 shows the electromyographies of the synapse after nervous stimulation. The radioactivity appears in the form of black spots.



Document 2

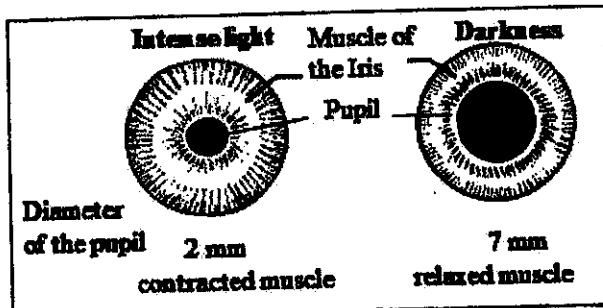
3. Specify the cause of muscle paralysis in the animal.

Exercise 36 (5 pts) Action of atropine

The diameter of the pupil, an orifice in the eye through which the light penetrates, is controlled by a muscle (the iris). This diameter varies with light intensity, document 1.

Ophthalmologists use medicine such as "atropine" which allows the examination of the eye.

A study is performed to determine the mode of action of atropine.

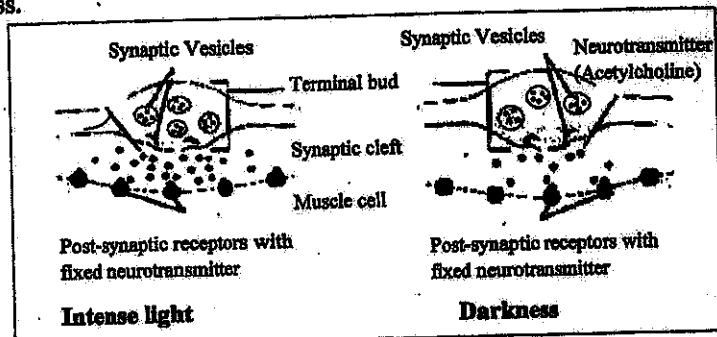
**Document 1**

- 1.1. Compare the aspect of the pupil and the muscle of the iris in light and in darkness.

- 1.2. Draw out the effect of light on the muscle of the iris.

At the level of the iris, the muscle fibers form excitatory cholinergic synapses with the ends of motor neurons.

Document 2 shows the functioning of these neuromuscular synapses in intense light and in darkness.

**Document 2**

2. List the steps of the synaptic transmission.

3. Justify, referring to document 2, the amplified muscle contraction in the presence of light.

Document 3 shows the amplitude of contraction of the muscle of the iris, in the presence and absence of atropine, as a function of the concentration of acetylcholine in the synaptic cleft.

- 4.1. Analyze the obtained results.

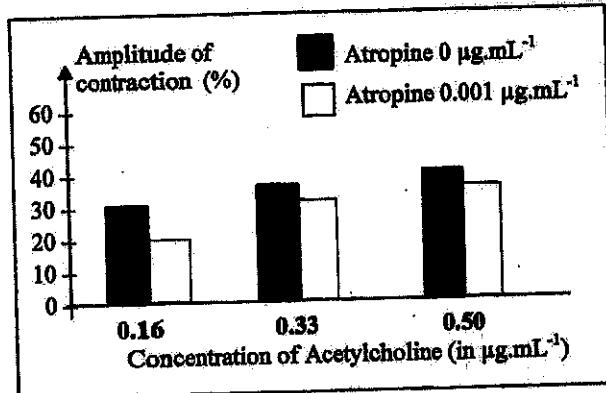
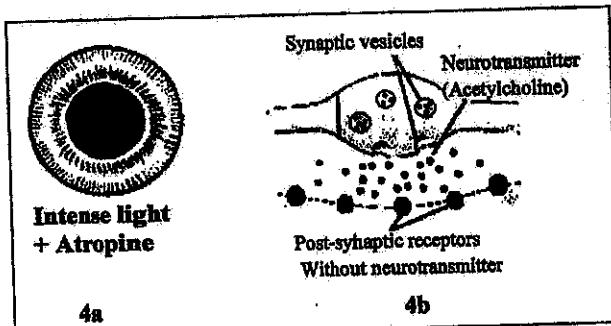
- 4.2. Conclude the effect of atropine on muscle contraction.

Document 4 shows the aspect of the pupil (4a) and the functioning of the neuromuscular synapse (4b) in intense light, after the application of a droplet of atropine in the eye of an individual.

5. Compare the aspect of the pupil in document 4a to each of the two aspects shown in document 1.

6. Draw out the step of the synaptic transmission at the level of which atropine acts.

7. Explain, based on what precedes, the use of atropine by ophthalmologist to provoke dilation of the pupil even in the presence of intense light.

**Document 3****Document 4**

Nervous system
Official exercises answer key

Exercise 1 (4.5 pts) Transmission of nervous message

1. After stimulation of F1 we observe a slight hypopolarization in the motor neuron (M1) without generating an action potential in A. The PSP obtained has lower amplitude (20 mv) than threshold depolarization.
Stimulation F1 + F2 provokes a greater hypopolarization still not enough to generate an action potential in A.
There is spatial summation, but the EPSP is still below the threshold of depolarization.
Stimulation F1 + F2 + F3 provokes a hypopolarization in M1 followed by an action potential of amplitude 80 mv in A. There is spatial summation; the EPSP obtained has a higher amplitude than the threshold depolarization. (1½ pt)
2. The interneuron has an inhibitory role because there is registration of hyperpolarization at M2 whose amplitude is of 4 mv. (1 pt)
3. In myotatic reflex, the motor neuron M1 is connected to the muscle that is stretched and that contracts whereas the motor neuron M2 is connected to the antagonistic muscle that relaxes. (1 pt)
4. The arrival of nerve impulse at the presynaptic membrane (1) provokes the liberation by exocytose of the neurotransmitter molecules present in the synaptic vesicles (2). Then these molecules bind to the postsynaptic membrane receptors (3) which trigger a postsynaptic potential (PSP) (4). Finally, the neurotransmitter is destroyed by a specific enzyme (5) and reabsorbed by the presynaptic neuron (6). (1 pt)

Exercise 2 (4 pts) Number of synapses in a circuit

1. The response D2 shows a prolonged form with variations in amplitude. Since we have one excitation (there is one artifact of stimulation), the obtained response is caused by the fact that the pulling action on the muscle gives rise to action potentials which are transported with different velocities, probably by different nerve fibers types (different diameters or myelination). The response D2 corresponds then to a global potential of a nerve. (1 pt)
2. L1 and L2 represent the delay necessary for the response to appear respectively in D1 and D2 (latency period for response) (1/2 pt)
L3 represents then the time necessary for a nerve message to pass from the dorsal root to the ventral root. (1/2 pt)
3. From document 2 we can estimate this delay:
 $L3 = L1 - L2 = 3.2 \text{ ms} - 1.9 \text{ ms} = 1.3 \text{ ms}$. (1/2 pt)
4. We can verify that this time (1.3 ms) permits the passage only by one synapse. Therefore, the myotatic reflex is monosynaptic. (1/2 pt)

Exercise 3 (2 ½ pts) Types of synapses

1. After the stimulation of A, E1, placed near to the synapse of the fiber A, recorded a hypopolarization having an amplitude of 5 mV; E2, placed at the implantation cone of the postsynaptic neuron, recorded a hypopolarization with an amplitude of 2 mv lower than E1. On the contrary E3, placed on the axon of the postsynaptic neuron, recorded no response.
After Stimulation of B, E'1, placed near to the synapse of the fiber B, recorded a hyperpolarization wave of amplitude 5 mV while E2 recorded a hyperpolarization of amplitude 1mv lower than E'1, on the contrary E3 recorded no response. (1 pt 1/2)
2. SA: is excitatory synapse; since it leads to hypopolarization that gets the membrane potential near the threshold of depolarization.
SB: is inhibitory synapse, since it leads to a hyperpolarization that gets the membrane potential far from the threshold of depolarization. (1/2 pt)
3. E3 can record a response if:
A is stimulated many successive times separated by short time interval permitting the postsynaptic neuron to make a temporal summation in order to reach the depolarization threshold and generate an action potential. (½ pt)

Exercise 4 (4 ½ pt) Synapses and integration**Session 2002-1**

1. - The stimulation by S1 generates an action potential recorded by R1 on neuron 1 and hyperpolarization by RM (IPSP) of 15 mv of amplitude that does not propagate because it is far from the threshold of depolarization. That's why there is no response that appears at RN. (1/2 pt)
 - The stimulation by S2 generates an action potential of 100 mv of amplitude recorded by R2 on neuron 2 and a local hypopolarization of 35 mv of amplitude at RM (EPSP) recorded on the motor neuron. This EPSP is enough to generate an action potential of 100 mv of amplitude recorded at RN since the recorded EPSP is above threshold. (1/2 pt)
 - The simultaneous stimulations by S1 and S2 generate a slight hypopolarization of 20 mv of amplitude in the motor neuron recorded at RM and whose amplitude is less than that of S2 alone, this is due to a spatial summation of the IPSP generated by the stimulation S2 and the IPSP generated by the stimulus S1. The hypopolarization obtained at RM is less than the threshold needed to produce an action potential that could be recorded at RN. (1/2 pt)
 - The simultaneous stimulations by S1 and S2 followed by the stimulation by S2 generate a post synaptic potential whose first part is the same as the preceding recordings and whose second part is due to the hypopolarization caused by the second stimulation with S2. Hence, there are temporal and spatial summations at the level of the cell body and the resulting potential has amplitude of 25 mv of amplitude sufficient to generate an action potential.
 - The motor neuron is capable of receiving afferent messages from many synapses, and integrating them by temporal and spatial summation of EPSP and IPSP to produce an efferent message. (1 pt)
2. The injection of GABA has the same effect of stimulation at the synapse neuron 1- motor neuron but has no effect on the synapse neuron 2 - motor neuron. Hence, GABA is the neurotransmitter at the synapse neuron 1 motor neuron.

The injection of acetylcholine has the same effect as stimulation on the synapse neuron 2 motor neuron but not on the synapse neuron 1 - motor neuron. Hence acetylcholine is the neurotransmitter at the synapse neuron 2 - motor neuron. (1 pt)

Exercise 5 (3 ½ pt) Synaptic transmission**Session 2002-2**

1. • First experiment 1: The injection of Ach in small increasing amounts, C1 and C2, provokes a local hypopolarization in the postsynaptic element (EPSP) of increasing amplitudes from 25 mv to 55 mv. Starting from C3 the EPSP generates an action potential of a constant amplitude 100 mv. This means that Ach is an excitatory neurotransmitter. The increase of its amount is modulated in amplitude of hypopolarization) until a certain threshold where it generates an action potential of constant amplitude. (1/2 pt)
 - Second experiment: The presence of radioactivity on the postsynaptic membrane indicates that there is a link between the neurotransmitter Ach and a receptor on the postsynaptic membrane. (1/2 pt)
 - Third experiment: The absence of EPSP in the postsynaptic element shows that curare blocks the action of Ach. The appearance of radioactivity on the postsynaptic membrane shows that curare blocks the action of Ach by binding on the postsynaptic membrane. (1/2 pt)
2. 3: Binding of Ach to its receptors. 5; Generation of action potential. (1 pt)
3. When we increase the amount of GABA injected into the synapse from C1 to C3, there is an increase of hyperpolarization amplitude IPSP from 3 to 7 mv. This implies that GABA is an inhibitory neurotransmitter and its amount is modulated in amplitude of IPSP. (1 pt)

Exercise 6 (4 pts) Action of morphine

1.1. Document 2a shows a frequency of 6 action potentials between 0 and 50 ms that corresponds to rapid pain, but no more recordings are done during the next 150 ms. Starting from approximately 200ms, a series of action potentials with a frequency that decreases between 500 and 700 ms, which corresponds to slow pain.

Document 2b shows the recordings obtained after the application of morphine. It reveals a frequency of action potentials similar to that of the recording in document 2a, between 0 and 50 ms (for rapid pain), after which one action potential is recorded between 200 and 700 ms (for the slow pain). (1 pt)

1.2. Thus, morphine is an analgesic that inhibits slow pain. (1/2 pt)

2. Hypothesis: Morphine inhibits the liberation of substance P by binding to the enkephalin receptors. Or

Morphine blocks the receptors of substance P. (1/2 pt)

3. The substance P is the messenger of pain since the stimulation of neuron S provokes the liberation of substance P since the number of the vesicles of substance P decreases, which is linked to pain sensation. (1/2 pt)

Enkephalin is a natural analgesic since the stimulation of neuron I and neuron S provoke the liberation of enkephalin and the decrease of the liberation of substance P, which is the messenger of pain. (1/2 pt)

4. Yes (for the first hypothesis) (No for the second hypothesis), because when morphine is added at the level of synapse I-S, it inhibits the liberation of substance P (the pain message). (1 pt)

Exercise 7 (6 pts) Synapses of myotatic reflex network

1. The stimulation of F1 provokes action potential in E1 and E2. This potential was recorded in E5, but not in 4. The nervous message did not pass from F1 to M2 this implies that one out of the two synapses S2 or S3 is inhibitory or its response is ineffective (document 2). (1/2 pt)

The injection of acetylcholine in the three synapses S1, S2 or S3 provoked action potentials in E5, E3 and E4. This implies that Ach is an excitatory neurotransmitter for the three synapses (1/2 pt) The injection of GABA in the synapses S1, S2 and S3 did not cause any response. This implies that GABA has no effect in the three synapses (1/2 pt)

The injection of Ach in S2, caused a response in E3 and nothing in E4; the message was not transmitted from the interneuron motor neuron M2. This implies that synapse S3 is an inhibitory one or ineffective, and that the blocking of the nervous message from F1 to M2 was done at S3 and not at S2 (document 3) (1/2 pt)

E1 and E2 record in the terminals of the same fiber F1. These terminals would liberate the same excitatory neurotransmitter; so, acetylcholine is the neurotransmitter of synapses S1 and S2, whereas GABA is the inhibitory neurotransmitter of synapse S3 (1/2 pt)

2. The expected movement is extension because the stimulation of F1 provokes a response in M1 but not in M2.

The extensor muscle stimulated by M1 contracts, while the flexor muscle connected to M2 will not be stimulated and it relaxes

3. The two muscles are qualified as antagonists. (2 pts)

Exercise 8 (5 ½ pts) Properties of the motor neuron**Session 2004-1**

- 1.1. The stimulation at S_2 of neuron 2 produces an AP of 100mv amplitude recorded at R_2 and that reaches the motor neuron M where an AP of same amplitude 100mv is recorded at R_N .
- 1.2. We can conclude that the synapse of the neuron 2 and the motor neuron M is an excitatory synapse.
2. When we simultaneously stimulate S_1 and S_2 , we observe an EPSP at R_M of low amplitude and nothing at R_N , this signifies that the synapse (neuron 1-M) is inhibitory and produces an IPSP. This IPSP generated by S_1 and the EPSP generated by S_2 are added, a spatial summation and give an EPSP having low amplitude which is incapable to generate an AP that can propagate to the motor neuron.
When we stimulate S_1 and S_2 then S_2 again, we observe a spatial summation for (S_1 and S_2) then temporal summation (S_2, S_2) this will increase the EPSP enough to trigger an AP that can propagate.
Since the neuron is capable to make a summation of the PSP, this indicates that the property of the motor neuron is integration.
3. Experiment 1 shows that barbiturates promote the action of GABA, we observe an increased hyperpolarization upon injecting GABA together with barbiturates.
Experiment 2, radioactivity is found on the postsynaptic membrane after injection of radioactive barbiturates. This indicates that barbiturates act at the level of the postsynaptic membrane.
The barbiturates amplify the action of GABA, they are amplifying for this synapse.
4. The GABA produces an IPSP at the level of the postsynaptic membrane, preventing the formation of AP in the motor neuron. These AP are responsible for motor functions by provoking the contraction of the muscles. The GABA prevents this motor function. Then, the barbiturates, which promotes the effect of GABA, favor muscular relaxation (relaxant, tranquilizers).

Exercise 9 (4½ pts) Muscular dysfunction**Session 2004-2**

1. For a frequency of 3 per second before the injection of prostigmine, the electromyogram reveals that the amplitude of the first response is about 10 mV then this amplitude decreases gradually to become very weak at the 5th stimulation. While, after the injection of prostigmine, the amplitude of the electrical response is if 10 mv then it decreases very slightly.
The same for the mechanical response the amplitude is high (1kg) at the beginning then, it decreases progressively. While, after the injection of prostigmine and for the same frequency of stimulations, the mechanical responses decrease very slightly. (1pt)
2. The acetylcholinesterase is an enzyme that degrades acetylcholine in the synaptic cleft and thus prevents its prolonged fixation on the postsynaptic membrane. After the inhibition of the acetylcholinesterase by the prostigmine, a prolonged fixation of acetylcholine on the receptors is observed which explains the obtained recording and the maintain of the electrical and mechanical response of the muscle. (½ pt)
3. The muscle fibers are not affected because at the maximum of fatigue, the muscle remains excitable directly by an electric stimulation while it cannot be excited by its nerve. (½ pt)
4. 1st Hypothesis: The quantity of Ach, which fixes on the postsynaptic receptors, is not sufficient. (½ pt)
2nd Hypothesis: The number of Ach receptors are not sufficient. (½ pt)
5. It is an autoimmune disease. (½ pt)
6. The second hypothesis is valid because the researches have revealed the presence of antibodies, which block or destroy the Ach receptors. (½ pt)
7. At birth, some maternal antibodies are present in the infant's blood which disappears at the age of some months. These maternal antibodies are at the origin of myasthenia in the infant and the disease disappears with the disappearance of these antibodies. (½ pt)

Exercise 10 (4 ½ pts) Myotatic reflex

1. Myotatic reflex. (1/4 pt)
because the muscle responds to its own stretching by contraction.
2. The recordings in document 2 during extension reveal that when the triceps sural is in action with maximal amplitude of 4 mv of electric activity, the anterior tibialis is at rest (0 to 2.5 seconds) and during flexion the triceps sural is at rest, and the anterior tibialis is in action with maximal amplitude of 5 mv (2.5 to 5 seconds). This implies that the two muscles are antagonistic and the triceps sural is responsible for extension (extensor) and the anterior tibialis is responsible for flexion (flexor). (2 pts)
- 3.1. In the Achillean reflex (Case A), the recordings reveal the action potentials by oscilloscopes 1 and 3 and no recordings by oscilloscopes 2 and 4, which leads to the contraction of triceps sural and the relaxation of anterior tibialis. On the other hand, when we ask for the voluntary activity for the contraction of the anterior tibialis while stretching of the triceps, the recordings reveal action potential in 1, 2, and 4, and no recording in 3, which leads to the relaxation of the triceps sural that should have contracted and to the contraction of the anterior tibialis that should have relaxed.
- 3.2. We can conclude that when neuron 2 of the cerebrum is active, it modifies the activity of motor neurons 3 and 4, which stops the reflex act. Or, the cerebrum controls the reflex activity. (2 pts)

Exercise 11 (5 pts) Motor end plate

1. The stimulation at S1 has provoked the recording of an AP at the level of R1 placed in the nerve fiber and R2 placed in the muscle fiber. These action potentials are of the same amplitude 100 mv but the depolarization of R1 takes place 0.1 ms after the stimulation while the recording needed more time, 0.7 ms, to take place at R2. (1 pt)
2. When the stimulation took place at the level of S2, situated at the level of the postsynaptic membrane, the muscular action potential took 0.1 ms to reach R2. We can estimate that the distance that the nervous than muscular action potentials should cover according to the pathway S1-R1 on one hand and S2-R2 on the other hand has required a period of time equal to 0.2 ms and since the time to pass from S1 to R2 is 0.7 ms, then the time needed for the nervous message to traverse the synapse is 0.5 ms. (1 pt)
3. The addition of a small drop of acetylcholine G1 of a low concentration at the level of the synapse does not provoke any response at R1 nor at R2. However, the addition of a small drop of acetylcholine G2 of a concentration higher than G1, leads to the generation of a muscular action potential of 100 mv of amplitude at R2, placed in the postsynaptic muscle fiber and not at the level of R1 placed in the presynaptic membrane. This indicates that acetylcholine is the neurotransmitter of this synapse and that the response is induced starting from a given concentration; moreover, the transmission at the level of the synapse is unidirectional. (1 1/2 pts)
4. After the stimulation of a nerve fiber, one action potential is triggered which provokes the entry of Ca^{2+} ions by opening of Ca^{2+} voltage dependent channels at the axon's terminal, the exocytosis of the synaptic vesicles that pour their content (acetylcholine) into the synaptic cleft.

Upon contact with the postsynaptic membrane (muscle fiber), acetylcholine binds on specific receptors of the Na^+ chemical dependent channels and provokes the entry of Na^+ ions that leads to the depolarization of the membrane, which leads to the creation of a muscular action potential and to the contraction of the muscle fiber. At the end, acetylcholine molecules are degraded by inactivation enzymes and recaptured to the presynaptic terminal bud. (1 pt)

Exercise 12 (8 pts) Site of action of neurotransmitters**Session 2006-1**

1. In the experiment 1, the injection of acetylcholine at the level of the synaptic causes an action potential of an amplitude that reaches 100 mV, While the injection of acetylcholine into muscle fiber produces no variation of potential. This means that acetylcholine acts at the level of the synaptic cleft. (1½ pt)

Document 2 reveals that in normal synapse, acetylcholine binds to the postsynaptic receptors provoking generation of postsynaptic action potential. On the other hand, in the case of poisoned synapse, acetylcholine does not bind on the postsynaptic receptors and there is no generation of postsynaptic action potential. This means that the fixation of acetylcholine of the postsynaptic membrane is indispensable for the generation of an action potential. (1½ pt)

2. After the stimulation of a nerve fiber, one action potential is triggered which provokes the entry of Ca^{2+} ions by opening of Ca^{2+} voltage dependent channels at the axon's terminal, the exocytosis of the synaptic vesicles that pour their content (acetylcholine) into the synaptic cleft.

Upon contact with the postsynaptic membrane (muscle fiber), acetylcholine binds on specific receptors of the Na^+ chemical dependent channels and provokes the entry of Na^+ ions that leads to the depolarization of the membrane, which leads to the creation of a muscular action potential and to the contraction of the muscle fiber. At the end, acetylcholine molecules are degraded by inactivation enzymes and recaptured to the presynaptic terminal bud. (1 pt)

Exercise 13 (4 pts) Global potential**Session 2006-2**

1. Why does the recording present two global potentials in response to a unique stimulus? (½ pt)
2. Hypothesis: The nerve is constituted of two lots of nerve fibers of different diameters. (½ pt)
Or: The nerve is constituted of two lots of nerve fibers of different nature.
3. Yes (or no). Document 3 reveals that the nerve is constituted of many nerve fibers and that these nerve fibers are of different diameters. Document 4, confirms the information in document 3 and reveals the presence of two lots of nerve fibers in the nerve. Document 5 indicates that nerve fibers of big diameters favor a more rapid propagation of action potential which leads to the appearance of two global potentials spaced by around 1 ms. (1 ½ pt)
4. The amplitude of the response of the nerve depends on the number of the stimulated nerve fibers, since the number of the nerve fibers of 14 μm in diameter (30) is greater than the number of the nerve fibers of 4 μm in diameter (10), then the first global potential recorded is the result of the activity of all the nerve fibers of diameter 14 μm and the second global potential recorded corresponds to the nerve fibers of 4 μm in diameter. (1pt)

Exercise 14 (4½ pts) Action of cocaine

1. The arrival of action potential to the nerve endings of the presynaptic neuron allows the release of dopamine in the synaptic cleft. Dopamine fixes on its postsynaptic receptors, then it is recaptured by the presynaptic neuron. (1 ½ pt)
2. At the beginning of the experiment, the percentage of dopamine concentration in the two lots of rats is 100%, this percentage remains almost constant in the control rats of lot 1, while after the injection of cocaine at time $t = 0$ min. it increases in the rats of lot 2, to become more than twice (225%) after 40 minutes. This percentage starts to decrease to become normal again 100% after 120 minutes.

This means that, cocaine amplifies temporarily, the quantity of dopamine in the synaptic cleft. (1pt)

3. Hypotheses: Cocaine increases the release of dopamine in the synaptic cleft.

Cocaine prevents or decreases the recapture of dopamine by the presynaptic neuron. (½ pt)

4. The validated hypothesis that cocaine prevents the recapture of dopamine because it blocks the pump that allows its recapture. (1 pt)
5. Under the repeated effects of cocaine, the neurons adapt to the abnormally elevated concentration of

this substance. The brain is thus, forced to maintain an increased production of this neurotransmitter. This production can only be maintained by the frequent consumption of the drug. (½ pt)

Exercise 15 (5 ½ pts) Characteristics of the synaptic transmission

1. The stimulation of the nerve fiber by S_1 caused an AP recorded in R_1 then in R_2 . On the contrary, the stimulation by S_2 caused the recording of an AP only in R_2 . This means that the nervous message passes from N_1 towards N_2 , and not in the opposite direction. or, the nervous message is unidirectional from neuron N_1 to neuron N_2 . (1pt)
2. The deposition of the micro-drop of acetylcholine in zone S caused an AP recorded in R_2 that is attached to the postsynaptic neuron, and no recording in R_1 that is attached to the presynaptic neuron. On the contrary, the injection of the micro-drop of acetylcholine in N_1 or N_2 caused no AP in R_1 or R_2 . This indicates that acetylcholine acts on the postsynaptic neuron and does not act on the presynaptic neuron; and this action takes place only when the acetylcholine is deposited in the synaptic cleft. (1.5pt)
3. Synapse at rest (document 2a); synapse in action (document 2b). Because the synapse in action reveals vesicles that liberate, by exocytosis, their neurotransmitter into the synaptic cleft. This appears only in document 2b. (1pt)
4. For a frequency of presynaptic AP equal to 1 a.u, the number of vesicles that release their neurotransmitter is 1 thousand, and the quantity of released acetylcholine is 100 a.u. When the frequency of presynaptic AP increases reaching 6 a.u, the number of vesicles that release their neurotransmitter increases also and reaches 6 thousand, and the quantity of neurotransmitter increases also and reaches 600 a.u. This permits us to say that the quantity of released acetylcholine into the synaptic cleft increases with the frequency of the presynaptic AP, and that the nervous message at the level of the synapse is coded by modulation of the concentration of neurotransmitter. (1pt)
5. Following the arrival of the nervous message at a presynaptic neuron, the voltage-dependent calcium channels of the presynaptic membrane open and allow the entrance of calcium ions. This leads to the fusion of the synaptic vesicles with the presynaptic membrane. The synaptic vesicles pour by exocytosis their content, a neurotransmitter, which is acetylcholine in this case, into the synaptic cleft. This neurotransmitter binds to specific receptors located in the postsynaptic membrane and causes the opening of chemical-dependent sodium channels in the postsynaptic membrane, which causes depolarization at the level of this membrane that will be at the origin of a postsynaptic potential. (1pt)

Exercise 16 (5 pts) Muscles involved in myotatic reflex**Session 2008-1**

- 1- Myotatic reflex: Each time the muscle is stretched it contracts.
Voluntary motor activity: Certain movements follow an intention. (0.5pt)
- 2- Myotatic reflex: The nerve center is the spinal cord.
Voluntary motor activity: The nerve center is the cerebrum. (0.5pt)
- 3- The stimulation of a nerve fiber issued from the neuromuscular spindle of the triceps surae allowed the recording of a hypopolarization at E₁ placed at the level of motor neuron M₁. This excitatory message leads to the appearance of many AP recorded at E₃ placed at the level of the efferent fiber of this motor neuron linked to the extensor (triceps surae). On the contrary, at the level of E₂, placed at the level of motor neuron M₂, a hyperpolarization is recorded and no recording was obtained on E₄, placed at the level of the efferent fiber linked to the anterior tibialis.
- 4- Therefore, the activity of the motor neuron M₁ and the absence of the activity of the motor neuron M₂ leads to the contraction of the extensor and the relaxation of the flexor. (2pt)
- 5- The nerve message transmitted by the afferent nerve fiber took approximately 0.7ms to reach motor neuron M₁. Since the time needed to cross a synapse is 0.5ms, then one synapse exists along this pathway. The neuron circuit of M₁ is monosynaptic.
The nerve message took 1.2ms to reach M₂. This delay noticed at the level of M₂ versus M₁ is equivalent to the time needed to cross an additional synapse. Therefore, on pathway M₂ we have two synapses. The neuron's circuit of M₂ is then polysynaptic. (1pt)
- 6- Anterior tibialis contracts while triceps surae relaxes. The message coming from the superior nerve centers inhibited the nerve message arriving to the triceps surae, since no recording was noticed on E₃, however a nerve message at E₄, located at the level of the efferent fiber linked to the anterior tibialis, was observed. (1pt)

Exercise 17 (5pts) Myotatic reflex**Session 2008-2**

1. With a weak stretching of the neuromuscular spindle, 6 AP of 100mV amplitude each, are obtained. As the stretching increases, the number of AP (34) increases while their amplitude remains constant of 100 mv. This means that, the nerve message is coded by the frequency of action potentials (1pt)
2. Recording (A) obtained on the afferent fiber of the extensor muscle shows a frequency of AP of same amplitude. These AP start at 1 and end at 2. This recording is identical to that obtained in B at the level of the efferent fiber of the extensor muscle. On the other hand, recording (C) at the level of the efferent fiber of the flexor muscle shows the absence of AP during the period of stimulation. (1pt)
3. At the level of the spinal cord, the afferent fiber of the extensor muscle synapses directly with the motor neuron of the extensor muscle. This synapse is excitatory; it transmits the nerve message to the efferent fiber to the extensor muscle. Also, this afferent fiber of the extensor muscle synapses at the level of the spinal cord, with an interneuron through an excitatory synapse and the interneuron synapses with the motor neuron of the flexor muscle along an inhibitory synapse; as a result, the nerve message is inhibited. The spinal cord, via its different synapses, is able to coordinate the activity of the different motor neurons and the muscles on which these synapses depend. (1.5 pts)
4. Stretching (extension) movement (0.25 pt) Tension increase of the extensor muscle is an indication that the muscle contracts after the arrival of the nerve message through the efferent nerve fiber. The decrease in the tension of the flexor muscle is an indication that the muscle is relaxed and that this muscle is not receiving any nerve messages. (0.75pt)
5. Antagonistic muscles (0.25pt). Because as one muscle contracts, the other muscle relaxes (at the same time), favoring the extension of the foot. (0.25pt)

Exercise 18 (5 pts) Action of benzodiazepine

- 1- Document 1 reveals that the injection of GABA alone provokes hyperpolarization (I_{psp}), of an amplitude ~ 5mv that deviate the membrane potential away from the threshold of depolarization. This means that the synapse is inhibitory. (1 pt)
- 2- Benzodiazepine enhances the action of GABA, while picrotoxin inhibits its activity, because the injection of GABA and benzodiazepine provokes hyperpolarization of a higher amplitude (~6 mv) compared to that produced after the injection of GABA alone.
picrotoxin increases the muscular contraction and the signs of anxiety, it favors the transmission of the nervous message and consequently it is excitatory. (1 pt)
- 3- Variation of the percentage of the fixation of GABA on its receptors as a function of the quantity of benzodiazepine injected in the synaptic cleft. (1 pt)

Quantity of benzodiazepine in the synapse (nanomoles)	1	10	100	1000
Fixation of GABA (% in presence of benzodiazepine)	100	120	140	145

- 4- The percentage of GABA fixation increases from 100 to 145% as the quantity of benzodiazepine increases from 1 to 1000 nanomoles, while it becomes constant beyond 1000 nanomoles on 145%. This indicates that benzodiazepine favors the fixation of GABA and its action is enhanced by the increase of its concentration till a certain limit.
- 5- Document 3 that schematizes the structure of the postsynaptic receptor, reveals that the sites of fixation of GABA and benzodiazepine are close to each other and these sites are located on the same membrane structure: Cl⁻ channel. The presence of benzodiazepine decreases the quantity of GABA indispensable for the opening of Cl⁻ channel / or favors the opening of great number of Cl⁻ channels that increases the entering of Cl⁻ and consequently the inhibition of the nervous message, therefore the muscular contractions decrease.

Exercise 19 (5 pts) Role of morphine**Session 2009-2**

- 1- The role of enkephalin is to decrease pain sensation ($\frac{1}{2}$ pt), because we observe in O2 a pain message of 4 AP after the stimulation of the sensory nerve fiber and the enkephalin interneuron (experiment 2), which is lower than the message recorded in O2(9 AP) after the stimulation of the sensory nerve fiber only (experiment 1). This shows that the enkephalin liberated after the stimulation of the interneuron inhibited partially the transmission of the pain message. (1 pt)
- 2- The nociceptive neuron has an integrating role. It performs spatial summation for two nerve messages coming from the presynaptic fibers, the first is an EPSP caused by the sensory neuron (11 AP in O1) and the second is an IPSP caused by enkephalin neuron (10 AP in O3). The result of this summation is a message of weaker frequency (4 AP in O2). (1 1/2 pt)
- 3- The frequency of action potentials at the level of the medullary neuron is high, it varies from 5 AP/s till a maximum of 65 AP/s after the stimulation of the sensory nerve fiber in the absence of morphine (doc. 3a). On the other hand, this frequency decreases sharply and fluctuates between 2 and 12 AP/s after the stimulation of the sensory nerve fiber with the injection of morphine (doc. 3b). This indicates that morphine inhibits the activity of the nociceptive neuron by decreasing the frequency of AP that leads to the decrease in pain sensation. (1 1/2 pt)
- 4- They are agonists because morphine and enkephaline have the same effect of decreasing pain sensation at the level of the nociceptive medullary neuron. (1/2 pt).

Exercise 20 (5 pts) Properties of motor neuron**Session 2010-1**

- 1- Two hypopolarizations, of the same amplitude are obtained after two successive stimulations separated approximately with a time difference of 16 ms. These two hypopolarizations get closer and sum up, producing an action potential with an amplitude 100 mV when the time difference between the two stimulations decreases to 6 msec. This means that the motor neuron M1 integrates the successive messages arriving from the same afferent neuron Fa1. (1 1/4 pt)
- 2- The recordings obtained by O1 show a global potential of the nerve fibers with amplitude that increases from 100 mV to 250 mV as intensity of stimulations increases from 1-3. In each stimulation, a certain number of nerve fibers is activated, this explains the increase in the amplitude of potential as intensity increases from 1-3. However, the global potential remains constant at 250 mV following the fourth stimulation which corresponds to the stimulation of all nerve fibers. (3/4 pt).
- 3-1-We observe hypopolarization in O2, on the contrary, we observe no response in O4 following the first stimulation(1/2pt). We observe an AP of a constant amplitude of 100 mV in O2 and O4, following stimulations 2, 3 and4 of increasing intensities. (1/2 pt).
- 3-2-The nerve message propagates starting at a certain intensity, ($\frac{1}{4}$ pt) with the same amplitude irrespective of the distance.(1/4 pt)
- 4- Spatial summation ($\frac{1}{4}$ pt) because as intensity of stimulation increases, more nerve fibers are activated and the motor neuron receives many nerve messages, EPSP, from many fibers simultaneously. The motor neuron integrates (sums up) these messages ($\frac{1}{2}$ pt)
- 5- The motor neuron M2 innervates the flexor muscle ($\frac{1}{4}$ pt) because during an achillian reflex, the flexor muscle is relaxed. Document 3 shows a hyperpolarization recorded by O3 which is connected to the cell body of the motor neuron M2 innervating the flexor muscle. This hyperpolarization can't propagate so the flexor muscle relaxes.($\frac{1}{2}$ pt)

Exercise 21 (5 pts) Neuromuscular disease

1. For the healthy individual the frequency of the action potentials is high: 15 AP of same amplitude (120mV) during 3 seconds, while for the myasthenic individual this frequency is lower: 10 AP with similar amplitude during the 3 seconds, with an absence of AP for certain moments. (1 pt)
2. For the healthy individual, the regular presence of AP induces the continuous contraction of the muscle. While for the myasthenic individual, the absence of AP for certain moments induces a decrease in the contraction of the muscle. (1/2 pt)
3. 1- Presynaptic membrane (1/4 pt)
2- Secretory vesicles containing neurotransmitters (1/4 pt)
3- Synaptic cleft (1/4 pt)
4- Postsynaptic membrane (1/4 pt)
4. The autoradiography of the fiber of the healthy individual reveals an important concentration of black grains; on the other hand, this concentration is less important for the myasthenic individual. (1/2 pt)
5. The comparison shows, for individual A, an important fixation of α -bungarotoxin molecules to the acetylcholine receptors and a less important fixation for individual B. The presence of these molecules on the muscle fiber reveals the presence of acetylcholine receptors. Thus for the healthy individual A there is a great number of acetylcholine receptors at the level of the postsynaptic membrane of the neuromuscular junction while this number is less important for the myasthenic individual B.
Therefore the myasthenia is due to a lack in acetylcholine receptors at the level of the motor end plate. (1 pt)
6. In the myasthenic individual there is a release of acetylcholine neurotransmitters following the stimulation of the motor neuron. However only few of these neurotransmitters bind to the post synaptic membrane of the muscle fiber due to the lack in post synaptic membrane receptors (doc 3)
This lack in binding inhibits the appearance of a regular train of AP (doc 1) and is thus responsible for the weak muscular activity, the muscular contraction is not maintained.
(1 pt)

Exercise 22 (5 pts) Achillean reflex and voluntary movement

Session 2011-1

1	The amplitude of the electric activity of the extensor muscle is 0.18 mV during an achillian reflex, in the absence of voluntary flexion of the foot. However, this amplitude decreases to 0.6mV upon the voluntary flexion of the foot. This means that the voluntary command inhibits the achillian reflex.	1
2	case B corresponds to curve 2, because oscilloscope number 2 connected to the motor nerve fiber, issued from the encephalon and which is responsible for the voluntary command, shows 3 AP action potential only in case B revealing a voluntary intervention. Case A corresponds to curve 1 because oscilloscope number 2 connected to the motor nerve fiber, issued from the encephalon and which is responsible for the voluntary command, shows resting potential revealing no voluntary intervention. OR The student may refer to the activity of the motor neuron innervating the extensor muscle: the decrease in the frequency of action potential from 5 AP to 3AP indicates a decrease in the electric activity of the muscle.	1
3	Oscilloscope number 3 shows a decrease in the frequency of action potential from 5 AP in case A to 3AP in case B and this could be explained by the fact that the motor neuron innervating the extensor muscle receives in case A only one excitatory nerve message from the T-shaped sensory neuron and records a series of 5 Ap., while the motor neuron in case B receives in addition to the excitatory message from the T-shaped sensory neuron an inhibitory message from the encephalon through the interneuron. The motor neuron integrates these two messages, by spatial summation, this results in a decrease in the frequency of AP .	1
4	Oscilloscope no. 1:  Because the same stimulation at the level of achillian tendon records the same frequency of AP in the sensory fiber. Oscilloscope no.2:  (Any drawing showing a frequency > 3 AP is accepted) Because the strong voluntary flexion reveals an increase in the frequency of AP in the nerve fiber coming from the superior centers responsible for voluntary command. Oscilloscope no. 3:  (Any drawing showing a frequency < 3 AP is accepted) Because the Inhibitory message is stronger than that in the case of slight flexion of the foot leading to a decrease in the excitatory message transmitted through the motor neuron innervating the extensor muscle. Oscilloscope no. 4:  (Any drawing showing a frequency > 3 AP is accepted) Because the excitatory message is stronger than that in the case of slight flexion of the foot what leads to an increase in the the excitatory message transmitted through the motor neuron innervating the flexor muscle.	2

Exercise 23 (5 pts) Neurotransmitters and pain

1	<p>Role of the enkephalin: inhibitory (0.25 pt) Site of action: synapse between cell I and cell D (0.25 pt) Because following the application of enkephalin we observe a hyperpolarization having an amplitude of 25mV only at the level of R2 while we observe a resting potential of -70mV at the levels of R1, R3 and R4. (0.5 pt)</p> <p>Role of substance P: excitatory(0.25 pt) Site of action : synapse between the cell D and cell L(0.25 pt) Because following the application of substance P we observe a hypopolarization having an amplitude of 20mV only at the level of R4 while we observe a resting potential of -70mV at the levels of R1, R2 and R3. (0.5 pt)</p>	2
2	<p>A nervous message of 3AP/6ms having the same amplitude (100mV) is recorded at the levels of R2 and R3. This shows that the stimulation is efficient and that the action potential propagates in the same cell keeping the same amplitude and the same frequency. (0.25 pt)</p> <p>Similarly, we observe a nervous message of the same amplitude as R2 and R3 at the level of R5 but with a lower frequency of 2AP/6ms following a depolarization of the membrane that reaches the threshold. This shows that the synapse between the cells D and L is excitatory and attenuates only the frequency of the nervous message and not its amplitude. (0.25 pt)</p> <p>However we observe always a resting potential of -70 mV at the level of R1. This shows that the nervous message triggered by the nociceptor doesn't propagate from cell D to cell I. (0.25 pt)</p>	0.75
3	<p>3-1- A single AP is recorded at the level of R1 in the presence of serotonin (case B) while no action potential is recorded in case A. (0.25 pt) A hyperpolarization is recorded at the level of R2 in the presence of serotonin (case B) while 3 AP/ 6ms is recorded in case A. (0.25 pt) No response is recorded at the level of in R3 and R5 in the presence of serotonin (case B) while 3AP/ 6ms is recorded at the level of R3(0.25 pt) and 2AP/ 6ms at the level of R5(0.25 pt) in case A.</p> <p>3-2- We can conclude that serotonin excites only the cell I thus inhibiting the propagation of the pain nervous message in the cell D(0.25 pt) It acts between the axon of the cell S and the cell I. (0.25 pt)</p>	1.5
4	<p>The encephalon sends a nervous message through the cell S and provokes the release of serotonin at the level of the synapse between the cell S and the cell I. This triggers a nervous message at the level of cell I. This message propagates and induces the release of enkephalin at the level of the synapse I-D provoking a hyperpolarization at the level of the postsynaptic membrane of the cell D. Thus the propagation of the nerve message at the level of cell D is inhibited and the release of substance P is prevented thus stopping transmission of the pain nerve message.</p>	0.75

Exercise 24 (5 pts) Activity of antagonistic muscles

Session 2012-1

1	Myotatic reflex (1/4pt) because the muscle responds by a contraction due to its own stretching. (1/2pt)	3/4
2	<p>The intensity of the contraction of the extensor muscle increases from 0.2 AU to 2 AU between 0 s and 0.6 s, then it drops slightly to 1.8 AU at 1.8 s as long as the extensor muscle is stretched. This shows that the muscle contracts due to its own stretching. However, this intensity decreases from 1.8 AU to 1AU between 1.8 s and 3.2 s following a simultaneous stretching of the extensor muscle and muscle X (moment S). This shows that the extensor muscles and muscle X are antagonists or the activity of muscle X attenuates that of the extensor muscle.</p> <p>Similarly, there was a greater decrease in the intensity of the contraction from 1 AU to 0.2 AU, following the simultaneous stretching of the two preceding muscles and muscle Y (moment B). This means that muscle Y is also antagonist to the extensor muscle and agonistic to muscle X or the activity of muscle Y attenuates that of the extensor muscle.</p>	1 ½
3	<p>Fiber 1 is the afferent fiber of the extensor muscle because it is connected directly to the motor neuron of the extensor muscle M (monosynaptic circuit). Similarly, the recording 1 shows a hypo polarization (EPSP) of amplitude 2mV which means that the synapse is an excitatory synapse (1pt).</p> <p>Fiber 2 is the afferent fiber of muscle X or Y is connected via an interneuron to motor neuron M (polysynaptic circuit) which has an inhibitory role in this reflex. Also, the recording 2 shows a hyperpolarization (IPSP) of amplitude 2 mV, which means that the synapse is inhibitory. (1pt).</p>	2
4	At moment S, the motor neuron M receives an excitatory message from the afferent fiber 1 and at the same time, an inhibitory message from the interneuron attached to the sensory fiber 2. This motor neuron M integrates the two messages in a spatial summation (or spatial-temporal summation + frequency of AP). The resultant is an attenuated efferent nerve message which leads to a decrease in the intensity of the contraction of the extensor muscle.	3/4

Exercise 25 (5 pts) Protection reflex

Session 2012-2

	The popliteal nerve is afferent (sensitive). (1/4pt) because the flexion of the lower limb disappears following the sectioning of popliteal nerve and the stimulation of its peripheral end however the flexion appears following the stimulation of its central end; This shows that the nervous message is transmitted by this nerve from the periphery to spinal cord (centripetal direction). (1/2pt)	
1	<p>The crural nerve is efferent. (1/4 pt) because there is no more contraction of the biceps crural following the sectioning of the crural nerve and the stimulation of its central end while the biceps crural contracts following the stimulation of its peripheral end; This shows that crural nerve transmits the nervous message from the spinal cord toward the biceps crural. (1/2pt)</p> <p>The nerve of the quadriceps is efferent. (1/4pt) because there is no more contraction of the quadriceps femoral following the sectioning of the quadriceps nerve and the stimulation of its central end while the quadriceps femoral contracts following the stimulation of the peripheral end of this nerve; This shows that quadriceps nerve transmits the nervous message from the spinal cord toward the quadriceps femoral. (1/2pt)</p>	2 1/4
2	<p>From 0 to 5 and from 10 to 15 s, the electromyogram of the quadriceps femoral has a amplitude that fluctuates between -1 and 1mv greater than that of the electromyogram of the biceps crural that is almost null.</p> <p>From 5 to 10 mv, the electromyogram of the biceps crural has as amplitude that fluctuates between -2 and 2.5 mv greater than that of the electromyogram of the quadriceps femoral which is almost null. (1/2pt)</p> <p>Thus, the flexion of the lower limb is due to the activity of the biceps crural and the relaxation of the quadriceps femoral and that these two muscles are antagonistic. (1/2pt)</p>	1
3	<p>Functional diagram of the structures implicated in the protection reflex</p>	11/4
4	In the protection reflex, the receptor is the skin and the effector organ is the muscle, while in the myotatic reflex, the stretched muscle is, at the same time, the receptor organ and the effector organ.	1/2

Exercise 26 (5 pts) Ecstasy, euphoria or depression?

Session 2013-1

1	a- After 1h : euphoric, very talkative, and feels extreme happiness. After 5h: a "descent" period marked by exhaustion, and even a strong depressive syndrome... b- Irreversible destruction of neurons.	0,75
2	Following a nervous message propagated through the serotonin presynaptic neuron we observe at the level of E2 a hypopolarization of 10mV (EPSP) or an action potential of 100 mV at the level of the postsynaptic membrane. Thus the synapse F is excitatory.	0,5
3	Temporal summation since following the two successive stimulations separated by a long time interval, 2 AP separated by a long time interval are recorded at the level of the presynaptic neuron generating two distinct hypopolarizations (EPSP) of 10 mV each at the level of the postsynaptic neuron which didn't reach the threshold of depolarization. While following two stimulations separated by a short time interval, 2 AP separated by a short time interval are recorded at the level of the presynaptic neuron generating two hypopolarizations that add up reaching the threshold of depolarization and leading to an AP of 100 mV as amplitude. This shows that the postsynaptic neuron has summed the two EPSP.	0,75
4	In the case where the two stimulations are separated by a long time interval the EPSP recorded at the level of the cell body (E2), is not recorded at the level of the axon (E3) of the same neuron. Thus EPSP doesn't propagate. However, in the case where the two stimulations are separated by a short time interval the AP recorded at the level of E2 propagates and is recorded at (E3). Therefore, only the action potential propagates at the level of a neuron.	0,5
5-1	The euphoria sensation: Ecstasy consumption doesn't modify the frequency of AP at the level of the serotonin neuron (2+) nor the synthesis of serotonin (2+). Whereas, it increases the amount of serotonin released (from 2+ to 4+) and reduces the activity of the serotonin recapture pump (from 2+ to 1+). This leads to a more important concentration and more persistent presence of serotonin in the excitatory synapse. Thus the activity of the dopaminergic neuron that is modulated by serotonin concentration increases (frequency of AP increases from 2+ to 4+), leading to a more important release of dopamine which explains the euphoria sensation 0 to 4 hours after ecstasy consumption.	1,25
5-2	State of depression : The serotonergic neuron stops the synthesis and release of serotonin, thus leading to a decrease in the activity of the dopaminergic neuron (frequency of AP decreases from 4+ to 1+). Since in absence of serotonin, the dopaminergic neuron is no more stimulated, thus the release of dopamine which is responsible for pleasure sensation drops leading to exhaustion and to a state of depression.	1,25

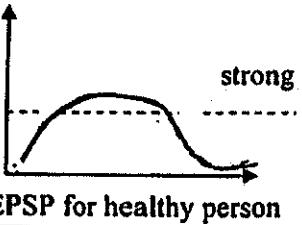
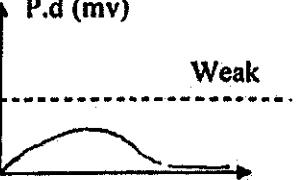
Exercise 27 (5 pts) LSD and hallucinations

Session 2013-2

1	"Every sound generated a corresponding animated image". The "acoustic perceptions such as the sound of a door handle or a passing automobile became transformed into optical perceptions". Sounds are generating visual perceptions. Thus LSD is a powerful hallucinogen since it provokes perceptions without objects to perceive.	0,5								
2	LSD doesn't modify only the visual sensations, but it also causes general disturbance. Hoffmann didn't realize that the bike was moving, he felt dizzy and weak he couldn't stand up and was forced to lie down on a sofa bed.	0,5								
3	The nerve message coming from the retina towards the extremity of the axon of presynaptic neuron A, leads to the influx of calcium in the terminal knob, this causes vesicles filled with neurotransmitters, serotonin, to migrate to the cell's surface and to release their contents of serotonin into the synaptic cleft by exocytosis. Then, serotonin fixes on specific receptors of the membrane of the postsynaptic neuron B. the binding of the neurotransmitter to its specific receptor generates an EPSP in the postsynaptic neuron inducing the birth of a nerve message that is transmitted by neuron B towards the nerve centers.	1								
4	Histogram : variation of the amount of serotonin as a function of the intensity of stimulation	1,25								
	<table border="1"> <caption>Amount of serotonin (in AU)</caption> <thead> <tr> <th>Intensity of stimulation</th> <th>Amount of serotonin (in AU)</th> </tr> </thead> <tbody> <tr> <td>I1</td> <td>~1.5</td> </tr> <tr> <td>I2</td> <td>~2.5</td> </tr> <tr> <td>I3</td> <td>~3</td> </tr> </tbody> </table>	Intensity of stimulation	Amount of serotonin (in AU)	I1	~1.5	I2	~2.5	I3	~3	
Intensity of stimulation	Amount of serotonin (in AU)									
I1	~1.5									
I2	~2.5									
I3	~3									
5	5-1-The frequency of AP at the level of the neuron A and at the level of neuron B increases respectively from 5 to 12 AP and from 8 to 18 AP as the intensitiy of stimulation increases from I1 to I3. Meanwhile, the amount of serotonin increases from 1.5 to 3 au. 5-2-Thus the nerve message at the level of neuron is modulated by frequency of AP and at the level of the synapse by the amount of neurotransmitter.	0,75								
6	the molecule of serotonin and that of LSD have an identical part in their molecular structures, and this common part allows the serotonin to fix on its specific receptor. We can suggest that molecules of LSD fix on serotonin receptors due to their complementary form. Being agonist to serotonin, the LSD fixation on serotonin receptors generates an EPSP at the level of neuron B in absence of any message at the level of the presynaptic neuron A, and consequently the induced nerve message propagates towards the visual centers even though eyes don't detect any object. This explains the visual hallucinations described by Hoffmann.	1								

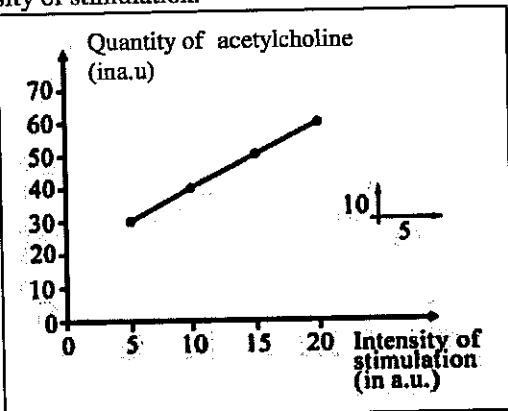
Exercise 28 (5 pts) Myasthenia

Session 2014

1)	<p>Since identical muscle contraction is detected in the healthy and diseased individual upon giving an effective stimulation directly to the muscle, and since same number of potential 16 AP/time with same amplitude 100 mV is also detected in the two individuals as a result of stimulating effectively the motor neuron. However the recording of the electric response at the level of muscle upon effective stimulation of motor neuron is weak with less amplitude decrease from 4 mv in the diseased individual that becomes nil, than in the healthy individual that reveals normal response with same amplitude.</p> <p>We can say that the muscle itself is capable of contracting normally in the diseased individual and that the motor neuron reveals normal function in transmitting neuron message. Thus the disease is due to a synaptic malfunction.</p>	
2)	<p>Since healthy animal shows a temporary myasthenia after its injection with antibodies X, that are detected in the blood of myasthenic one only. Therefore the origin of the disease is due to presence of antibodies X, which is auto immune disease.</p>	
3)	<p>Hypothesis 1: Antibodies X act at the level of the pre synaptic membrane inhibiting the release of acetylcholine neurotransmitter. Hypothesis 2: Antibodies X bind to the post synaptic acetylcholine receptors inhibiting the binding of Ach.</p>	
4)	<p>Both structures show normal amount for the synaptic vesicles in the terminal of axon. However the acetylcholine receptors in the post synaptic folds of the muscle fiber that are presented in the healthy individual are absent in the myasthenia individual.</p>	
5)	<p>Healthy person P.d (mv)</p>  <p>EPSP for healthy person</p>	<p>Diseased person P.d (mv)</p>  <p>EPSP for myasthenic ind.</p>
6)	<p>In the myasthenia individual, Ach is released into the synaptic cleft, after an effective stimulation of the motor neuron, however only few of Ach will bind to the postsynaptic membrane of the muscle, due to the fact, that the antibodies are blocking the Ach receptors. This will inhibit the appearance of normal frequency of A.P.s and is thus responsible of the weak muscle activity ant the muscular contraction is not maintained (doc. 1)</p>	

Exercise 29 (5 pts) Mode of action of Botox

Session 2015-1

1	The recording obtained at the level of the axon of the presynaptic neuron of document 1 shows APs of same amplitude. However, the frequency of AP increases from 4 APs to 17 when the intensity of the stimulation increases from 5 a.u. to 20 a.u. This shows that the response of the axon is modulated by the frequency of APs as function of the intensity of stimulation.	1/2
2	Curve showing the variation of the quantity of acetylcholine as a function of the intensity of stimulation. 	1
3	At the level of the presynaptic neuron, the nervous message is modulated by the concentration of calcium as function of the intensity of stimulation, since document 2 shows that the concentration of calcium in the presynaptic element increases from 1 a.u. to 6 a.u when the intensity of stimulation increases from 5 a.u. to 20 a.u. At the level of the synapse, the nervous message is modulated by the concentration of the released acetylcholine as function of the intensity of stimulation, since document 3 shows that the quantity of acetylcholine released increases from 30 a.u to 60 a.u when the stimulation intensity increases from 5 a.u to 20 a.u.	1
4	Hypothesis: Botox inhibits the synthesis of Acetylcholine. Botox inhibits the exocytosis of Acetylcholine. Botox neutralizes Acetylcholine. Botox blocks the postsynaptic receptors.	1/2
5	The fluorescence in the presynaptic bud decreases from 50 a.u before stimulation to 5 a.u after stimulation in a medium without Botulinum toxin. However, in a medium containing Botulinum toxin, it remains almost constant at 50 a.u before and after stimulation. Thus, Botox blocks the release of neurotransmitters by exocytosis of the presynaptic vesicles. Hence, in medium containing Botox, the quantity of released acetylcholine should be null.	1
6	Botox blocks the transmission of the nervous message at the level of neuromuscular synapses by blocking the release of acetylcholine. Thus preventing the permanent muscular contractions that are responsible for the signs of aging. When Botox is injected in small doses, its action will be limited on the treated zone. However, when it is used in high doses, its action is generalized on other muscles especially respiratory ones which will be permanently relaxed leading to death by asphyxia.	1/2

Exercise 30 (5 pts) Nervous coding and sensory information

Session 2015-2

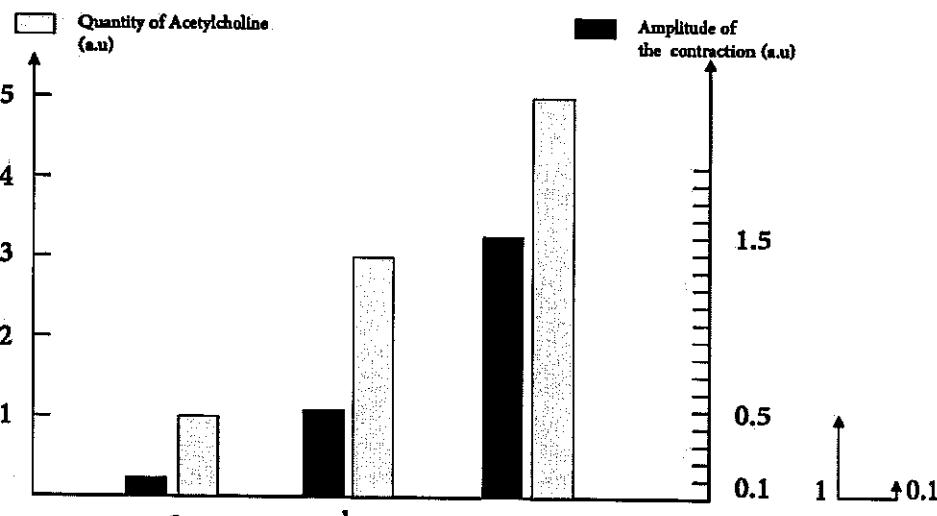
1	A myotatic reflex is the contraction of a muscle in response to its own stretching, in this case (exp 1) the stretching of the muscle of the arm leads to its contraction. Thus it is a myotatic reflex.	1/2												
2.1	Document 2 shows that the amplitude of the receptor potential increases from 4 to 35 a.u. when the used mass increases from 5 to 200g. Document 3 shows that the frequency of APs per second increases from 4 to 35AP/s when the used mass increases from 5g to 200g.	1/2												
2.2	The coding of the nervous message at the level of the neuromuscular spindles is modulated in amplitude. The coding of the nervous message at the level of the fiber is modulated in frequency of APs.	1/2												
3	The response is modulated in frequency of APs as a function of the velocity in which the stretching is performed.	1/2												
4	Curve showing the variation of the amount of released acetylcholine as a function of the intensity of stretching.	1												
	<table border="1"> <caption>Data points estimated from the graph</caption> <thead> <tr> <th>Intensity of stretching (a.u.)</th> <th>Amount of released acetylcholine (a.u.)</th> </tr> </thead> <tbody> <tr><td>5</td><td>20</td></tr> <tr><td>10</td><td>30</td></tr> <tr><td>15</td><td>35</td></tr> <tr><td>20</td><td>40</td></tr> <tr><td>25</td><td>50</td></tr> </tbody> </table>	Intensity of stretching (a.u.)	Amount of released acetylcholine (a.u.)	5	20	10	30	15	35	20	40	25	50	
Intensity of stretching (a.u.)	Amount of released acetylcholine (a.u.)													
5	20													
10	30													
15	35													
20	40													
25	50													
5	The amount of the released neurotransmitter increases from 20 to 60 a.u. when the intensity of stretching increases from 5 to 25 a.u. Hence, the coding at the level of the synapse is modulated in concentration of neurotransmitters.	1/2												
6	The traction performed by the dog stretches the neuromuscular spindles of the arm muscle ensuring the contraction of the same stretched muscle (it's a myotatic reflex). Since the response of the sensory neuron: the receptor potential and the APs as well as the response at the level of the synapse, the amount of neurotransmitters, are modulated in function of the intensity and the velocity of the traction. This allows to adapt the response to each traction.	3/4												
7	<p>The neuronal circuit and the structures involved in this reflex.</p>	3/4												

Exercise 31 (5 pts) Analgesia without morphine

1	Morphine is agonist to enkephalin. Morphine has a shape complementary to that of enkephalin receptors. It binds to the enkephalin receptors and inhibits the release of substance P. Thus, it stops the transmission of nerve message associated with pain.	1/2
2	The duration of staying in zone P by animals that haven't received any injection or by animals that have received 6 mg/kg of endorphin is the same 5 sec. This duration is 14 times less than 72 sec which corresponds to the duration of staying in zone P of animals that have been injected by morphine. Therefore, endorphin seems not to have an analgesic effect in comparison with morphine which is a strong analgesic.	3/4
3	Hypothesis: Endorphin cannot cross the blood brain barrier to act at the level of endorphin synapses. OR Endorphin is decomposed rapidly before reaching the endorphin synapse. OR Endorphin has a short term effect.	1/2
4	Experiment 2: shows a rapid transformation of endorphin into other substances. This leads to its rapid disappearance in blood and prevents its arrival to the spinal cord and the brain. Similarly, it shows that the radioactivity remains at the level of the blood which explains the inability of endorphin to cross the blood brain barrier that is neither permeable to this substance nor to its products. This explains the ineffectiveness of endorphin as an administered exogenous analgesic.	3/4
5	The duration of staying in Zone P of animals injected by opioid is 62s which is less than 72s which is the duration of staying of animals injected by morphine, despite the injection of 1 mg/kg of opioid. This quantity is 6 times less than 6 mg/kg which corresponds to the amount of injected morphine. Thus opioid even in small doses is an efficient analgesic.	3/4
6	Since opioid seems to protect enkephalin from the effects of the enzyme NEP that is present in the cleft of enkephalin synapses, this analgesic decreases the degradation of this neurotransmitter after its fixation on its corresponding postsynaptic receptors. This leads to an increase in the concentration of enkephalin and its persistence in the synaptic cleft and on the receptors. Thus, the action of enkephalin that consists of inhibiting the transmission of pain messages is enhanced. That is why, the analgesic effect, observed in doc 2, revealed by the duration of staying, 62s, is close to that of morphine, 72s.	3/4
7	Opioid acts at small doses (6 times < than that of morphine) to have a certain analgesic effect. The secondary effects of opioid are reduced compared to that of morphine: no constipation, no addiction... Opioid is a natural substance secreted by the body unlike morphine which is exogenous. It acts by amplifying the natural analgesic capacities of the organism (amplifies the action of enkephalin that is also a natural endorphin), contrary to morphine which reduces them.	1

Exercise 32 (5 pts) Synaptic transmission

Session 2016-2

1	The depressions observed at the level of the presynaptic membrane correspond to the fusion of vesicles with the membrane, exocytosis. Since the number of depressions increases between cases b and c as the intensity of stimulation increases from I1 to I2, thus the number of vesicles undergoing exocytosis increases with the intensity of stimulation. This justifies that the exocytosis of neurotransmitters at the level of a synapse is amplified with the increase of the intensity of stimulation.	3/4												
2	Variation of the amplitude of the muscle contraction as a function of Acetylcholine dose.  <table border="1"><thead><tr><th>Case</th><th>Quantity of Acetylcholine (a.u.)</th><th>Amplitude of the contraction (a.u.)</th></tr></thead><tbody><tr><td>a</td><td>~0.8</td><td>~0.1</td></tr><tr><td>b</td><td>~2.8</td><td>~0.4</td></tr><tr><td>c</td><td>~5.0</td><td>~1.1</td></tr></tbody></table>	Case	Quantity of Acetylcholine (a.u.)	Amplitude of the contraction (a.u.)	a	~0.8	~0.1	b	~2.8	~0.4	c	~5.0	~1.1	1 3/4
Case	Quantity of Acetylcholine (a.u.)	Amplitude of the contraction (a.u.)												
a	~0.8	~0.1												
b	~2.8	~0.4												
c	~5.0	~1.1												
3	The nervous message at the level of the synapse is coded in concentration of neurotransmitters. Since the amplitude of the muscle contraction increases from 0.5 to 1.5 a.u. when the amount of acetylcholine increases from 3 a.u up to 5 a.u. which corresponds to an increase in the intensity of stimulation from I1 to I2.	3/4												
4	The amplitude of the contraction increases to a maximum of 1.1 a.u within 15 s in the presence of 0.1 µg of Norcuron and 5 au of acetylcholine. On the contrary, this amplitude decreases and become almost constant at 0.45 a.u when we increase the amount of injected Norcuron up to 2 µg with the same injection of 5 au of acetylcholine. Thus, Norcuron inhibits the action of acetylcholine and decreases the amplitude of the muscle contractions and its action varies in parallel to its concentration.	3/4												
5	Since Norcuron reduces the muscle contraction while acetylcholine provokes the muscle contraction. Thus Norcuron has an opposite (reverse) effect to acetylcholine. Hence they are antagonistic substances. TEPP provokes the permanent contraction of muscles like acetylcholine. Thus it has the same effect as acetylcholine on the muscle. They are agonistic substances.	1												

Exercise 33 (5 pts) Action of an insecticide**Session 2017-1**

1.	Table showing the variation of the amplitude of the contraction and the duration of the contraction as a function of the presence and the absence of pyrethrum.	1									
	<table border="1"> <thead> <tr> <th></th> <th>Amplitude of the contraction (a.u.)</th> <th>Duration of the contraction (s)</th> </tr> </thead> <tbody> <tr> <td>Without pyrethrum</td> <td>2</td> <td>2</td> </tr> <tr> <td>With pyrethrum</td> <td>4</td> <td>10</td> </tr> </tbody> </table>		Amplitude of the contraction (a.u.)	Duration of the contraction (s)	Without pyrethrum	2	2	With pyrethrum	4	10	
	Amplitude of the contraction (a.u.)	Duration of the contraction (s)									
Without pyrethrum	2	2									
With pyrethrum	4	10									
2.1.	Without pyrethrum, the amplitude of the contraction is 2 a.u. less than 4 a.u. obtained with pyrethrum. The duration of the contraction is 2 s in the medium without pyrethrum less than 10 s obtained in the presence of pyrethrum.	0.5									
2.2.	The organophosphorus insecticides amplify the muscular contractions and make them permanent.	0.5									
3.	Hypotheses: - Pyrethrum amplifies the exocytosis of neurotransmitters. - Pyrethrum neutralize the Ach. Esterase. - Pyrethrum blocks the recapture.	1.5									
4.	The presynaptic neuron corresponds to the structure 1, since this structure is situated close to the synaptic cleft and it is rich in synaptic vesicles.	0.5									
5.	Pyrethrum exerts an excitatory effect on the nerve while increasing the duration and the amplitude of the muscular contraction. It binds on acetylcholinesterase at the level of the synaptic cleft of neuromuscular synapses. This blocks the action of acetylcholinesterase enzyme that degrades normally the acetylcholine and stops the depolarization of the postsynaptic membrane and the muscular contraction. Then, this will block the respiratory movements ensured by the permanent contractions of the respiratory muscles. Thus, the respiratory gas exchanges stop and the individual dies by asphyxia.										

Exercise 34 (5 pts) Control of reflex

Session 2017-2

1	The myotatic reflex is the contraction of the muscle due to its own stretching	1/2
2	<p>The two circuits posses the same sensory neuron . Each of the two circuit have a unique motor neuron α. The circuit of the flexor muscle possesses an interneuron between its sensory neuron and its motor neuron α. However the circuit of the extensor muscle does not have interneuron .</p> <p>The number of synapses in the extensor muscle(1) circuit is less than that in the circuit of the flexor muscle (2).</p>	1
3	<p>The muscle which receives the excitatory nerve message, contracts. Since the fiber issued from the motor neuron α which innervates the extensor muscle shows the propagation of the nerve message of frequency equals to 15 A.Ps. Hence the muscle which contracted is the extensor muscle.</p> <p>The muscle which does not receive any nerve message does not contract. Since the fiber issued from the α motor neuron and which innervates the flexor muscle shows resting potential (or absence of action potential). Hence this muscle stays at rest.</p>	1
4	The interneuron plays an inhibitory role on the α motor neuron of flexor muscle.	1/2
5	<p>The recordings at the level of the fibers issued from the motor neuron of the extensor muscle shows disappearance of the frequency of action potential (previously shows recording at the level of the same fiber in the absence of contraction of the flexor muscle). Although there exists sensory nerve message of frequency of 8 APs at the level of the sensory neuron, and this is explain by the spatio-temporal summation of an excitatory nerve message coming from sensory neuron and an inhibitory message coming from the flexor muscle resulting in the disappearance of the recording (algebraic sum).</p>	1
6	<p>Only the frequency of APs of the fiber issued from the motor neuron innervating the extensor muscle decreases from 15 to 3 APs after the voluntary contraction of flexor muscle. (On the contrary, sensory nerve message stays at the same frequency of 8 Aps in the two cases with and without voluntary contraction of flexor muscle) .</p> <p>Thus the superior nerve center inhibits only the motor nerve message at the level of the motor neuron innervating the extensor muscle. This results in decreasing the stimulation of the muscle consequently its contraction and attenuates the myotatic reflex. .</p>	1

Exercise 35 (5 pts) Cause of a paralysis

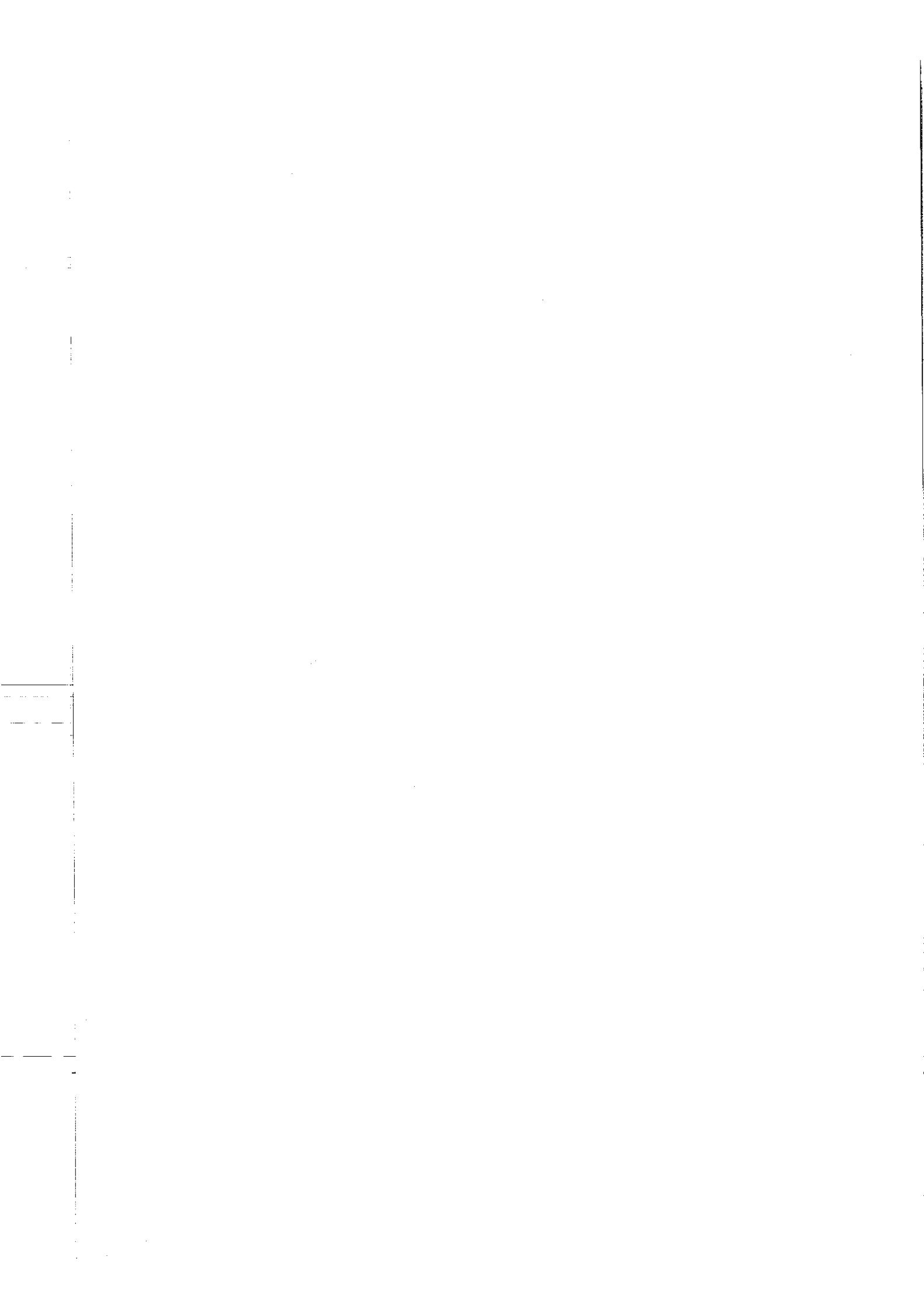
Session 2018-1

1.1	In intense light, the diameter of the pupil is more dilated, 7mm , a value greater than 2 mm obtained in darkness. Furthermore, the muscle of the iris is contracted in the presence of intense light, but it is relaxed in darkness.	0,5
1.2	Light stimulates the contraction of the muscle of the pupil.	0,25
2	The steps of the synaptic transmission are : 1. Arrival of the nervous message to the terminal bud of the presynaptic neuron. 2. Opening of the voltage dependent calcium channels and entrance of calcium ions into the terminal bud. 3. Exocytosis of the neurotransmitter from the synaptic vesicles 4. Fixation of the neurotransmitter on its specific receptors found on the post-synaptic membrane. 5. Generation of PSP 6. Recapture or degradation of neurotransmitter.	1,25
3	In the presence of intense light, the quantity of neurotransmitter in the synaptic cleft and that fixed on the post-synaptic receptors is higher than the quantities obtained in darkness. Since these synapses are excitatory, the muscle will be more contracted in light.	0,5
4.1	In the presence of atropine, the amplitude of muscle contraction increases from 17% to 25% as the concentration of acetylcholine increases from 0.16 microg.mL ⁻¹ to 0.50 microg.mL ⁻¹ , which is less than the amplitude recorded in absence of atropine which increases from 24% to 40%.	0,5
4.2	Thus, atropine reduces (attenuates) the muscle contraction.	0,25
5	In the presence of atropine and intense light, the pupil shows a diameter similar to that obtained in darkness, and which is larger than that obtained in intense light without atropine.	0,5
6	The step of the synaptic transmission at the level of which atropine acts is the binding of acetylcholine on its post-synaptic receptors.	0,25
7	Atropine is antagonistic with acetylcholine (document 3), it prevents the fixation of acetylcholine on its specific receptors (document 4). Consequently, the muscle of the iris does not contract in the presence of light. When it is relaxed, the pupil dilates, allowing the passage of more light which permits the ophthalmologist to examine the eye even in the presence of intense light.	1

Exercises 36 (5 pts) Action of atropine

Session 2018-2

1.1	In intense light, the diameter of the pupil is more dilated, 7mm , a value greater than 2 mm obtained in darkness. Furthermore, the muscle of the iris is contracted in the presence of intense light, but it is relaxed in darkness.	0,5
1.2	Light stimulates the contraction of the muscle of the pupil.	0,25
2	The steps of the synaptic transmission are : 1. Arrival of the nervous message to the terminal bud of the presynaptic neuron. 2. Opening of the voltage dependent calcium channels and entrance of calcium ions into the terminal bud. 3. Exocytosis of the neurotransmitter from the synaptic vesicles 4. Fixation of the neurotransmitter on its specific receptors found on the post-synaptic membrane. 5. Generation of PSP 6. Recapture or degradation of neurotransmitter.	1,25
3	In the presence of intense light, the quantity of neurotransmitter in the synaptic cleft and that fixed on the post-synaptic receptors is higher than the quantities obtained in darkness. Since these synapses are excitatory, the muscle will be more contracted in light.	0,5
4.1	In the presence of atropine, the amplitude of muscle contraction increases from 17% to 25% as the concentration of acetylcholine increases from 0.16 microg.mL ⁻¹ to 0.50 microg.mL ⁻¹ , which is less than the amplitude recorded in absence of atropine which increases from 24% to 40%.	0,5
4.2	Thus, atropine reduces (attenuates) the muscle contraction.	0,25
5	In the presence of atropine and intense light, the pupil shows a diameter similar to that obtained in darkness, and which is larger than that obtained in intense light without atropine.	0,5
6	The step of the synaptic transmission at the level of which atropine acts is the binding of acetylcholine on its post-synaptic receptors.	0,25
7	Atropine is antagonistic with acetylcholine (document 3), it prevents the fixation of acetylcholine on its specific receptors (document 4). Consequently, the muscle of the iris does not contract in the presence of light. When it is relaxed, the pupil dilates, allowing the passage of more light which permits the ophthalmologist to examine the eye even in the presence of intense light.	1



Ch. 8 Regulation of female sex hormones

Regulation of female sex hormones

Course abstract

1. The organs of the female reproductive system

1.1. The ovary: it is the organ responsible for the release of the female gamete, the oocyte II. Each oocyte II is included in a follicle that develops, releases the oocyte II, then turns into a yellow body (corpus luteum). Finally, the corpus luteum degenerates after 14 days of the ovulation.

1.2. The uterus: it is composed of three layers: serous external layer, muscular layer (myometrium that contracts during delivery) and internal layer (endometrium: site of uterine variation and target of ovarian hormones).

1.3. The cervix of the uterus: The cervix of the uterus secretes cervical mucus in two different aspects according to the phases: loose, fluid and permeable during ovulation facilitating the passage of sperm cell; dense, thick and sticky outside of ovulation during the post ovulatory phase.

2. The sexual cycle:

The sexual or menstrual cycle is manifested by synchronized changes in the ovaries, the endometrium (uterus) and the cervix. These variations last 28 days in average and are decomposed into two phases separated by ovulation, the pre-ovulatory phase and the post-ovulatory phase.

The following table summarizes these variations:

Genital Organ Phase	Ovaries	Uterus	Cervix of the uterus
Pre-ovulatory phase	Follicular phase: the cavitary follicle develops and becomes Graafian follicle (mature)	Proliferation Phase: the first 5 days are characterized by a flux of blood due to desquamation of the endometrium. Afterwards, the endometrium begins to thicken and becomes rich in blood vessels and uterine tube-like glands	Thick and sticky cervical mucus
Ovulation	The mature follicle envelope ruptures and releases the Oocyte II which survives for 24 hours and then degenerates in the absence of spermatozoa	The endometrium continues its development	Loose, fluid and permeable cervical mucus
Post-ovulatory phase	Luteal phase: the ruptured follicle turns into a yellow body that degenerates 14 days later.	Secretory phase: the endometrium becomes very thick; the uterine glands secrete glycogen and the blood vessels become spiral. It takes the appearance of uterine laces.	Thick and sticky cervical mucus

Cyclic variations in the menstrual cycle are also accompanied by variations in the vagina and body temperature.

At the level of the vagina, it is manifested by the percentage variation of differentiated cells that reach their maximum differentiation during ovulation.

The body temperature oscillates around 36.8°C during the follicular phase, it increases the day of ovulation to exceed 37°C and remains high during the luteal phase.

3. Regulation of the amount of female sexual hormones:

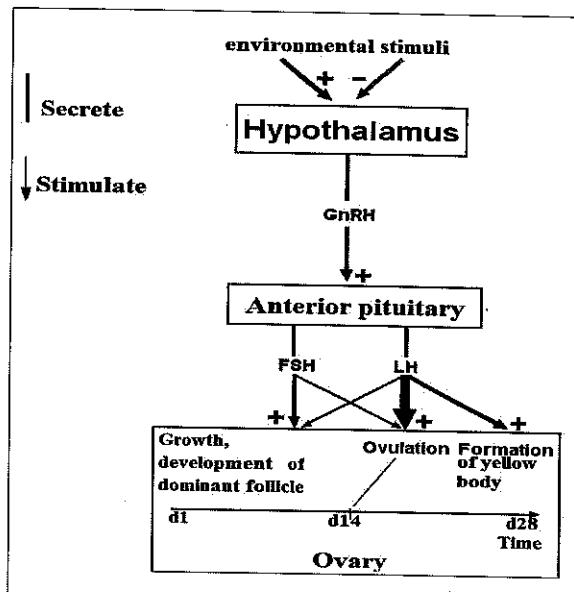
The maintenance of a menstrual cycle in a woman requires a control by the secreted hormones in a pulsatile and synchronized manner by the posterior hypothalamus, anterior pituitary and ovaries. In another way, the activity of each of these organs is not autonomous.

3.1. The control of the ovaries activity by the hypothalamo-pituitary axis:

The hypothalamus, containing neurons, secretes neuro-hormone GnRH (also called gonadotropin releasing hormone) which stimulates the anterior pituitary gland to secrete FSH and LH (gonadotropin-stimulating hormones or gonadotropic hormones).

FSH is characterized by a slight increase at the beginning of this phase, promotes the development of follicles and stimulates their secretion of estrogen.

On the other hand, LH is characterized by a slight increase at the beginning of this phase and also by a peak a few hours before ovulation, it triggers the ovulation and the transformation of the ruptured follicle into a yellow body.

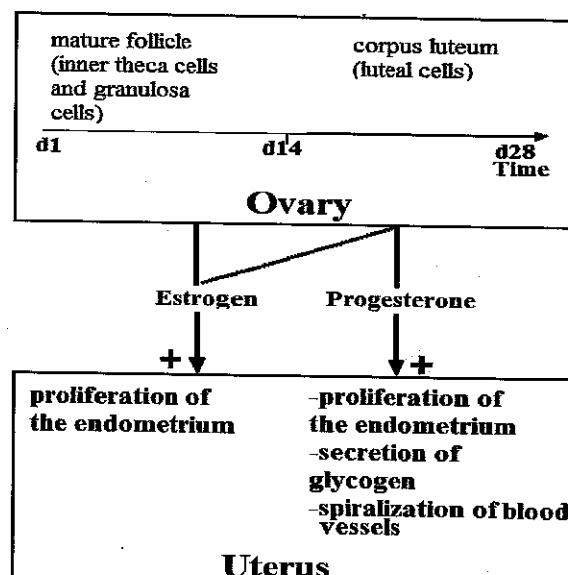


3.2. The control of the activity of the endometrium by the ovarian hormones:

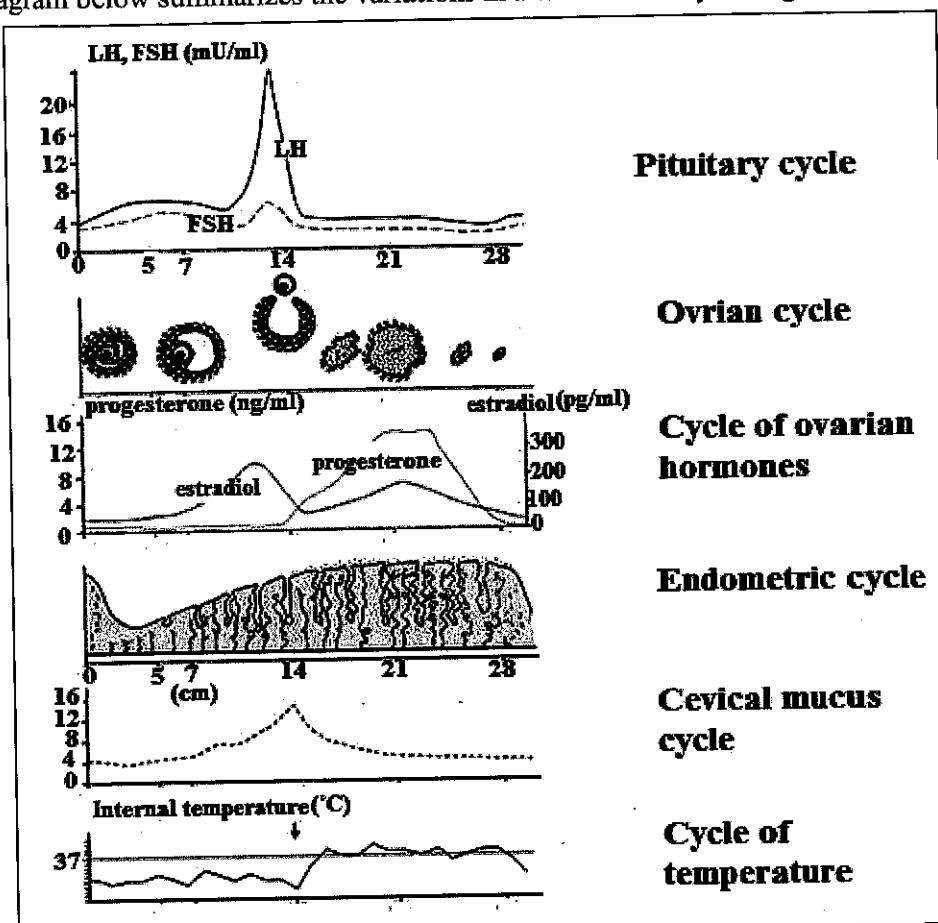
During the follicular phase, follicles secrete estrogen from internal theca cells and granulosa cells; estrogen stimulates the proliferation of the endometrium, development of tube glands and blood vessels

During the luteal phase, the corpus luteum is responsible for the secretion of two ovarian hormones: estrogen and progesterone. Progesterone is responsible for the formation of uterine lace and the glycogen secretion, as well as the elevation of body temperature and the inhibition of uterine contractions. It amplifies and completes the action of estrogen.

At the end of the cycle, the decrease in hormone levels of the ovary causes desquamation of the endometrium, which causes menstruation, and marks the beginning of a new cycle.



The diagram below summarizes the variations in a woman's body during a normal menstrual cycle.

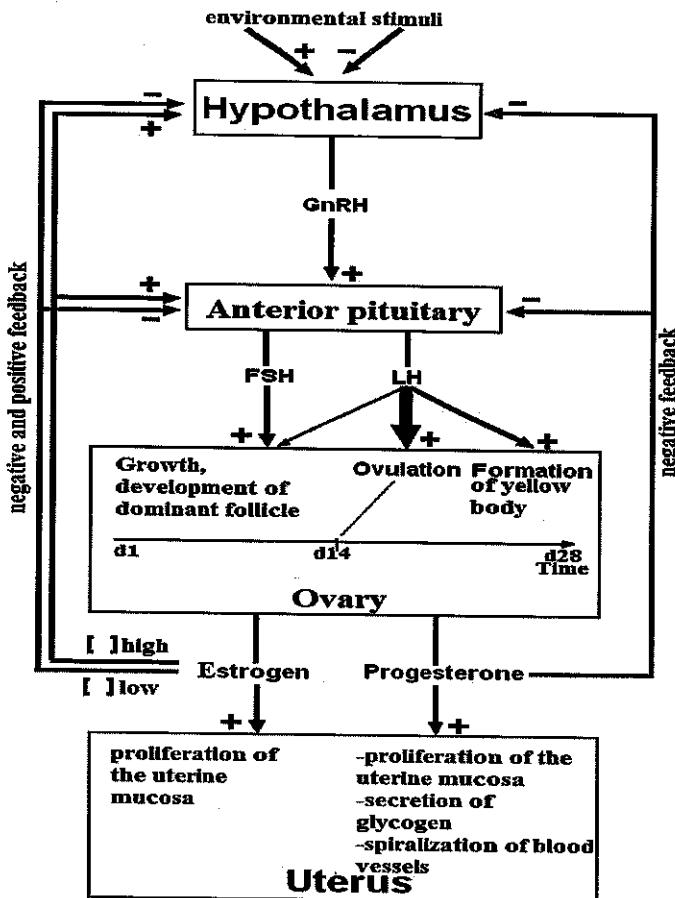


3.3. Ovarian feedback control on the activity of hypothalamo-pituitary axis:

The activity of the hypothalamic-pituitary axis is modified according to the quantity of the ovarian hormones: This is the feedback control (positive or negative):

- During the follicular phase:
 - The moderate rate of estrogen causes a decrease in levels of FSH and LH starting from the middle of the follicular phase: negative feedback.
 - The high rate of estrogen 48 hours before ovulation causes an increase (peak) of FSH and LH levels that occur 24 hours before ovulation: positive feedback
- During the luteal phase:
 - High levels of estrogen and progesterone cause a decrease in FSH and LH levels: negative feedback

The diagram below summarizes the control and feedback of the activities of the female reproductive organs.



4. The menstrual cycle and the pregnancy:

During pregnancy, the corpus luteum persists for 3 months; it continuously secretes estrogen and progesterone, which takes the pregnancy in a long luteal phase where estrogen and progesterone exert a negative feedback on the secretion of FSH and LH. Thus, the endometrium does not slough. In addition, there is neither development and maturation of the follicles, nor ovulation during this period. On the other hand, in a postmenopausal woman, ovaries are depleted of oocytes and follicles, estrogen and progesterone levels are almost nil, FSH and LH levels are high due to the absence of ovarian feedback on the pituitary gland. The endometrium does not develop and there are no menses.

5. Birth control methods

- Contraceptives methods:
 - Prevention of fertilization: **male condom, vasectomy, diaphragm, tubal ligation and mini pill.**
 - Inhibition of ovulation: **combination pill.**
 - Prevention of implantation: **IUD.**
- Contraceptive methods:
 - mechanical method: aspiration of embryo
 - Chemical method: **RU486**
 - Artificial insemination (**AIH et AID**)
 - **IVF and ET**
- MAP:

The contraceptive method is used to prevent conception while the contraceptive method is used to interrupt a pregnancy (gestation).

5.1. Role and mode of action of combination pill:

The combination pill contains estrogen and progesterone, which together exert negative feedback on the hypothalamic-pituitary axis, which results in inhibition of FSH and LH secretion at the beginning of the cycle, since FSH and LH are responsible for follicle development and as a result of the secretion of estrogen then their absence causes an absence of estrogen peak and also absence of the LH peak responsible for triggering ovulation. In addition, the combination pill prevents the formation of uterine lace making implantation difficult (anti-implantation) and also it makes cervical mucus dense preventing the passage of spermatozoa (anti-mucus action), all these actions are contraceptive. In a woman under the effect of a combination pill, the interruption of the pills use is necessary to eliminate their hormones which ensure a moderate development of the endometrium and consequently a weak flux of blood.

5.2. Role and mode of action of mini pill

The mini pill contains synthetic progesterone alone, it disturbs the development of the endometrium, and modify the aspect and the volume of the cervical mucus that blocks the passage of sperm cells; it has a contraceptive action.

5.3. Role and mode of action of RU486

Normally progesterone binds to its receptors present in the nucleus of an endometrial cell; this binding causes the formation of proteins of endometrial thickening by transcription and translation. RU 486 is a competitive substance for progesterone with a greater affinity for it, it blocks their receptors in the endometrial cells, thus prevents the action of progesterone on them and also the protein synthesis, which will stop the development of the endometrium and the formation of the uterine lace. This will prevent the implantation of the embryo and consequently there will be a desquamation of the endometrium and an appearance of menses.

5.4. MAP: medically assisted procreation:

This is the help offered to a couple with infertility problems.

5.4.1. Artificial insemination:

There are two types:

- AIH (from husband) in cases where women have cervical mucus problem (always dense) or the husband has oligospermia (low numbers of spermatozoa).
- AID (From a donor) in the case where the husband has azoospermia (absence of spermatozoa).

5.4.2. IVF

This technique involves injecting the woman having blocked tubules with FSH that is responsible for the maturation of the follicles followed by an oocyte research after aspiration of the mature follicles. Oocytes are put in contact with spermatozoa to undergo fertilization. The transfer of selected embryos, from 8 to 16 cells, takes place 3-4 days after fertilization in the uterine cavity ready for implantation.

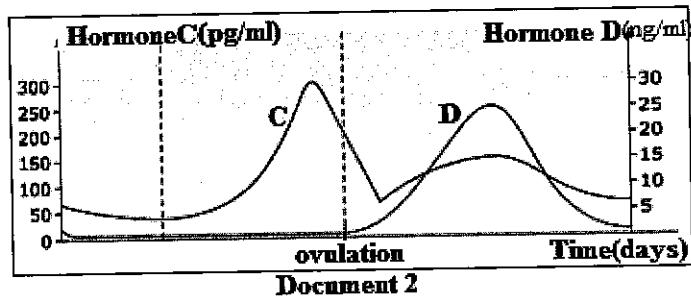
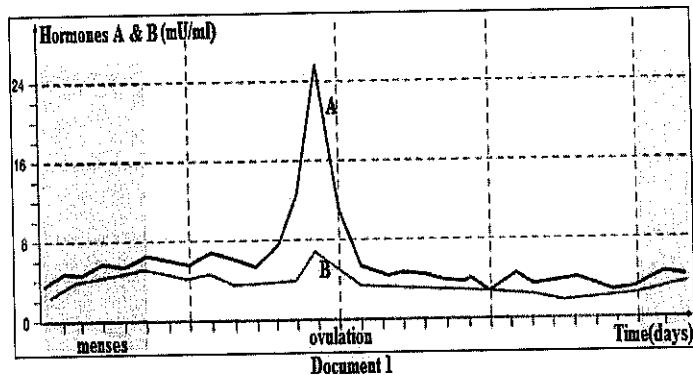
Regulation of female sex hormones

Training exercises

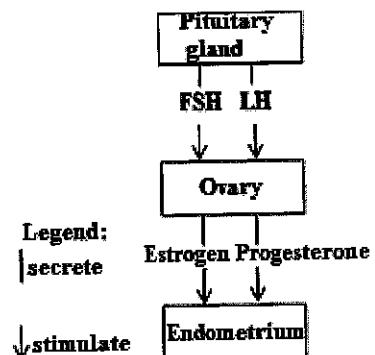
EXERCISE I Sexual hormones

Documents 1 and 2 shows the variations of the plasma amounts of four hormones A, B, C et D which interfere during the normal sexual cycle in a woman.

- 1 Identify the hormones A, B, C and D
- 2.1 What are the cells responsible for the secretion of hormones C and D?
- 2.2. What are the effects induced by these hormones?
- 3 Indicate the role of hormones A and B.
- 4 Knowing that ovulation occurs on the 14th day of this cycle; what are the ovarian and pituitary hormonal variations responsible for triggering ovulation?
- 5 Establish a simplified functional diagram, using all the information provided by questions 1, 2, 3 and 4, showing the functional and chronological links between the organs involved in the determinism of female sexual cycles.

**Solution**

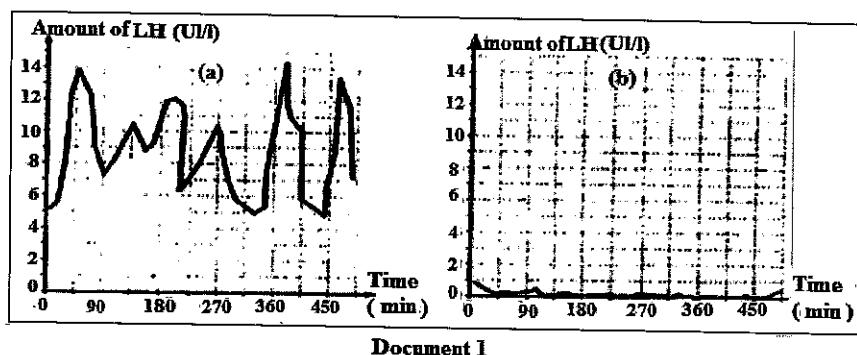
- Both hormones A and B show a slight increase during menses and a peak just before ovulation for each one of these two hormones, these characteristics correspond to pituitary hormones. On the other hand, the peak of the hormone A (24 UI / l) is greater than that of the hormone B (6 UI / l). So, hormone A is the pituitary hormone FSH and that of B is the pituitary hormone LH. For hormone C, there are two peaks, one before ovulation and the other smaller after ovulation. This variation corresponds to estrogen ovarian hormone. For hormone D, which has only a peak after ovulation and is almost nil before ovulation, its variation corresponds to the ovarian hormone progesterone.
- The cells that secrete hormone C: follicular granulosa cells, internal theca cells and luteal cells of the corpus luteum.
- The cells that secrete the hormone D: the luteal cells of the corpus luteum.
- Estrogen stimulates the proliferation of the endometrium, the development of tube-like glands and blood vessels. Progesterone is responsible for the formation of uterine lace and glycogen secretion, as well as the increase of body temperature and the inhibition of uterine contractions.
- Hormone B (FSH) promotes follicle development and stimulates estrogen secretion. Hormone A (LH) also supports triggers ovulation and ensures the transformation of the ruptured follicle into a corpus luteum
- The optimal conditions of ovulation: estrogen peak which by positive feedback ensures a peak of LH.
- Simplified functional diagram, showing the functional and chronological relation between the organs involved in the determinism of female sexual cycles.



EXERCISE 2 An abnormal cycle in a girl

In some adult women there is an absence of pubertal development and the complete absence of menstruation. Document 1 shows the results of LH dosage every 10 minutes for 8 hours in a normal woman (a) and in the patient (b) with a pubertal delay.

1. Compare the evolution of the LH concentration in a pubertal woman and in a patient, woman suffering from pubertal development disorders, presented in document 1.

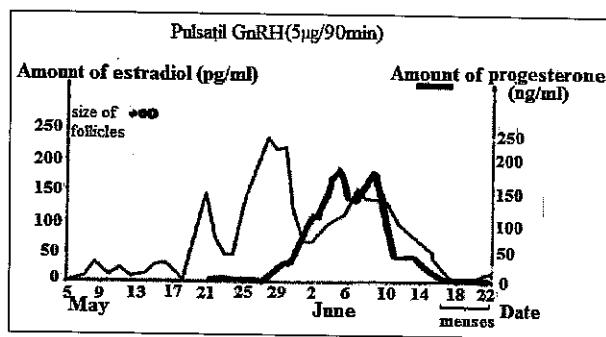
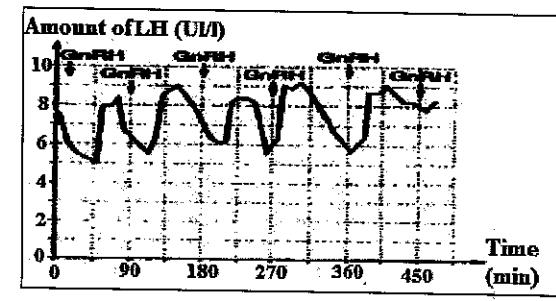


Document 2 shows the measurement of LH concentration in the patient (b) during pulsatile administration of GnRH (5 µg every 90 minutes).

2. Specify, from the result obtained in document 2, whether the pulsatile injection of GnRH is effective or not.

Document 3 represents the variations of estradiol and progesterone amount during prolonged and pulsatile administration of GnRH in the patient (b).

3. Determine, from document 3, whether the prolonged and pulsatile administration of GnRH is capable of leading to a complete normal cycle.
4. Draw out, according to document 3, the origin of the pubertal delay of this patient.

**Solution**

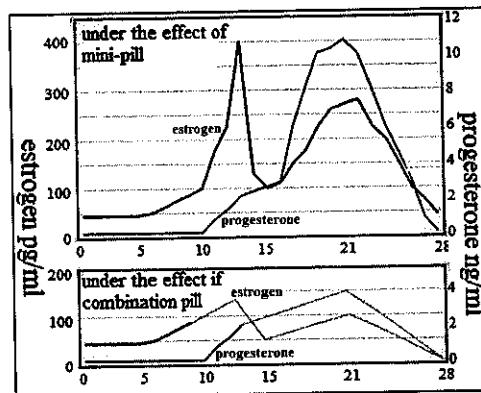
1. In the patient woman suffering from pubertal developmental disorders, the amount of LH is zero during the 450 min of dosage. On the other hand, the level of LH in the normal woman is not constant; it fluctuates between 5 and 14 IU / l during the same minutes of dosage.
2. The pulsatile injection of GnRH (5 µg every 90 minutes) in the patient woman is effective because this injection causes a secretion as pulsatile and has an amount of 7 IU / l quite similar to the secretion of a normal woman presented in document 1 (a).
3. From May 17 to June 16, a normal cycle is observed:
 - From May 17th to May 31st: increase of the estradiol amount and absence of progesterone, it is the follicular phase
 - From June 1st to June 16th: increase of progesterone and estradiol with a maximum secretion around June 8th, it is luteal phase.
 After, a new cycle begins on June 17 characterized by the appearance of menses. So, the pulsatile injection of GnRH allowed a normal cycle.
4. The origin of the pubertal delay of the patient woman is a deficiency of GnRH secretion.

EXERCISE 3 The contraceptive pills

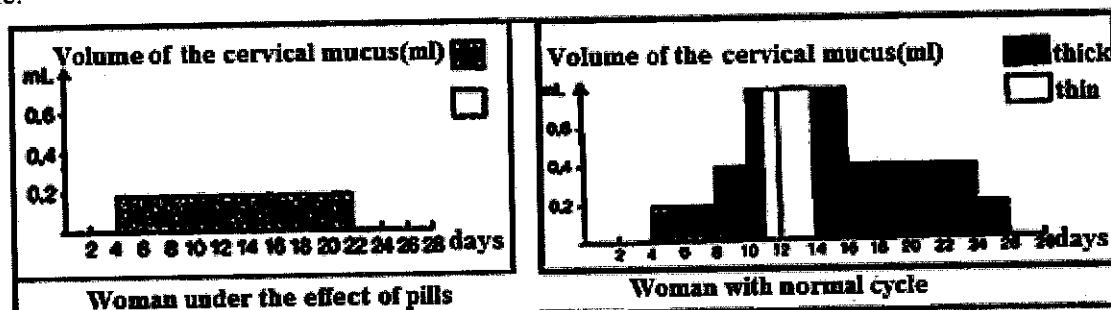
In order to study the difference between the effect of two different contraceptive pills, document 1 represents the variation of the estrogen and progesterone amount in two women, the first taking a mini-pill and the other taking a combination pill

1. Compare the variation of estrogen and progesterone in both women.
2. Determine the pill that does not prevent ovulation.

Document 2 represents the variation in the volume and the aspect of cervical mucus in a woman under the effect of a pill and in another woman using no pill during the normal cycle.



Document 1

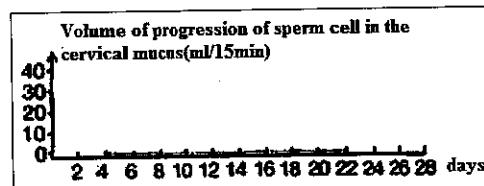


Document 2

1. Explain, based on acquired knowledge and the results in document 2, the contraceptive action of the mini-pill used in document 1.
2. Propose a graph which represents the variation of the sperm speed in the cervical mucus in a woman under the effect of mini-pill during the days of a cycle.

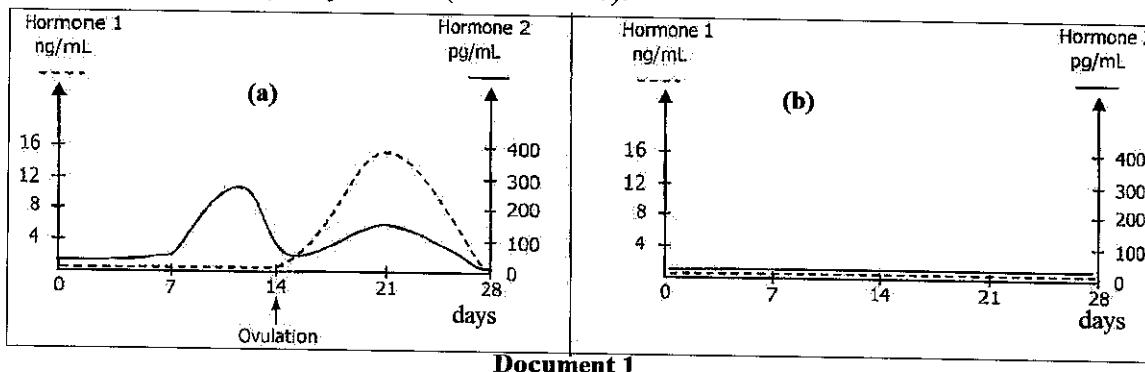
Solution

1. In a woman under the effect of mini-pill, the estrogen amount has two peaks of 400 pg / ml and 375 pg / ml respectively at the 13th day and the 21st day, likewise in a woman under the effect of a combination pill but with smaller peaks of 150 pg / ml and 100pg / ml on the same days. With regard to progesterone, it is almost nil before 10 days in both women and has a peak on the 21st day but it is higher in the woman under the effect of mini-pill (11ng / ml) than that in the woman using combination pill in the same day.
2. The high amount of estrogen during the first phase in the woman under the effect of mini-pill causes a peak of LH by positive feedback, this peak triggers ovulation. On the other hand, the low amount of estrogen in a woman under the effect of combination pills does not ensure this peak of LH and consequently there is no ovulation. So, the mini-pill does not prevent ovulation.
3. Document 2 shows that the cervical mucus is thin and abundant (0.8 ml) in a woman under normal cycle between 11 and 14 days (ovulation period) and it is dense outside this period. On the other hand, in a woman under the effect of combination pills or mini-pills the cervical mucus is dense during all days of the cycle with a constant and low volume (0.2ml). Because the dense cervical mucus prevents sperm progression in the female genital tract and hence the meeting between male and female gametes.
4. Graph representing the variation of the speed of progression of sperm cells in the cervical mucus in a woman under the effect of mini-pill during the days of a cycle.



EXERCISE 2) Human reproduction

It is proposed to study certain aspects of the reproductive function in women. Document 1 presents 28 days of ovarian hormone dosages in two women: woman X of 25-year-old (document 1a) and another woman (Y-woman) 50 years old (document 1b).

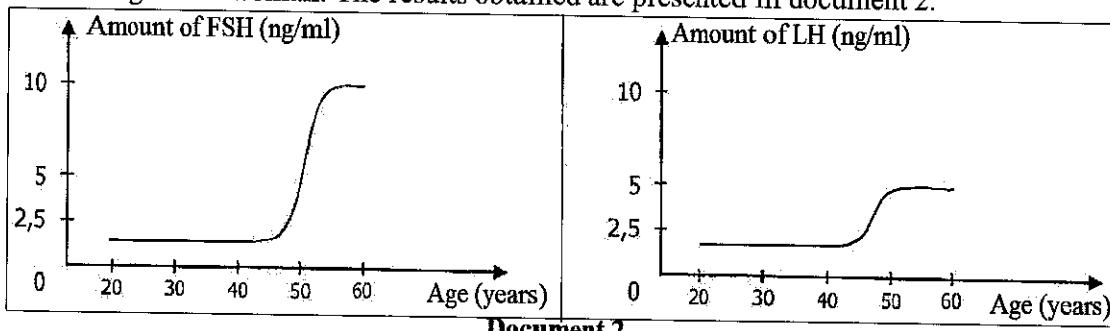


1. Name the hormones 1 and 2.
2. Draw out, from document 1, two differences that distinguish the ovarian activity of woman X from that of woman Y.

To explain the origin of the modifications observed in the woman Y (50 years old), the following hypotheses are proposed:

- **Hypotheses 1:** these modifications are related to an abnormality in the hypothalamic-pituitary axis that does not produce its hormones.
- **Hypotheses 2:** these modifications are related to a phenomenon of ovarian origin.

To verify the validity of these hypotheses, we follow the evolution of the average amounts of pituitary hormones during life in woman. The results obtained are presented in document 2.



3. Determine the rejected hypothesis starting from these givens.
4. Explain, based on the given in document 1b and the acquired knowledge, the origin of the hormonal modification observed in a woman 50 years old (document 2).

We try to locate the action of hormone 1 (see document 1a) in the uterus and specify the utility of this action during pregnancy. For this we use a substance X whose structure is similar to that of hormone 1 and which causes the interruption of pregnancy following its administration to the pregnant female. In addition, the endometrium of a rabbit is taken and crushed. The ground material is centrifuged. The supernatant which contains proteins from the endometrium is distributed in two tubes. Two experiments are carried out by adding to the supernatants of the two tubes certain elements as indicated in the table in document 3.

Tube	Elements presented in the tubes	Percentage of binding Hormone 1 - proteins present in the supernatant
1	Supernatant + radioactive hormone 1	100%
2	Supernatant + non-radioactive substance X and after a certain time we add the radioactive hormone 1	0%

Document 3

5. Specify the physiological role of the proteins present in the supernatant.
6. Explain, based on the previous information and the acquired knowledge, the mode of action of the substance X on the progress of the pregnancy.

Solution:

1. Hormone 1: progesterone, hormone 2: estrogen
2. The ovaries of a woman Y do not produce oocytes and therefore they do not secrete ovarian hormones: estrogen and progesterone, contrary to the woman X.
3. Document 2 shows that from the age of 50 years, FSH and LH levels increase rapidly to 10 mU / ml (4 times greater) for FSH and 5 mU / ml (2 times greater), for LH only in years with normal cycles. This indicates that the hypothalamic-pituitary axis secretes its hormones normally and it has no abnormalities. So, the most likely hypothesis is the presence of modifications in the ovaries.
4. The ovaries of a 50-year-old woman are almost exhausted from the follicles and consequently from oocytes, so no secretion of ovarian hormones (estrogen and progesterone) which by negative feedback reduces GnRH secretion by the hypothalamus and also the secretion of FSH and LH by the anterior pituitary gland. This explains the increase of FSH and LH amounts from the age of 50 years.
5. These proteins are progesterone receptors and since progesterone plays a role in the development of the uterine lace necessary for the implantation and development of the embryo, then they maintain the pregnancy by ensuring its normal development.
6. The percentage of binding of radioactive hormone 1 (progesterone) to the receptors present in the endometrial supernatant is 100%. On the other hand, this percentage becomes zero in the presence of non-radioactive substance X, that means that substance X having a structure close to hormone 1 binds to its receptors and prevents its action in the development of the endometrium, which causes desquamation and termination of pregnancy. It is competitive with the hormone 1 it is a contraceptive substance.

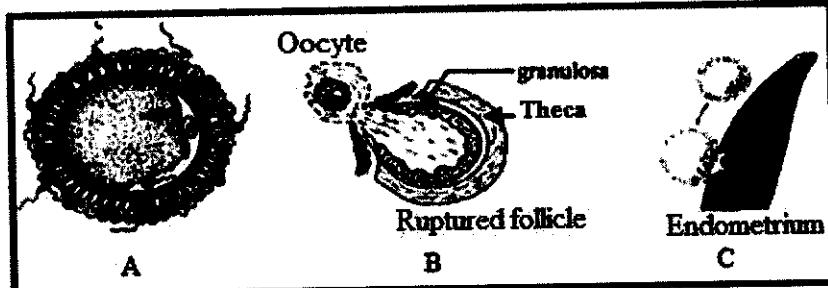
Regulation of female sex hormones

Solved exercises

EXERCISE I Contraception

The birth of a new individual in the mammals necessitates the intervention of the male and the female. The role of the male is to produce male gametes: the sperm cells. The role of the female in this birth requires many processes. Some of these processes are indicated by A, B and C shown in document 1.

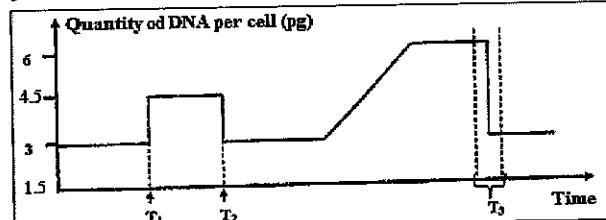
- 1.** Identify the phenomena A, B and C.



Document 1

Document 2 represents the evolution of the quantity of the DNA in the oocyte II of a female X. This oocyte is taken and fertilized *in vitro* by a capacitated sperm cell of Mr. X.

- 2.** Show that the time T_1 corresponds to the entry of the sperm cell to the oocyte and the time T_2 corresponds for the expulsion of the second polar body.

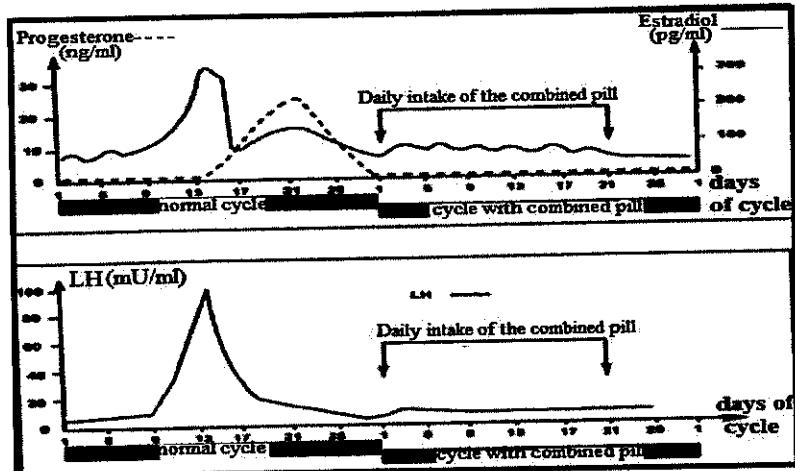


Document 2

Document 3 shows the results of the measurements of the gonadotropin hormones and the natural ovarian hormones during normal cycle and a cycle under combined pill.

By referring to document 3 and to the acquired knowledge:

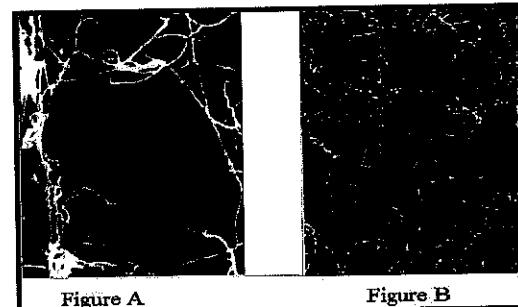
- 3.** Explain how the combination contraceptive pill is able to prevent the process B in document 1.
4. Show, starting from the results in document 3, that the combination pill prevents the process C in document 1.



Document 3

Document 4 shows the state of the cervical mucus in a female in two different states, before and after the administration of the combined pill.

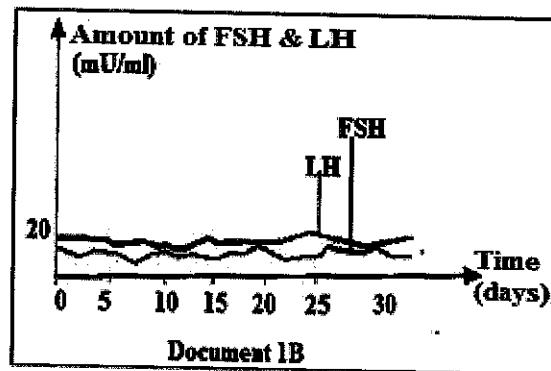
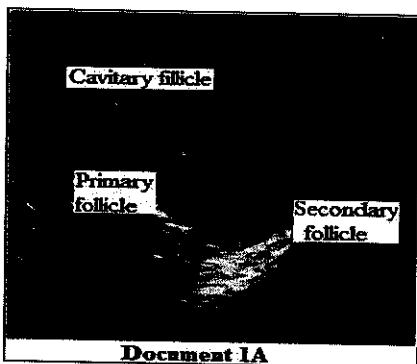
- 5.1.** Specify the figure that represents the cervical mucus after the administration of the combination pill.
5.2. Indicate, starting from document 1, the process that is prevented by the action of the contraceptive pill shown in document 4.



Document 4

EXERCISE 2 A problem of sterility

A woman consults a doctor for a problem of infertility. In order to understand the cause of her problem, the doctor practiced repeated bilateral echography during one month. The two ovaries always appear as shown in document 1A. In addition, he dosed her pituitary hormone levels. The results are shown in document 1B.

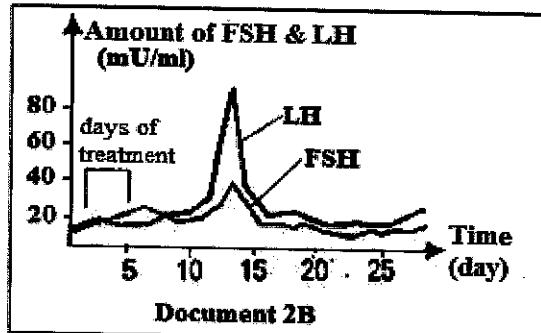
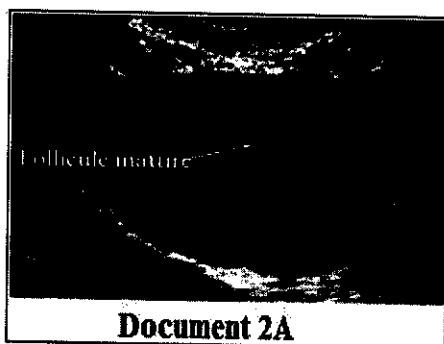


1. Show, by referring to document 1A, that the infertility of this woman originates from a problem of ovarian development.

Based on the echography, the doctor made two hypotheses about the origin of the woman's infertility, the first concerns a pituitary hormone and the second concerns a hypothalamic hormone.

2. Justify the choice of the two hypotheses emitted by the doctor.

In order to treat the infertility problem in this woman, the doctor prescribes clomiphene citrate (Clomid) as a tablet for a period of five days. Treatment begins between the 2nd and 5th day after the first day of menstruation. After administration of high doses of clomiphene citrate, the doctor performed scans at two-day intervals. On the sixth day after he stopped the administration of clomiphene citrate, the ultrasound shows the aspect shown in document 2A. In addition, he dosed her levels of pituitary hormones, the obtained results are shown in document 2B. A dosage of plasma estrogen is made at the same day; it shows a value close to that obtained in a normal woman between days 12 and 13 of the cycle.



3. Determine the effect of clomiphene citrate on the ovaries of women A.
4. Explain, by referring to documents 1B and 2B, the choice of the interval of treatment between the 2nd and 5th day after the first day of the menstruation.

Clomiphene citrate is a synthetic molecule capable of binding to estrogen receptors in the hypothalamus.

5. Explain, from all the above information, the mode of action of clomiphene citrate leading to ovulation in woman A.

EXERCISE 3 A contraceptive: the morning pill

At the woman, ovulation is obligatorily caused by a massive discharge of pituitary hormone (LH).

The mechanism of this discharge is well known and it is based on the effect of the ovarian hormones on the hypothalamo-pituitary axis. Some scientists aim to block the discharge of LH in order to inhibit the ovulation process as in the case of rape or unprotected sexual relation possibly fertilizing.

In castrated guenon, slightly impregnated of estrogens starting from t_0 , they measured the amount of LH in the two following experimental situations:

- After injections of strong amounts of estrogens at t_1 ;
- After injections of strong amounts of estrogens at t_3 , preceded by a continuous plasmatic progesterone impregnation close to 10 micrograms per liter, starting from t_2 .

The results are represented in document 1.

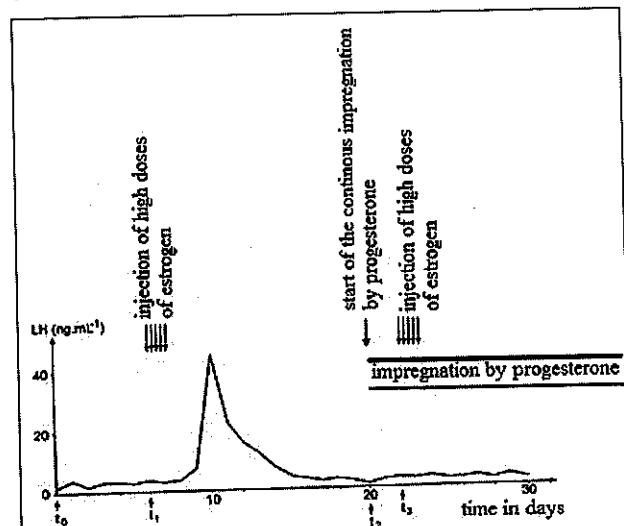
- 1.** Explain the variation of the amount of LH between the intervals $[t_0-t_2]$ and $[t_2-30 \text{ days}]$.

An emergency contraceptive whose active molecule is the levonorgestrel avoids, in 85% of the cases, a not desired ovulation. After taking this contraceptive, the plasmatic concentration of levonorgestrel is close to 10 micrograms per liter. Document 2 shows the condensed structural formula of progesterone molecule and that of levonorgestrel

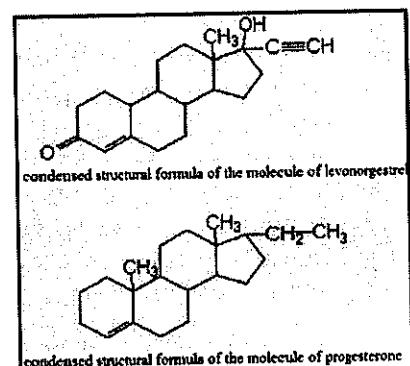
- 2.** Formulate an explanatory hypothesis of the mode of action of levonorgestrel.

Some thin sections of the hypothalamus are treated by:

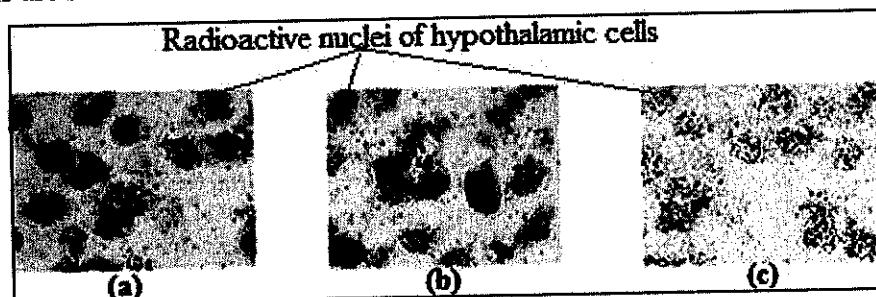
- Radioactive progesterone.
 - Radioactive levonorgestrel.
 - Radioactive progesterone in the presence of non-radioactive levonorgestrel.
- These sections are shown in document 3.



Document 1



Document 2



Document 3

- 3.** Deduce, by referring to document 3, the action of levonorgestrel on progesterone.
- 4.** Explain, by referring to the acquired knowledge and to the documents presented above, the mode of action of levonorgestrel in the prevention of a not desired pregnancy.
- 5.** Specify if we can use the levonorgestrel in the same mode of action as RU 486.

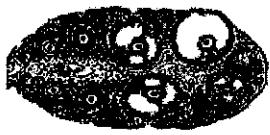
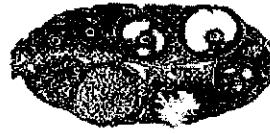
EXERCISE 2 Human Reproduction and Health

We propose to study the mastery of certain aspects of human reproduction through advances in the field of medicine and biotechnology.

Women A, B and C suffer from difficulties of procreation. In order to specify the possible causes of infertility of two women A and B, a gynecologist asks them to perform two types of tests:

Test 1: morphological and histological data of the ovaries

1. Determine the cause of infertility in women A and C.
2. Formulate a hypothesis concerning the possible cause of the infertility of Mrs. B.

Woman (patient)	Size of ovaries	Grouping of photographs of the patient's ovaries at different times during 2 successive months	Tubes: observation during 2 successive months of exam
A	Normal		
B	Normal		No oocyte II in the upper third of the fallopian tubes of patients A and C
C	Normal		

Document 1

Test 2: Hormonal dosages for 28 days

Dosed hormones	Woman A	Woman B	Woman C	Woman with normal sexual cycle
LH (UI/I)	10	Follicular phase: 10 Ovulatory peak: 90 Luteal phase: 10	10	Follicular phase: 10 Ovulatory peak: 90 Luteal phase: 10
FSH (UI/I)	Follicular phase: 2 to 17 Ovulatory peak: 26 Luteal phase: 2 to 8	Follicular phase: 2 to 17 Ovulatory peak: 26 Luteal phase: 2 to 8	0.5	Follicular phase: 2 to 17 Ovulatory peak: 26 Luteal phase: 2 to 8
Estradiol (pg/ml)	Follicular phase: 30 to 90 Ovulatory peak: 400 Luteal phase: 0.5	Follicular phase: 30 to 90 Ovulatory peak: 26 Luteal phase: 5 to 210	15	Follicular phase: 30 to 90 Ovulatory peak: 400 Luteal phase: 5 to 210

3. Explain, with reference to the result of test 2, the hormonal situations of the two women A and C.

The doctor prescribes to woman A during the follicular phase a hormonal treatment which consists of regular injections and normal doses of a substance LH at a specific day of the cycle. After 10 days of the treatment, the doctor detects the presence of the hormone HCG in the blood of woman A. A hormone that prevents the degeneration of the corpus luteum.

3.1 Pick out the role of HCG.

3.2 What does the appearance of the HCG hormone in woman A's blood show? Justify.

The doctor advises woman B to perform a technique called IVF.

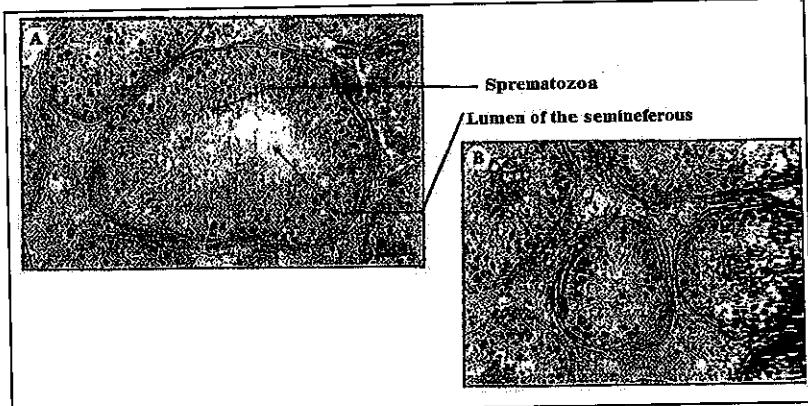
3. Explain, based on all the information concerning woman B, the advice of the doctor.

EXERCISES The mode of action Male Contraceptives

Since 1960, many experiments have been tried to develop male hormonal contraceptive methods. However, some attempts have undesirable side effects (acne, weight gain, aggression) studies are conducted to determine the mode of action of one of these male contraceptives and it is presented below:

Document 1 shows the microscopic observation of cross-sections of testis:

- Photography-A is for a man without treatment.
- Photography-B shows the structures that could be observed in the same man under the action of chemical contraceptive: testosterone undecanoate (TU). This molecule is similar to testosterone.



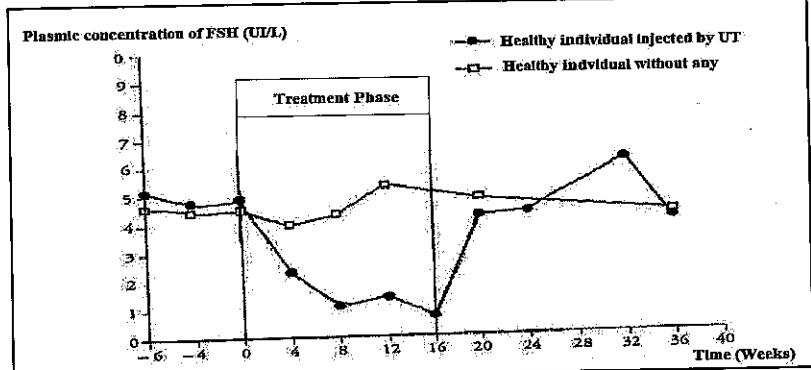
Document 1

1. Draw out, based on document 1, the effect of this treatment on the testicles.

To study the effect of contraception on the production of FSH, we measure the evolution of the plasma concentration of FSH over time in two men:

The first man receives no chemical contraceptive.
The second received the chemical contraceptive "TU."

2. Represent the data of the graph in document 2 in a table.
3. Deduce the effect of this contraceptive on the secretion of FSH.



Document 2

The table in document 3 shows the effects of various experiments on the production of sperm by a lot of rats. The mechanisms responsible for the continuous production of sperm in rats are similar to those of man. The experiments are independent.

Realized experiment	Obtained result
Non	Continuous production of spermatozoa
Injection of excess of testosterone	No production of spermatozoa
Ablation of the pituitary	No production of spermatozoa
Injection of pituitary extract (FSH) after the ablation of the pituitary	Continuous production of spermatozoa

Document 3

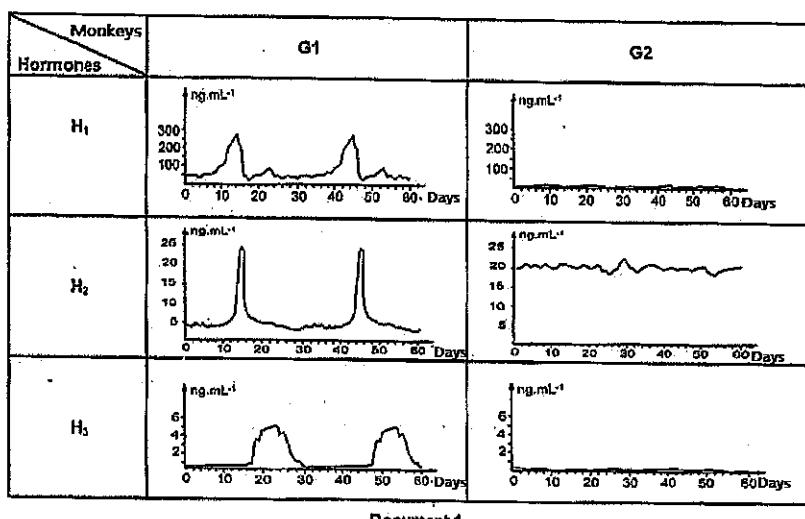
4. Interpret the results of the experiments shown in document 3.
5. Explain, based on what preceded, the mode of action on the TU contraceptive.

EXERCISE 6 Female Reproductive Function

In order to understand the hormonal interactions involved in the development of the uterine mucosa, some observations and experiments are made.

Document 1 represents the variations of three sex hormones H1, H2 and H3 in two mature guenon monkeys G1 and G2, one of which is normal and the other is ovariectomized (the sexual cycle of guenon is similar to that of the woman).

- 1.1.** Identify the hormones H1, H2 and H3.
- 1.2.** Specify which of the two monkeys is ovariectomized.



Document 1

In order to specify the nature of the relationship between H1 and H2 on the one hand and the role of H1 and H3 on the development of the endometrium, the following experiments are carried out:

First series of experiments:

Experiment 1: The injection of a low dose of the hormone H1 to the G2 monkey causes a fall in the secretion of the hormone H2.

Experiment 2: The injection at the beginning of the cycle and for a short time of a high dose of the hormone H1 to the monkey G1 causes a sudden secretion of the Hormone H2.

Second series of experiments

Experience 3: The injection of the hormone H3 at the beginning of the cycle with the G2 monkey does not show changes in the uterus.

Experiment 4: In the guenon G2, the following injections are carried out:

- * Injection of the hormone H1 during the first 30 days.
- * Injection of the hormone H3 from the 16th to the 30th day.

These injections show a development of the uterine mucosa and the appearance of uterine lace.

- 2.1.** Specify the nature of the relationship between the hormones H1 and H2.
- 2.2.** Explain the role of H1 and H3 hormones in the development of the uterine endometrium during a normal sexual cycle.

Document 2 represents the calendar of the sexual cycles in a woman during the months of January (without pill) of February and March. (with combination pill).

		1	2	3		5	6	7		4	5	6	7	Menses
		8	9	10										
11	12	13	14	15	16	17								
18	19	20	21	22	23	24								
25	26	27	28	29	30	31								
WITHOUT PILLS							WITH COMBINATION PILLS							Numbers in bold: Days of pills

Document

- 3.** Indicate the date of ovulation in this woman.
- 4.** Explain the mechanism that causes the presence or absence of ovulation during the months of January, February and March.
- 5.** Explain the appearance of menses during February and March.

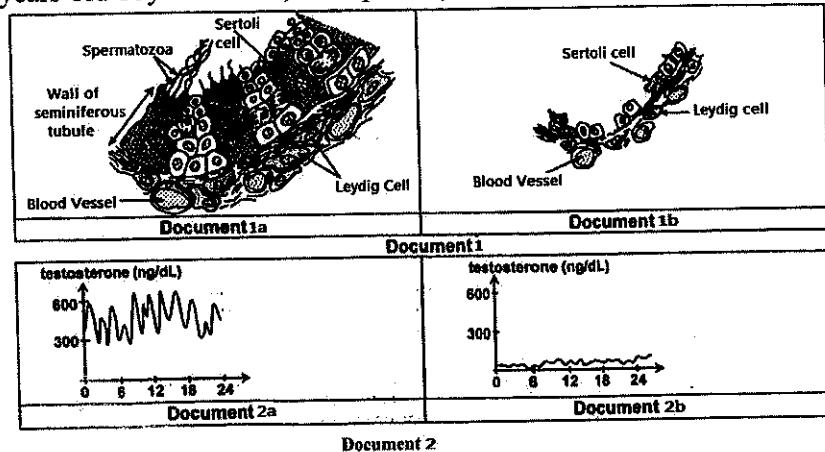
EXERCISE 7 Male fertility

The mechanisms of regulation of testicular functions in humans involve hormonal interactions between the hypothalamic-pituitary complex and the testes.

To understand these mechanisms, we refer to a medical study carried out in a normal mature boy and to a study carried out in two 19 years old boys X and Y, with puberty disorders.

Document 1 represents schemas, interpretation of microscopic testicular observations performed in the normal boy (doc 1a) and in both boys X and Y (document 1b).

Document 2 shows the results of testosterone levels secreted by Leydig cells for 24 hours in normal boy (Doc 2a) and in both boys X and Y (Document 2b).



- 1.1 Compare the testicular appearance observed in both boys X and Y to that of the normal boy.
- 1.2 Analyze testosterone test results in the three boys.
- 1.3 Draw out the relation between the microscopic structure of the testes and the secretion of testosterone observed in the two boys X and Y.
2. Formulate 2 hypotheses showing the origin of the disorders observed in boys X and Y

Document 3 shows the results of the LH and FSH levels in normal boy and in boys X and Y.

	Concentration of LH (UI/L)	Concentration of FSH (UI/L)
Normal mature boy	2 to 10	1 to 12
Boys X et Y	0.5 to 0.9	0.1 to 0.4

Document 3

Document 4 shows the results of two stimulation tests performed in boys X and Y.

3. Interpret the results of tests 1 and 2.

Document 5 shows test results for normal boys and boys X and Y.

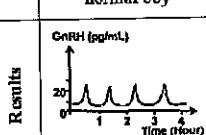
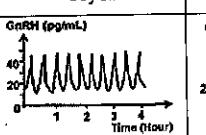
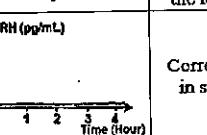
4. Determine by referring to the results of tests 3 and 4 and the data in document 2b:

4.1 The cause of disorders seen in boy X.

4.2 The cause of disorders seen in boy Y.

Stimulation tests	Results
Test 1: Injection of HCG, whose action is similar to that of LH, to boys X and Y	- Correction of disorders of testosterone secretion in both boys. - No change in the appearance of the wall of the seminiferous tube of the two boys.
Test 2: Injection of a mixture of HCG and FSH to the boys X and Y	- Correction of disorders of testosterone secretion in both boys. - Testicular appearance of the two boys becomes similar to that of the normal boy

Document 4

Test	Test 3	Test 4	Test 5	Test 6
Level of GnRH in normal boy	Level of GnRH in boy X	Level of GnRH in boy Y	Injection of GnRH to boy Y followed by measuring the level of LH and FSH	
			Correction of the troubles in secretion on LH and FSH	

Document 5

EXERCISE 3 Chemical substances affecting the endometrium

Many substances are known for their effect on the endometrium in direct or indirect way. The following documents explain some of these affections.

After fertilization, the egg cell begins its progression from the fallopian tubes to the uterus while dividing. At the end of the first week, an embryo consisting of several undifferentiated cells arrives in the uterus; it will implant in the uterine mucosa: this is the implantation.

From the beginning of implantation, the embryo releases the hormone HCG. This hormone has a structure and action similar to pituitary hormone LH; it is responsible for the maintenance of the corpus luteum. It is first of all the hormone HCG which makes it possible to detect a pregnancy, because it is present in the blood approximately eight days after the fertilization, and in the urine a few days later. It then permits to know the exact date of the beginning of the pregnancy by the dosage of its amount, because this one varies very precisely during the pregnancy.

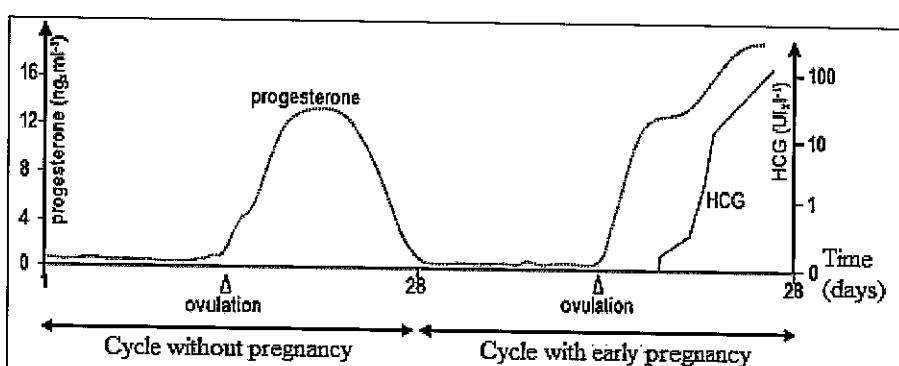
Finally, due to a dosage between the 15th and the 17th week, the hormone HCG is even an indicator of risk of trisomy 21! If its amount reveals a risk, an amniocentesis may be prescribed to confirm the abnormality, or not.

Document 1

- I. Pick out from document 1 the different interests of HCG dosage in urine or blood.

The concentration of HCG and progesterone is measured in a woman starting a pregnancy. The results of the measurement are shown in document 2.

2. Compare the evolution of these two hormones during the cycle without pregnancy and the cycle with early pregnancy.

**Document 2**

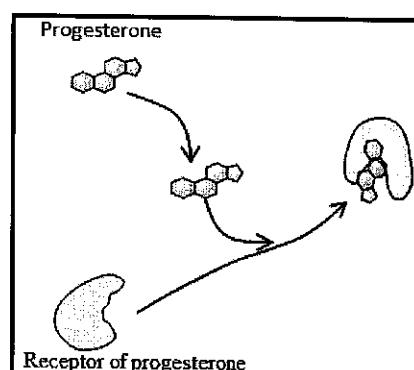
3. Explain, by referring to documents 1 and 2, the increase of progesterone amount after fertilization and its importance.

One of the observations in a pregnant woman is the absence of uterine and ovarian cycles. This absence is under the control of HCG.

4. Show, by referring to the above given, that the action of HCG is responsible for stopping ovarian cycles in a pregnant woman

Mifepristone (RU486), is another substance that affects the endometrium. In the year 2000, two out of three women used contraception, however, many unwanted pregnancies still occur, leading to a significant number of abortions. Document 3 shows the action of progesterone on a target cell receptor of the uterine mucosa.

5. Explain, by referring to the acquired knowledge and to document 3, the action of RU 486 on the endometrium.

**Document 3**

Regulation of female sex hormones

Solved exercises solutions

Exercise 1 Contraception

1. In A, one sperm cell crossed the corona radiata and the zona pellucida and reached periovulatory space, thus this is fertilization.
In B, a ruptured follicle releases the oocyte with the follicular fluid of the follicular cavity, thus this is ovulation.
In C, a blastocyst (an embryo) is bound on the endometrium so this is implantation.
2. The amount of DNA in the oocyte II corresponds to n chromosomes with 2 chromatids each; the amount of DNA contained in the sperm corresponds to half of this amount because the sperm contains n chromosomes of 1 chromatid each. At T1 after the entrance of sperm cell into the oocyte, the amount of DNA increases from the half of the original amount from 3 pg to 4.5 pg.
At T2, when the oocyte II resumes its meiosis II and releases the second polar body (n chromosomes with 1 chromatid each that correspond for 1.5 pg), so the DNA quantity decreases again to 3 pg.
Then the amount of DNA doubles due to the duplication of chromosomes of both male and female pronuclei. During mitosis at T3 chromatids of each chromosome separate between the two daughter cells leading for the decrease of the amount of DNA to the half.
3. The combined pill exerts a negative feedback by estradiol and progesterone on the hypothalamo-pituitary axis, which results the inhibition of LH secretion (constant value 10MU / ml) and FSH from the beginning of the cycle which prevents the development of the follicle, then it prevents the peak of LH which in turn prevents the ovulation.
4. The two graphs in document 3 show that the combined pill inhibits the secretion of estradiol and progesterone during the cycle (estrogen: low value 50pg / ml and almost zero of progesterone), this will result a very low endometrial development which will prevent implantation.

3.1. Figure B shows the cervical mucus after taking the pill because the mucus is very thick and prevents the entrance of spermatozoa.

3.2. The fertilization.

Exercise 2 A problem of sterility

1. Document 1A shows that during all the days of a month the ovaries present primary, secondary and cavitary follicles, i.e., there is absence of a mature follicle due to cavitary follicle development during the follicular phase in the ovaries. So, the problem of this woman lies in ovarian development.
2. The pituitary hormones FSH and LH ensure the development of cavitary follicles into mature follicles during the follicular phase. On the other hand, the hypothalamic hormone GnRH stimulates the activity of the pituitary to secrete FSH and LH. Then there is a close relationship between the pituitary hormones and the hypothalamic hormone and follicle development. For this, the doctor chooses these two hypotheses.
3. Normally, during menstruation the hormones FSH and LH show an increase to ensure the development of cavitary follicle in mature follicle. In this woman, the dosage of these hormones shows a constant and almost zero amounts (10 mU / ml: document 1B), which shows the absence or deficiency in the pituitary production and consequently they don't play any role in the development of follicles. But after treatment with clomiphene citrate the levels of these hormones are very close to normal values: increase at the beginning of the cycle and peaks on the 14th day of the cycle (40 mU/ml and 100mU/ml respectively for FSH and LH). The variations obtained explain the duration of treatment between the 2nd and 5th day after the first day of menstruation.
4. Since after the treatment with clomiphene citrate, the ovary contains mature follicle, thus it plays a role in the maturation of the follicles.
5. A woman's medical test A shows that before the use of clomiphene citrate there is no development of follicles and also the levels of FSH and LH are very low and these hormones are responsible for development of follicles into mature follicles containing the oocytes II blocked in metaphase II and which is ready for fertilization, which explains the infertility of this woman but the use of this medicine shows normal levels of these hormones and also a development of follicle accompanied by an almost normal estrogen level between days 12 and 13 of the cycle. This amount is responsible for a positive feedback on the pituitary and causes a peak of LH which triggers ovulation. So, this drug binds to estrogen receptors in the hypothalamus and promotes positive feedback to trigger ovulation in this woman.

Exercise 3 A contraceptive: the morning pill

1. Between t₀ and t₁ the level of LH fluctuates around 5 ng / ml but following the close injections of high doses of estrogen after t₁ the LH level shows a peak of 45 ng / ml (9 times greater). On the other hand, between t₂ and 30 days after the impregnation of progesterone and the injections of high doses of estrogen the level also fluctuates around 4 ng / ml like that in the absence of injection. No peak of LH is observed, but a fluctuation which is always around 5 ng / ml. This result is obtained because of the positive feedback of the high-dose estrogen on the hypothalamic pituitary axis which causes the increase of GnRH and LH but in the presence of progesterone the feedback is always negative while the LH level is always low.
2. Hypothesis: the levonogestrel by its configuration close to that of progesterone is fixed on its receptors in order to make a negative feedback control similar to that of progesterone.
3. After the injection of radioactive progesterone, black spots appear on the hypothalamus as well as with the injection of radioactive levonogestrel, but by injecting radioactive progesterone with non-radioactive levonogestrel the black spots become weak; therefore, levonogestrel binds to the same receptors of progesterone at the level of the hypothalamus and prevents the attachment of progesterone to these receptors.
4. The levonogestrel is fixed on the same progesterone receptors at the level of hypothalamus to make a negative feedback on it what will prevent the peak of LH responsible to trigger the ovulation and consequently it prevents the ovulation which is essential condition for pregnancy.
5. No, because the levonogestrel prevents the progesterone action to make the negative feedback and acts as a contraceptive whereas RU prevents the progesterone action and acts as contragestive.

Exercise 4 Human Reproduction and Health

1. According to the morphological and histological data of the ovaries, the two women A and C have a normal size of the ovaries with absence of the oocyte II in the upper third of the fallopian tubes, however the photograph of the ovaries at various times during 2 months shows that at woman A there are ovarian structures of the primordial follicles up to the mature follicle with absence of the corpus luteum, whereas in woman C there is not the presence of all the structures characterizing the phases of the cycle ovarian. woman A's ovary does not contain a corpus luteum, so ovulation did not occur to ensure corpus luteum formation from the wall follicle. Madam C presents a problem in the development of the follicles to become mature.
2. Hypothesis: Mrs B suffers from infertility of mechanical origin (bilateral obstruction of the fallopian tubes which prevents fertilization).
3. According to the hormonal assays for 28 days in a normal lady and ladies A and B, we note: A normal evolution of the pituitary hormones (FSH and LH) and ovarian (estradiol) at Mrs. B. A low and constant level of LH during the cycle (9 mIU / ml), a very low concentration of estrogen (0.5 µg / ml) compared to a high level in a normal cycle woman (5 to 210 µg / ml) during the luteal phase (normal level during the follicular phase and ovulation) and normal FSH levels in Mrs. A.
 - 4.1.HCG prevents the degeneration of the corpus luteum.
 - 4.2. The appearance of the HCG hormone in woman A's blood shows that she is pregnant, since this hormone prevents the degeneration of the corpus luteum that will persist and transform into pregnancy yellow body.
5. Exam 1 shows that Ms. B has no problem with either follicle development or ovulation. In addition to the hormonal dosages in this woman shows that there is no pituitary or ovarian hormone problem. Their values are identical to those of a woman with a normal sexual cycle. This means that the ovulation in woman B is normal and her problem is of mechanical origin that can be solved by IVF practiced in a woman in the case of bilateral obstruction of the fallopian tubes.

Exercise 5 The mode of action a male contraceptive

- Photograph B of a testicle after using contraceptive UT shows seminiferous tubules smaller than in a normal testis (A). The first have a diameter of 2 times smaller, in addition the lumen in tubules B is reduced lumen and much smaller.
- Table showing the action of UT on the production of FSH

Time (weeks)	Beginning of the injection of UT ↓						End of the injection of UT ↓				
	-6	-4	0	4	8	12	16	20	24	32	36
Plasmatic concentration of FSH in the individual injected by UT (UI-L)	4.8	4.8	4.8	4	5	6	5.8	5	5	4.5	4.5
Plasmatic concentration of FSH in the individual not injected by UT (UI-L)	5	4.9	4.9	2.5	1	1.5	0.9	4.8	5	7	4.5

- Since 6 weeks before till zero before the treatment the concentration of FSH is around 5 UI/L, but from the beginning of the treatment the concentration of FSH decreases from 5 to 1 during the 16 weeks to increase again following the arrest of the treatment to become 7 at week 32, then it return to its initial value at week 36, on the other hand the concentration of FSH, in the individual that is not subjected to TU treatment, fluctuates around 5 UI/L. Since the value of FSH decreases following the treatment, therefore, UT inhibits the secretion of FSH.
- Continuous production of spermatozoa is observed in the control lot, but no production of spermatozoa following the injection of testosterone. This shows that testosterone inhibits this production. In addition, no production of spermatozoa after the ablation of the pituitary gland indicating that the pituitary gland is responsible for the production of spermatozoa. But a continuous production of spermatozoa is reestablished when the ablation of the pituitary is followed by injection of pituitary extract FSH showing that the pituitary controls the production of spermatozoa by secretion of FSH in blood.
- UT is a molecule similar to testosterone, according to (doc.2) and following the treatment, FSH level decreased, moreover (doc.3) shows that the injection of testosterone and the ablation of the pituitary too provoke the arrest of spermatozoa production, and then testosterone exerts a negative feedback on the pituitary gland. The excess of testosterone should provoke the decrease of FSH by a negative retro control.
- UT induces the reduction of the lumen if the seminiferous tubules (doc.1) and the decrease of the FSH (doc.2) consequently the excess of testosterone or the deficiency in FSH block spermatogenesis (doc.3), then UT seems to affect using the negative feedback of testosterone on the pituitary. This later secret less of FSH that is responsible for stimulation of spermatogenesis that took place in the seminiferous tubules. The decrease of FSH explains the reduced aspect of the seminiferous tubules in photograph B of doc.1. The stop of spermatogenesis produces the contraception.

Exercise 6 Female Reproductive Function

- 1.1. A normal sexual cycle is characterized by two estrogen peaks, which corresponds to the H1 hormone, but a peak of LH in the middle of the cycle, which corresponds to the hormone H2. A post ovulatory peak of progesterone which corresponds to the H3
- 1.2. G2 is the ovariectomized monkey because it has the level of ovarian hormones H1 and H3 always zero which indicates the absence of the ovaries.
- 5.3. H1 exerts a negative feedback on H2 since a low dose of H1 inhibits the secretion of H2 but a high dose leads to a sudden secretion of H2. Then secretion of H2 depends on the amount of H1 in the blood and varies in the opposite direction.
- 5.4. H1 stimulates the proliferation of the endometrium by promoting the development of tube glands and increasing the number of blood vessels, in addition it prepares the progesterone receptors throughout the cycle.
H3 stimulates the development of spiral blood vessels and the formation of the lace aspect of the endometrium during the post ovulatory phase.
6. Since the post ovulatory phase is approximately 14 ± 1 days, then day 18 is the day of ovulation.
7. This woman has ovulation only during the month of January without pills, where the follicular cells stimulated by FSH develop and secrete estrogen in moderate amount, this exerts a negative feedback, but one day before ovulation the mature follicle secretes a large amount of estrogens that exert a positive feedback on the hypothalamo- pituitary complex causing the secretion of a large amount of LH triggering ovulation. But this ovulation is not observed in February and March because this woman takes every day a moderate amount of estrogen that exerts negative feedback on the hypothalamic-pituitary complex throughout the month, so no LH secretion and no ovulation.
8. The daily intake of a moderate amount of estrogen and progesterone causes limited development of the endometrium and as, at the end of each normal cycle, the fall in levels of these two hormones causes sloughing off of the upper layer of the endometrium and the appearance of the menses even stop taking pills causes the same effect hence the onset of menstruation during the months of February and March

Exercise 7 Male fertility

- 1.1.** The testicular microscopic observation shows in the normal boy many spermatozoa with a wall of the seminiferous tube developed where the Sertoli cells are well developed (1, a) but not in (doc 1-b) in the boys X and Y the Spermatozoa are absent, the seminiferous tubule wall is much weaker, and the Sertoli cell is much smaller, Leydig cells are smaller in boys X and Y than in normal boys, where they are more numerous and more developed.
- 1.2.** The testosterone level oscillates between 300 and 650ng / dl in the normal boy whereas this rate is very low \pm 50ng / dl and constant in the boys X and Y
- 1.3.** The origin of this testosterone is the cells of the testes and that this hormone is essential for the formation of spermatozoa.
- 2.** Hypothesis 1: The disorders seen in boys X and Y may be due to the absence of the pituitary hormones FSH and / LH.
 Hypothesis 2: The disorders seen in boys X and Y may be due to a lack of pituitary stimulation by the hypothalamus.
 Hypothesis 3: The disorders seen in boys X and Y may be due to an absence of GnRH or GnRH receptors in the pituitary gland
 Hypothesis 4: The disorders seen in boys X and Y may be due to absence of pituitary hormone or pituitary hormone receptors in the target organ (testis).
- 3.** The results of the LH and FSH test show that the concentration of LH is much lower 0.5 to 0.9 IU. L-1 in X and Y than in the normal boy of 2 to 10 IU.L-1 similarly the concentration of FSH is much lower 0.1 to 0.4 IU.L-1 in X and Y than in the normal boy from 1 to 2 IU.L-. This indicates a lack of stimulation of the pituitary gland that secretes an insufficient amount of FSH and LH. While a correction of disorders of testosterone secretion is observed, but without modifications of the aspect of the wall of the seminiferous tube following the injection of HCG to the boys X and Y. This shows that HCG which has an action similar to that of LH is responsible for the secretion of testosterone, while a correction of testosterone secretion disorders, and the testicular aspect of both boys which becomes similar to that of normal boy are observed following the injection of a mixture HCG and FSH in boys X and Y. This shows that FSH is responsible for the development of testicular structures (Sertoli cells, germ cells, spermatozoa formation) while LH stimulates the secretion of testosterone.
- 3.1.** GnRH level, in normal boy, shows a peak of 25pg / ml every an hour, whereas GnRH level in boy X shows a much higher rate 2 peaks each is 45pg / ml per hour, then hypothalamus secretes GnRH to stimulate the pituitary gland in a pulsatile way but, in greater frequency and in greater quantity, the pituitary that does not secrete a sufficient amount of LH and FSH responsible for development seminiferous tubules and Leydig cells and testosterone secretion by the testes which is confirmed by the results in document 2b showing a very low testosterone level in the boy X.
- 3.2.** Boy Y shows a very low and constant rate of GnRH about 2pg / ml, but once injected by GnRH a correction of FSH and LH secretion disorders is observed. Then the cause of disorders observed in boy Y is the absence (or the small amount) of GnRH. So, the lack of stimulation of the pituitary gland by the hypothalamus.

Exercise 8 HCG & pregnancy

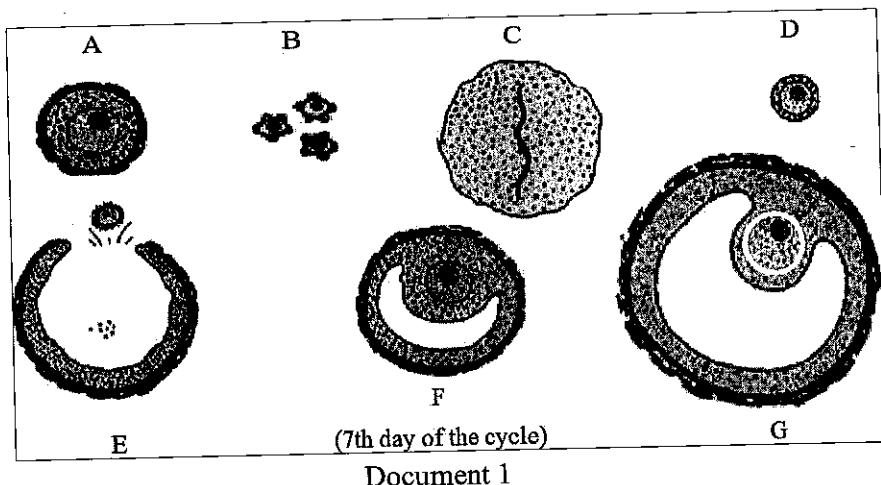
1. HCG has two interests: HCG detects a pregnancy and is a risk indicator for trisomy 21 between the 15th and 17th week.
2. Before ovulation the progesterone concentration is almost zero in both cycles without and with the beginning of pregnancy. On the other hand, after ovulation, this concentration peaks at 14 ng / ml before it returns to its initial value during a cycle without pregnancy which is identical to the value obtained during the cycle with early pregnancy but it continues to increase to 18ng / ml which is larger than that observed in the first cycle. For HCG, the concentration is zero during the cycle without pregnancy and up to a few days after ovulation during the cycle with early pregnancy where it becomes 100UI / 114days later.
3. HCG secreted by the embryo serves to maintain the corpus luteum which produces progesterone in large quantities which explains its increase (> 16 ng / ml) more than the peak observed during a cycle without pregnancy (14 ng / ml), this progesterone plays a role in the development and the maintain of the uterine mucosa which favors the implantation of the embryo and the normal progress of the pregnancy.
4. HCG has a function similar to LH which is responsible for the transformation of ruptured follicle into a corpus luteum, which secretes progesterone and estrogen together which triggers negative feedback on the hypothalamic-pituitary axis which in turn inhibits ovarian activity; since the ovaries control the development of the uterine mucosa then no variation in this mucosa. This confirms that HCG is responsible for the absence of uterine and ovarian cycles in a pregnant woman.
5. RU 468 has a structure similar to progesterone; it binds to progesterone receptors at the level of the endometrium and inhibits its action in the development of the uterine mucosa, causing desquamation and interruption of pregnancy. So, RU 486 has a contraceptive role.

Regulation of female sex hormones

Non-Solved exercises

EXERCISE 1 Ovarian cycle

Document 1 represents ovarian structures observed under a microscope during a 28-day sexual cycle.

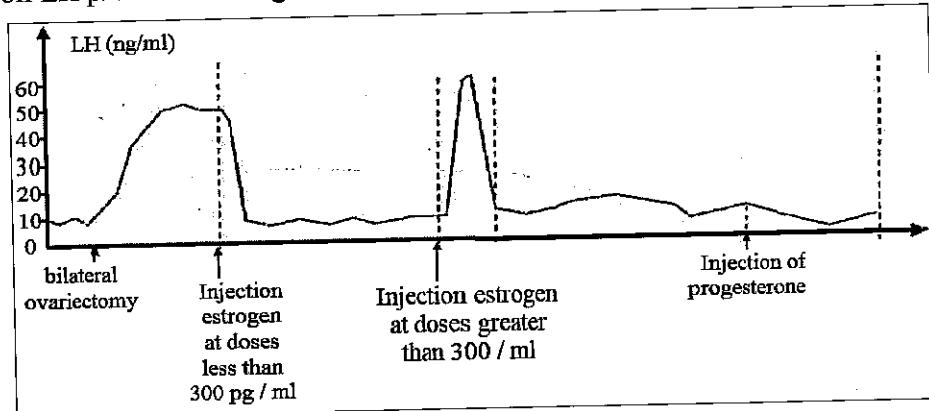


1. Identify these figures.
2. Classify these structures according to the chronological order of their appearances.

To study the action of the ovary on the pituitary gland, we carry out a series of experiments on a female macaque:

- Experiment 1: removal of the ovaries.
- Experiment 2: Increasing injections of estrogen; doses remain below 300 µg / ml. ($1\text{pg} = 10^{-9}\text{ g}$)
- Experiment 3: Estrogen injections at doses greater than 300 µg / ml.
- Experiment 4: Progesterone injections.

The effects on LH production are given in document 2.



Document 2

3. Interpret the results of each of these experiments.
4. Based on the analysis of the previous experiments and your acquired knowledge, reproduce and complete the following table:

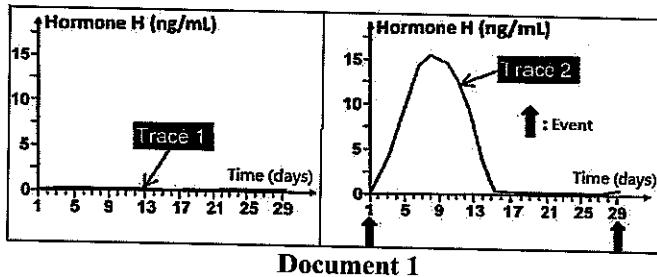
Structure	Type of feed back	Justification
Structure F		
Structure G		
Structure C		

EXERCISE 2 Female sterility

In order to determine certain causes of infertility in women, clinical tests are carried out in a 25-year-old sterile woman.

Test 1: Measurements of an ovarian hormone H are carried out in the sterile woman during a period of 29 days. The results obtained are represented by trace 1 in document 1.

Trace 2 corresponds to the evolution of hormone H in a fertile woman (control).



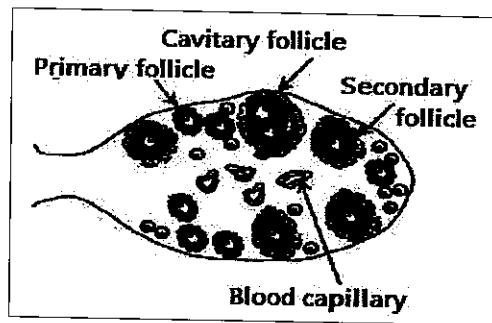
Document 1

1. Identify: -Hormone H and event X.

2. Propose 2 possible hypotheses that may be at the origin of the infertility of the woman.

Test 2: Ultrasound examination was performed in the sterile woman on the 27th day. Document 2 represents a diagram of interpretation of the ovary section observed under the microscope.

Test 3: The average level of LH is measured in the sterile woman before and after injection of a substance (S).



Document 2

	Period (days)	Control female	Sterile female before the injection of substance S	Sterile female after the injection of substance S
	Average rate of LH (UI/L)			
	From 2 to 16	10	10	10
	From 16 to 28	10	10	10
	From 28 to 30	90	10	84

Document 3

- 3.1. Justify, using the results of tests 2 and 3 and your acquired knowledge, the absence of the event X in the sterile woman.

- 3.2. Draw out the effect of substance S.

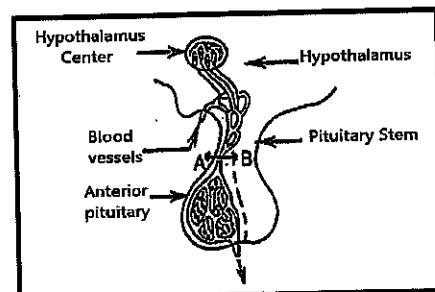
Knowing that the hypothalamo-pituitary complex of the sterile woman does not present structural and functional abnormalities.

4. Explain the cause of this infertility,

EXERCISE 3 Regulation of male hormonal secretions

In order to study the aspects of the hormonal regulation of testicular function (place of testosterone secretion and spermatogenesis) in men, the following experiments on animals were realized.

Document 1 presents the relationship between the pituitary gland and the hypothalamus.



Document 1

First series of experiments:

- In an adult rat, the pituitary gland is removed and then transplanted at any point on the same animal. The pituitary gland grafted in this way stops to secrete the gonadotrophic hormones (FSH and LH).
- In a second experiment, the pituitary gland is kept in place but the pituitary gland is cut at level AB, in document 1, and separated from the hypothalamus by impermeable membrane. It is found in this case that the pituitary gland does not produce gonadotrophic hormones.

1.1. Analyze the results of these two experiments.

1.2. Deduce the nature of the relationship between the pituitary gland and the hypothalamus.

Second series of experiments:

- The pituitary gland is removed from a mature rat and cultured on an appropriate nutritive medium. It is noted that it does not release gonadotrophic hormones.
 - The experiment is repeated by adding to the culture medium hypothalamic extracts from a normal rat. Secretion of gonadotrophic hormones occurs.
 - The previous experiment is repeated by adding to the culture medium this time hypothalamic extracts from a castrated rat. There is an abundant secretion of gonadotrophic hormones.
 - The experiment carried out in (d) is repeated, but hypothalamic extracts from a rat injected with a high dose of testosterone is added to the pituitary culture medium. The secretion of gonadotrophic hormones by the pituitary becomes very weak.
- 2.** Specify starting from these 4 experiments:
- If the activity of the pituitary gland is autonomous.
 - The mode of action of the hypothalamus on the anterior pituitary gland.
 - If testosterone exerts a negative feedback on the hypothalamus-pituitary complex.

Third series of experiments

To understand certain aspects of reproductive function in men, observations of testicular structures (seminiferous tubules) shown in experiments in document 2 are performed in mature mice; Testosterone secretion by Leydig cells is observed only in Experiment 4.

Experiment	Mature mouse (Control)	Experiment 1 Ablation of pituitary at puberty	Experiment 2 Repeared injections of FSH to the mouse with ablated pituitary gland	Experiment 3 Repeared injections of LH to the mouse with ablated pituitary gland	Experiment 4 Repeared injections of FSH and LH to the mouse with ablated pituitary gland
Observation					

Document 2

- Deduce the role of pituitary hormones in testicular functions.
- Establish, using experiments (1, 2 and 3) functional diagram showing the hormonal regulation of the reproductive function in the man.

Regulation of female sex hormones

Official exercises

Exercise 1 (4.5 pts) Role of progesterone

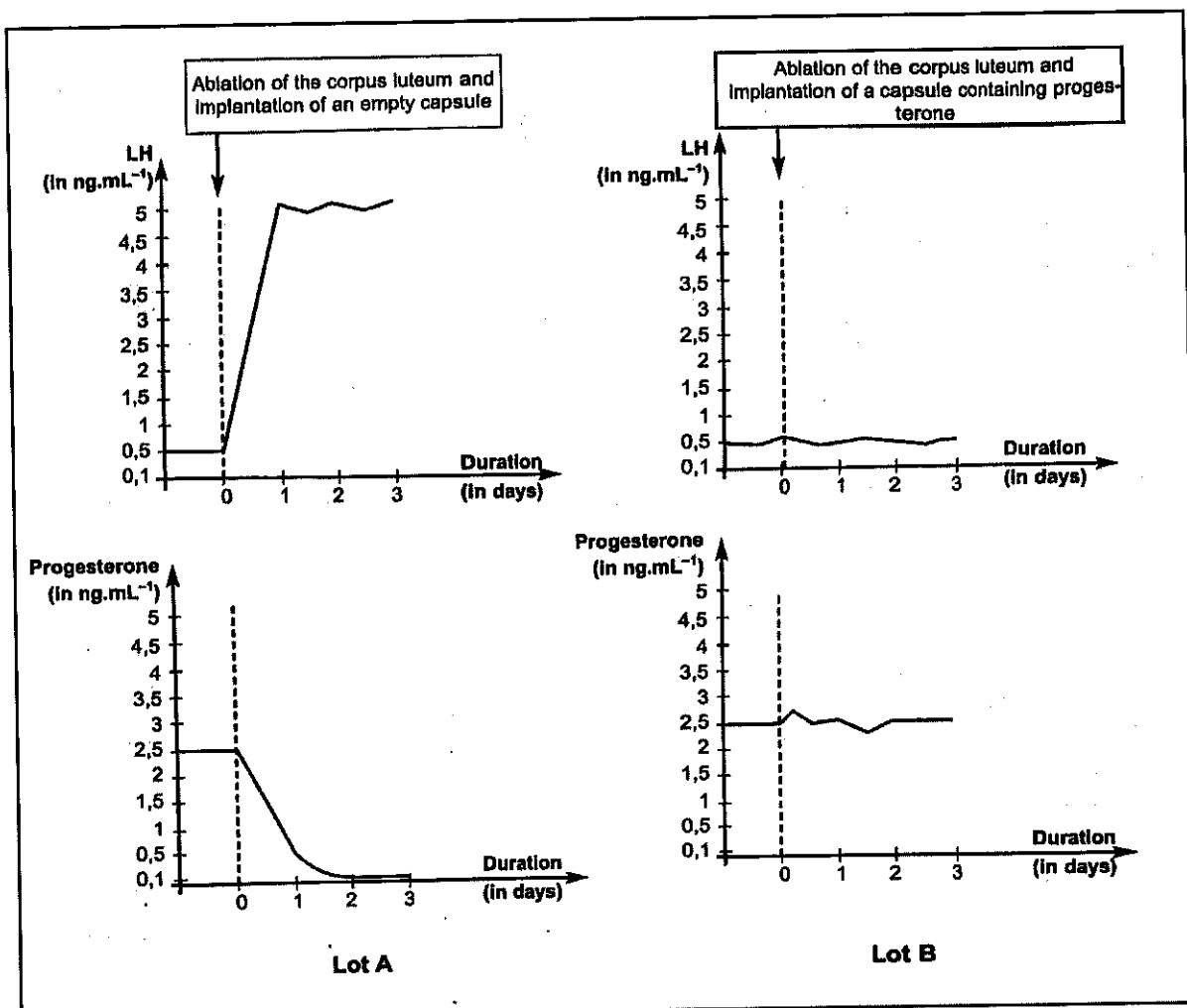
In the framework of studying the action of progesterone during the luteal phase of the estrous cycle of sheep, we perform the following experiment.

Three days before the end of the cycle (day 0), we ablate the corpus luteum of two lots of sheep, A and B. Immediately after the ablation, we implant under the skin of the animals of:

- lot A, an empty capsule;
- lot B, a capsule containing progesterone.

We measure the variation of the plasma concentration of two hormones, one is a pituitary hormone (LH), and the other is an ovarian hormone (progesterone), in the days that follow the implant.

The results are shown in the graphs of the following document.

**Document 1**

1. Construct one table that includes the variations of the concentration of the two hormones as a function of time, which are revealed by the graphs of the two lots A and B.
2. Interpret the curves of lot A and lot B.

Exercise 2 (4 pts) Organs of the sexual cycle and ovulation

Session 2002-2

A. We perform the following experiments on an adult female monkey.

Experiment 1:

The ablation of the hypophysis (pituitary) provokes multiple troubles, especially the disappearance of the ovarian cycle and the uterine cycle.

Experiment 2:

The repeated injections of the extract of the anterior lobe of the pituitary into this female restore the activity of the ovaries and the uterus.

Experiment 3:

The repeated injections of the extract of the anterior lobe of the pituitary into the same female after the ablation of the ovaries do not restore the uterine cycle.

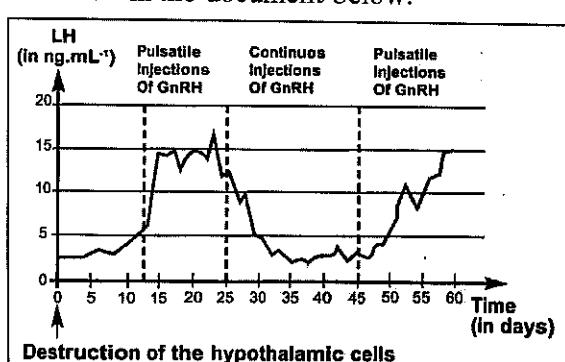
Experiment 4:

When the anterior lobe of the pituitary is isolated and placed in an appropriate nutritive medium, the medium becomes enriched with FSH and LH.

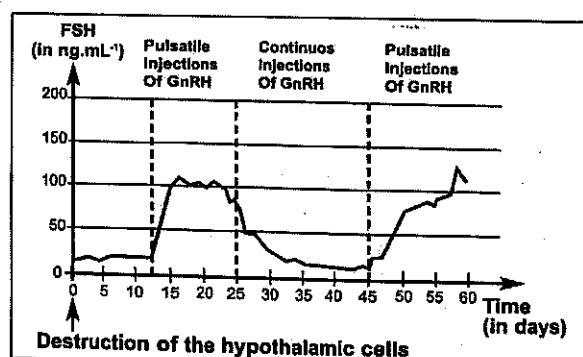
- Interpret the results of these experiments.

B. Experiment 5: In another female monkey, we destroy certain specific hypothalamic cells. This female does not present any ovarian cycle or ovulation.

Experiment 6: We inject this female monkey with a substance (GnRH) extracted from the specific hypothalamic cells of another female monkey. The results, concerning the secretion of the pituitary, are shown in the document below.



Document 1



Document 2

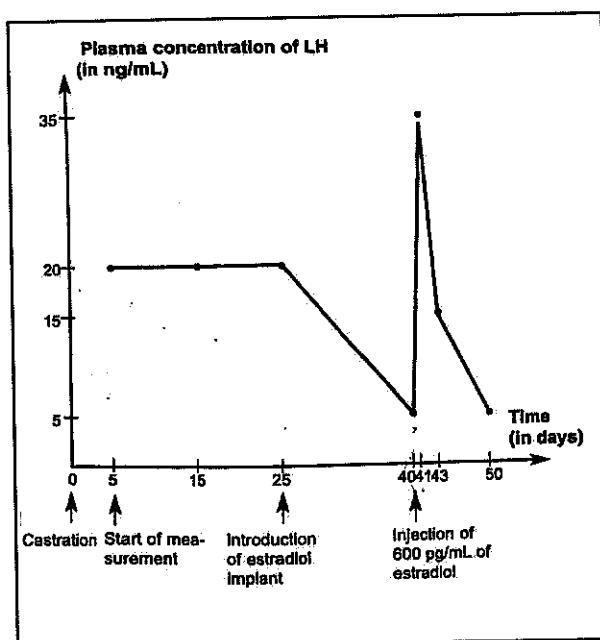
- Interpret the results of the experiments 5 and 6.
- Show, by referring to the information provided by the experiments of parts A and B, how the ovary is under the control of the hypothalamus.
- Explain how ovulation in women requires the intervention of ovarian hormones.
- Make a functional diagram to illustrate the hormonal mechanism that permits ovulation.

Exercise 3 (4 pts) Regulation of LH secretion

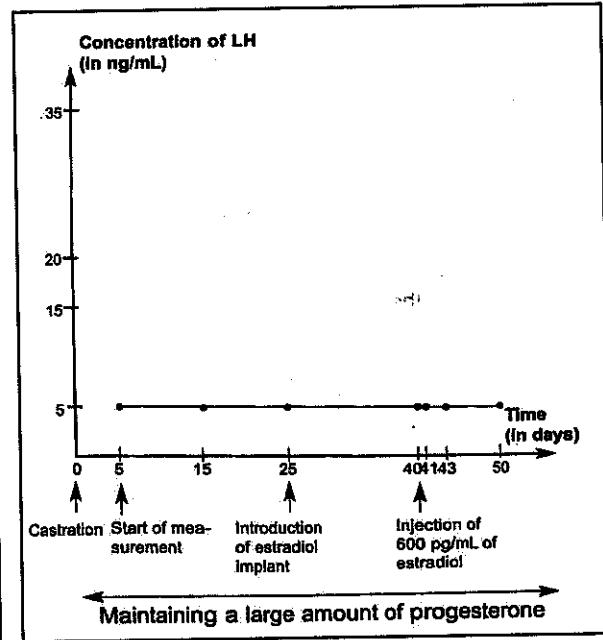
Session 2004-2

Experiment 1: After 25 days from the ovarioectomy (castration) of a female monkey, we introduce under the skin an implant of estradiol, which liberates continuously and in a small amount this hormone in the blood. In this way, the plasma concentration of estradiol is maintained during several days, at an amount near to that which normally exists at the start of the follicular phase of the menstrual cycle. We inject, 15 days after placing the implant, a large amount of estradiol (600 pg/mL). We measure the plasma concentration of LH in this female during the experiment; the results are shown in document 1.

Experiment 2: Another female monkey is subjected to the same treatment: castration, introduction of estradiol implant, and the injection of a large amount of estradiol. In addition, we maintain a large amount of progesterone in the blood starting from the beginning of castration. We measure the plasma concentration of LH during this second experiment; the results are shown in document 2.



Document 1

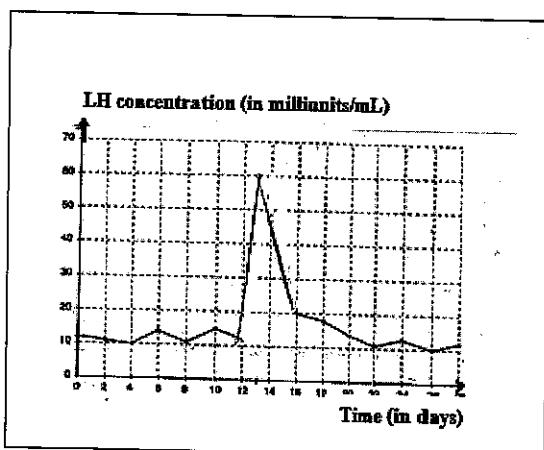


Document 2

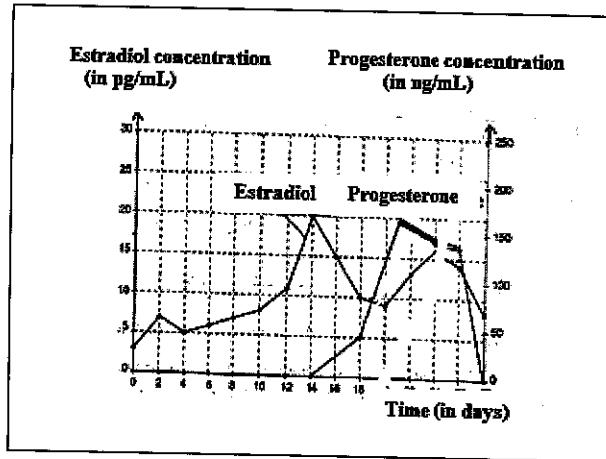
1. In the same table, represent the variation of the concentration of LH as a function of time, in these two females.
2. Interpret the results shown by each of these two documents.

Exercise 4 (4 pt) Action of contraceptive pill**Session 2005-1**

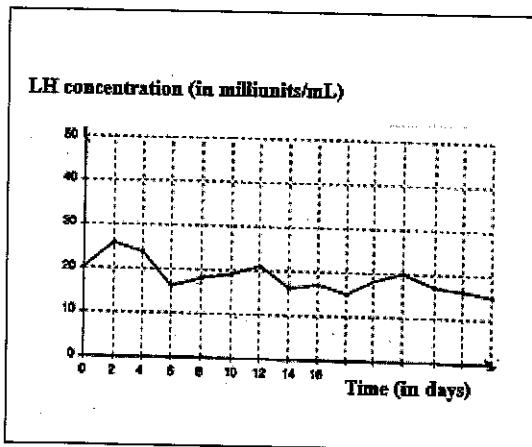
In the framework of studying the mode of action of a chemical contraceptive, we follow up the variation of the ovarian and pituitary hormonal secretion over time in two women having normal cycles, in two different situations: woman A, who does not take a contraceptive pill, and woman B, who takes an estro-progesterone contraceptive pill. The results are presented in documents 1 and 2 for woman A, and 3 and 4 for woman B.



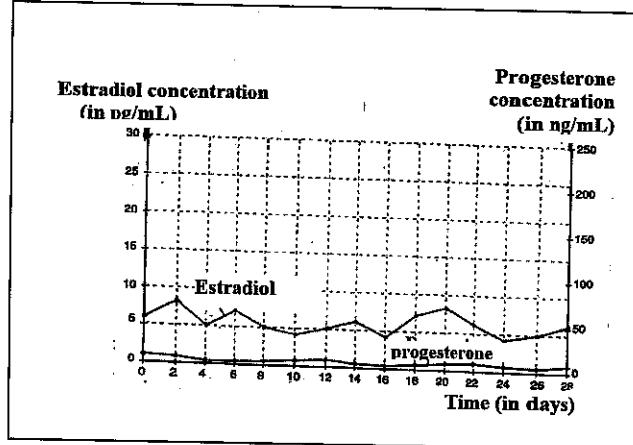
Document 1



Document 2



Document 3



Document 4

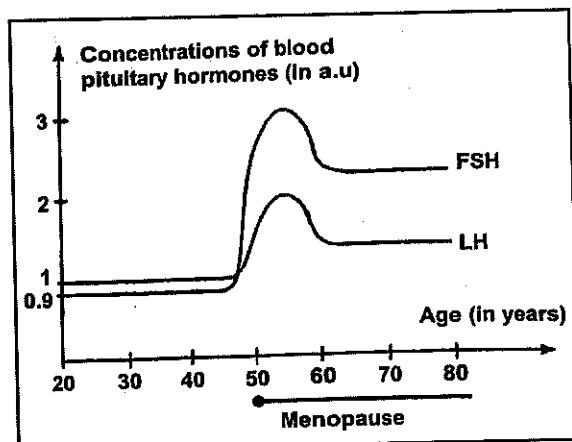
1. Compare the variations of the concentration of estradiol on one hand, and the variation of the concentration of progesterone on the other hand, in the two women.
2. Draw out the effect of the pill on the ovaries.
3. Explain, by referring to the acquired knowledge, the observed differences between the given two situations.

Exercise 5 (3 pts) Cause of the disappearance of follicles

The observation of the sections of a woman's ovaries at menopause reveals the depletion of the available follicles, structures into which we find the oocytes.

We search to know if the disappearance of the follicles is due to the aging of the ovary or to the stoppage of stimulation of the ovary by the pituitary.

Document 1 reveals the variation of the concentration of the pituitary hormones in the blood, during the life of a woman, and document 2 indicates the negative effects of menopause and how they are remedied.

**Document 1**

"The absence of estrogen, which is characteristic of menopause, has many effects because this hormone acts on a number of physiological systems. It causes a rapid decrease in the thickness of the skin which shrivels, and the dryness of the genital mucosa. It mainly provokes osteoporosis and arteriosclerosis which leads to cardiovascular troubles. The principle of treatment is relatively simple: restoring the previous hormonal levels by the administration of estrogen in different forms, most of the time in the form of pills. This permits a great improvement of the state of women patients. Moreover, the administration of estrogen leads to a decrease or a delay in the occurrence of neurodegenerative diseases such as Alzheimer."

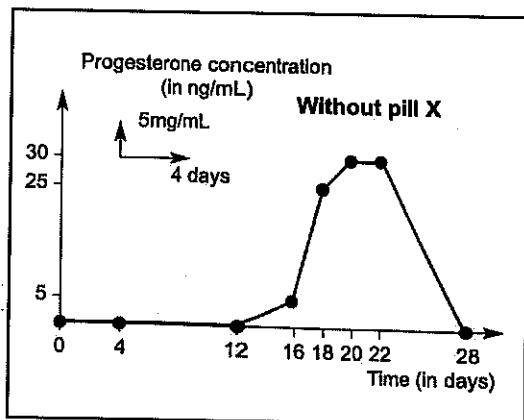
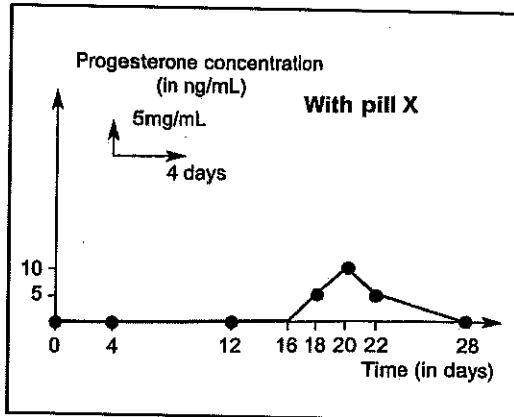
Document 2

1. Pick out, from the above text, the two hypotheses that are at the origin of the disappearance of the follicles.
2. Which of the two hypotheses is more probable? Justify the answer.
3. How can you explain the variation of the concentrations of pituitary hormones that appear at the beginning of menopause?
4. Pick out, from document 2:
 - 4.1.the consequences of estrogen deficiency at menopause.
 - 4.2.How this deficiency is remedied.

Exercise 6 (5 pts) Birth control methods

Session 2007-1

In the framework of studying birth control, two women A and B use two different types of pills. Mrs. A uses pill of type X. We measure the concentration of progesterone in this woman before and after taking pill X. The results are presented in documents 1 and 2.

**Document 1****Document 2**

N.B. Progesterone concentration is more than 20 ng/mL during the second half of the cycle, which indicates that ovulation took place

1. Construct a table that includes the variations of the concentration of progesterone of Mrs. A, before and after taking pill X.
2. Compare the variations of the concentration of progesterone, before and after this woman takes pill X.
3. What can you conclude concerning the effect of pill X?

Mrs. B uses another pill Y to interrupt her early pregnancy.

To understand the effect of pill Y, we perform experiments on three lots of rabbits that did not reach puberty. Document 3 shows the experimental conditions and the obtained results.

	Lot 1	Lot 2	Lot 3
Injection of estradiol	+	+	+
Injection of progesterone	-	+	+
Intake of an appropriate dose of pill Y	-	-	+
Results	Thickening of the endometrium, no formation of uterine lace	Thickening of the endometrium, formation of uterine lace	Thickening of the endometrium, no formation of uterine lace

(+/-) presence

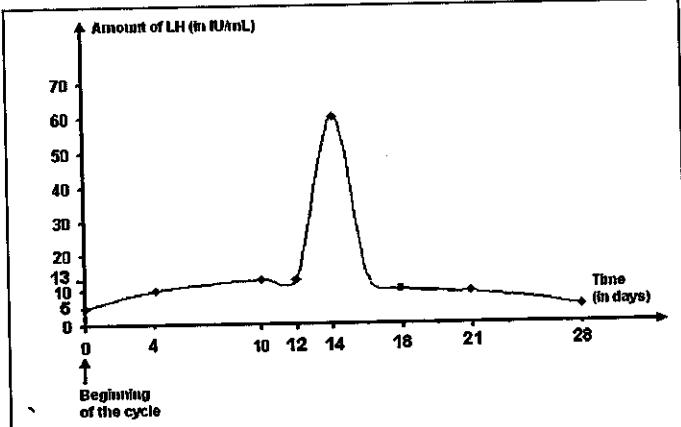
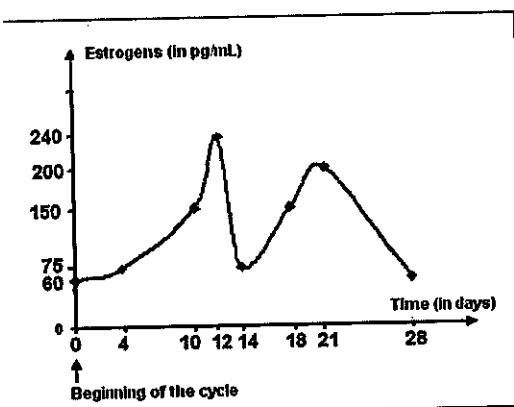
(-) absence

Document 3

4. Determine, from document 3 the target organ and the effect of pill Y.
5. Name the birth control method that corresponds to each of the two pills used.

Exercise 7 (7 pts) Problems of sterility

In order to determine the reason of sterility in a 30 years old woman, a gynecologist prescribed the determination of estrogens and LH hormones levels in the course of a sexual cycle. The obtained results are shown in documents 1 and 2.

**Document 1****Document 2**

1. Draw up in the same table, the variations of the plasma amount of estrogens and LH in this woman.

Advanced analysis showed a production of normal gametes. The doctor affirms that the results show an absence of disturbances in the functioning of the hypothalamus, pituitary, and ovaries and that the anomaly is mainly in the genital ducts.

2. By referring to documents 1 and 2 and to acquired knowledge, justify the doctor's affirmation.
3. Name the technique that allows treating this woman's sterility.

Document 3 reveals the amount of estrogens and LH in another woman of the same age, who also suffers from sterility.

Days after the beginning of menstruation	0	4	10	12	14	18	24	28
Amount of estrogens (pg/mL)	29.1	0	30.4	29.9	29.3	30.3	30	30.3
Amount of LH (mIU/mL)	5.3	6.8	6.3	7	6.2	6	7.3	6.5

Document 3

4. Interpret the obtained results.
5. Explain the probable origin of this woman's sterility.
6. Propose a treatment that may solve the problem of sterility in this woman.

Exercise 8 (5 pts) Mode of action of RU486

Session 2009-2

RU 486 (mifepristone) is a molecule that has a contraceptive action. It prevents the implantation of the embryo, and terminates early gestation.

Document 1 presents the time of the appearance of menses and the variation of the amount of progesterone in a control group of women and in women having absorbed RU 486.

- Determine, from document 1, the effect of RU 486 on the secretion of progesterone and on the appearance of menses.

We inject three lots of female rats with the same quantity of different molecules labeled with a radioactive element called tritium (^3H). Fifteen minutes following the injection, we remove the uterus of these female rats. Autoradiography was done on thin sections of the uterine mucosa. We count the silver grains that became black by radioactive emission and which reveal the concentration of radioactive molecules present in the nuclei of the uterine mucosa cells.

Document 2 shows the results obtained on 300 uterine mucosa cells.

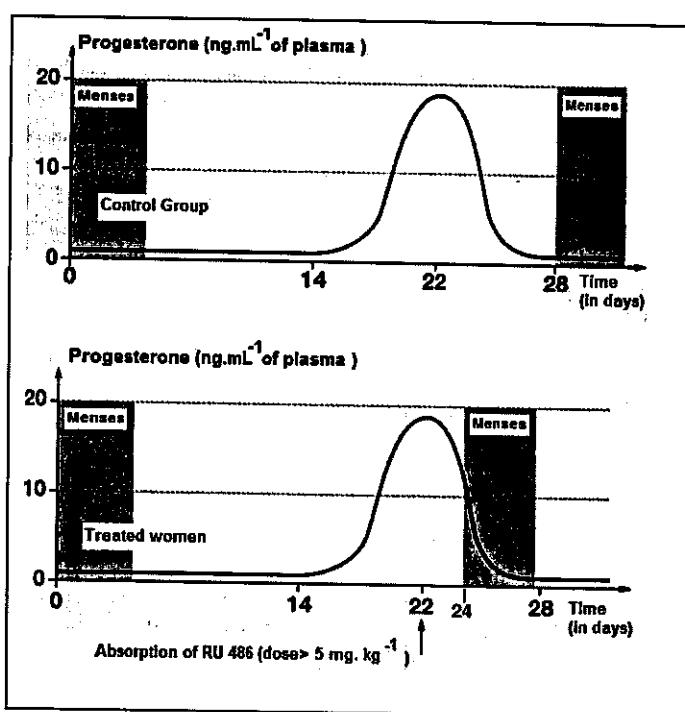
Lots	1	2	3
Injections done	RU486 labeled with tritium	Progesterone labeled with tritium	Equal quantities of non-labeled RU486 and progesterone labeled with tritium
Average number of silver grains (grains/per cell)	8	8	2

Document 2

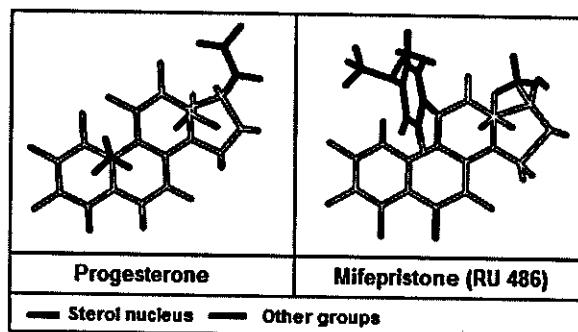
- Construct a histogram that represents the data of document 2.
- Interpret the results of document 2.

Document 3 reveals the structure of RU 486 and that of progesterone molecules.

- Explain, by referring to the information derived from documents 2 and 3 and to the acquired knowledge, the results obtained in the treated women (document 1).



Document 1



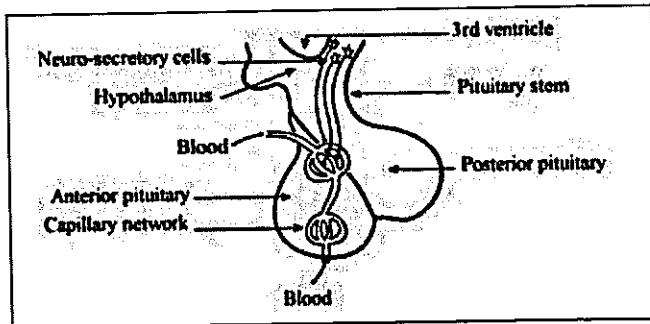
Document 3

Exercise 9 (6½ pts) Regulation of the sexual cycle

We aim to study the ovarian and uterine cycles by performing experiments on adult mammals.

Document 1 illustrates the hypothalamo-pituitary complex implicated in the regulation of these cycles.

Experiment 1: The ablation of the anterior pituitary is followed by the atrophy of both the ovaries and the uterus along with the disappearance of the cycles.

**Document 1**

Experiment 2: In animals submitted to the ablation of the pituitary gland and receiving regular injections of anterior pituitary extracts, we can observe a redevelopment of the ovaries and sometimes a reestablishment of the ovarian and uterine cycles. However in an ovariectomized animal, injected by anterior pituitary extracts, we never observe a reestablishment of the uterine cycle.

Experiment 3: Lesions of the posterior hypothalamus have the same effect as the ablation of the anterior pituitary.

- Interpret the results of each of the three experiments.

Experiment 4: Bilateral ovariectomy provokes a hypertrophy of the pituitary gland followed by an abnormal high production of gonadotropic hormones. This experiment allows us to admit the existence of a feedback mechanism exerted by the ovaries on the production of FSH and LH.

In order to determine the types of this feedback, an ovariectomized female monkey receives, for four periods of 15 days each, injections of ovarian hormones with different doses and composition. For each period the average level of FSH and LH production is measured (document 2).

Periods of 15 days	Characteristics of the injections		Plasmatic levels	
	Composition	Plasmatic levels	of FSH in ng/ml	of LH in ng/ml
1	Estrogen	0	> 15	> 50
	Progesterone	0		
2	Estrogen	70 pg/ml	Around 6	Around 4
	Progesterone	0		
3	Estrogen	300 pg/ml	Around 12	Around 40
	Progesterone	0		
4	Estrogen	300 pg/ml	< 4	< 3
	Progesterone	4 pg/ml		

Document 2

- State the types of the feedback revealed in document 2. Justify the answer.
- Establish, by referring to the four experiments, a functional diagram showing the relations between the different organs involved in the regulation of the sexual cycles.

Exercise 10 (5 pts) Relations between the pituitary gland and the testis

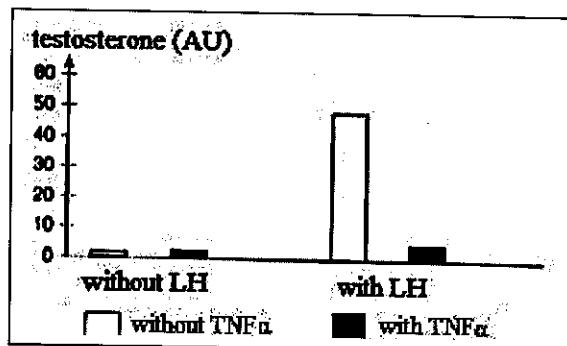
Session 2011-2

The testis produces testosterone in a constant manner due to a regulatory system that we aim to discover by performing the following experiments.

Experiment 1

We inject gonadotropins (anterior pituitary hormones) into a male animal that have not reached puberty and whose testicular cells are normally inactive. The consequences of these injections on three types of testicular cells are presented in document 1.

Testicular cells	Pituitary hormones	Injection of LH	Injection of FSH
Spermatogonia		inactive	activated
Sertoli cells		not developed	developed
Leydig cells		activated	inactive

Document 1**Document 2****Experiment 2**

Leydig cells are extracted from pig testes and cultured in vitro. We add different molecules, LH and/or TNF α , to the culture medium and we measure, at the same time, the production of testosterone. TNF α is a molecule that blocks the action of LH by binding the receptors of LH target cells. Document 2 shows the effects of LH on these cells.

- 3- Determine by referring to document 2, how are Leydig cells activated.

Experiment 3

In order to study the action of certain types of cells on the activity of pituitary cells, we prepare three appropriate culture media and we measure the level of gonadotropins released in these media after a period of incubation (document 3).

Experimental conditions	Medium 1	Medium 2	Medium 3
	Pituitary cells only	Pituitary cells + kidney cells or spleen cells	Pituitary cells + Leydig cells
Release of FSH	100%	100%	100%
Release of LH	100%	100%	60%

Document 3

- 4- Interpret the results of experiment 3.
 5- Specify the type of feedback control revealed by experiment 3.

Exercise 11 (5 pts) Functional relationships between ovaries and uterus

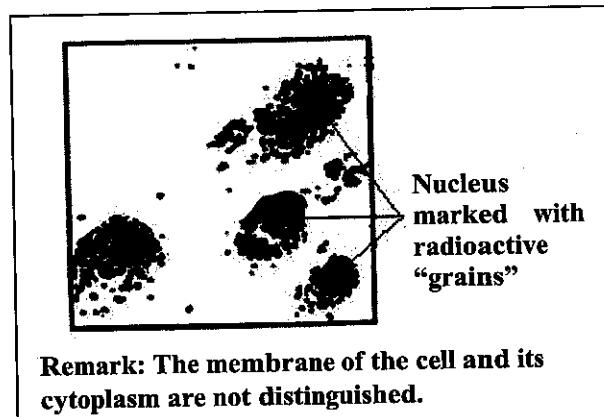
Session 2012-1

In the framework of studying the functional relationships between the ovaries and the uterus, many experiments are performed on female mammals.

Lots of mice	1 (control)	2	3	4
Amount of injected estradiol (in µg)	0	0.005	0.01	0.1
Average mass of the uterus (mg)	12	20	40	100

Experiment 1: We inject increasing amounts of estradiol, to lots of ovariectomized mice at puberty; the results are shown in document 1.

Experiment 2: We inject only physiological doses of progesterone to an ovariectomized female mouse. No significant changes were observed at the level of the uterus. In another ovariectomized female, 0.01 µg of estradiol is injected followed by an injection of the same previous doses of progesterone. We observe more amplified results than those represented in document 1.

Document 1

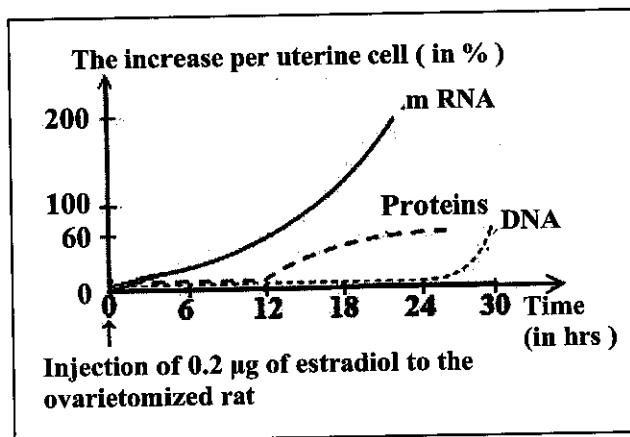
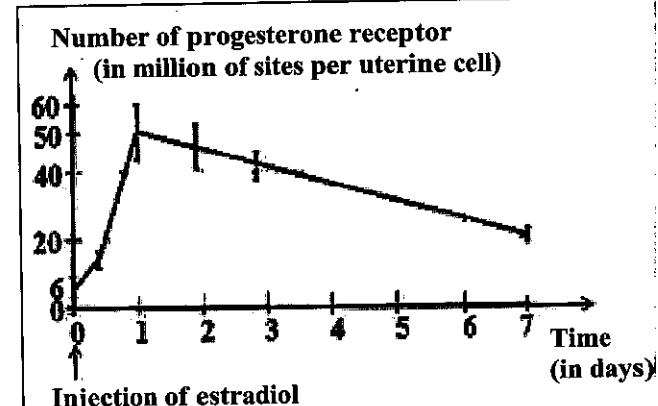
- 1- Interpret each of the above experiments.

Document 2

Experiment 3: We perform autoradiography on a cross-section of the uterine mucosa taken from an ovariectomized female after 1 to 2 hours of being injected with estradiol marked by tritium (radioactive isotope of hydrogen) as shown in document 2.

- 2- What can you draw out in document 2?

Experiment 4: We inject estradiol to an ovariectomized rat at time 0. Then we measure the rate of certain constituents of the uterine mucosa cells. The results are represented in documents 3 and 4.

**Document 3****Document 4**

- 3- Knowing that the development of the uterine mucosa is related to mitosis, determine, using document 3 and the acquired knowledge, the mode of action of estradiol on the uterus.
 4- Explain, based on document 4, the results of experiment 2.

Exercise 12 (5 pts) Ovaries and sexual cycles

Session 2013-2

Ovaries are active from puberty till menopause.

In order to understand the endocrine role of ovaries on the genital activity, the following experiments are performed.

Experiment 1:

Two lots of female rats which did not reach puberty, 2 and 3, are subjected to ovariectomy with or without injection of ovarian extracts: estradiol and progesterone. The conditions and the results of the experiment are presented in document 1.

- 1- Draw out the roles of ovaries and their mode of action as revealed in this experiment.

Experiment 2:

Four lots (A, B, C and D) of female rabbits that did not reach puberty receive daily injections of 5 µg of estradiol (E) and/or 200 µg of progesterone (P) during several days. Then, transverse sections of their uterus are prepared at the end of the experiment, at day 11. Document 2 presents the experimental conditions as well as the obtained results.

- 2- Interpret the results of document 2.

Experiment 3:

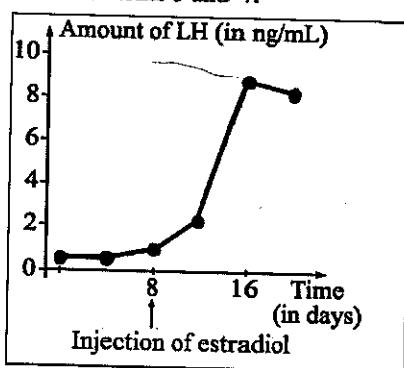
Protein receptors for progesterone were revealed at the level of endometrium cells. The injection of estradiol provokes an increase in the number of progesterone receptors in the day following the injection.

- 3- Explain the obtained results of lot A in experiment 2.

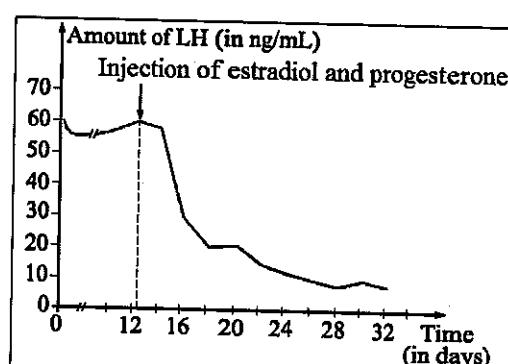
Experiment 4:

A lot of ovariectomized female mammals are subjected to injections of high amounts of estradiol with or without progesterone.

The evolution of the plasmatic concentration of the pituitary hormone LH is measured and the obtained results are shown in documents 3 and 4.



Document 3



Document 4

- 4- Show, by referring to documents 3 and 4, that "the activity of the pituitary gland is under the control of ovarian hormones".
- 5- Establish, by referring to all what precedes, a functional diagram showing the relations existing between the ovaries and the two other organs: the pituitary gland and the uterus.

Exercise 13 (5 pts) Retarded puberty

Nadia, a 16 years old girl, has a normal feminine phenotype. She shows certain signs of puberty such as the development of pubic hair but with no breast development and with absence of menses. Knowing that there are no cases of retarded puberty in her family, she consults a doctor who prescribes her many tests. The results are presented below:

- Echography reveals two ovaries of normal size.
- The biopsies performed on the ovaries of the patient, at different moments, present in addition to primordial and primary follicles, the follicles shown in the section presented in document 1.
- The karyotype shows 46 normal chromosomes distributed into 44 autosomes and 2 X gonosomes.

- 1- Determine, by referring to the results of the performed tests, the cause of Nadia's problem.

To complete his diagnosis, the doctor prescribes the following supplementary tests.

Test 1: the concentration of LH, FSH and estradiol hormones are measured in Nadia for a period of one month. These concentrations show no cyclic variation with a high level of LH and low levels of FSH and estrogens.

- 2- Name the organs that secrete each of the studied hormones.
- 3- Formulate two hypotheses explaining the probable origin of Nadia's problem.

Test 2: the patient receives an injection of 100 micrograms of GnRH, and then the levels of LH and FSH hormones are measured. The results are shown in document 2. In a second time, when the patient is injected by FSH, ovulation is induced.

Measured hormonal concentrations	Initial amount	Amount within 30 minutes	Amount within 60 minutes
LH IU/L	33	170	130
FSH IU/L	0.6	0.6	0.8

Document 2

- 4- Interpret the obtained results.
- 5- Explain, by referring to all what precedes, the clinical signs shown by Nadia.

Exercise 14 (5 pts) Female infertility

Session 2016-1

Fertilization is not an automatic phenomenon. Only 25% of the sexual intercourses occurring during the fertile period are followed by pregnancy.

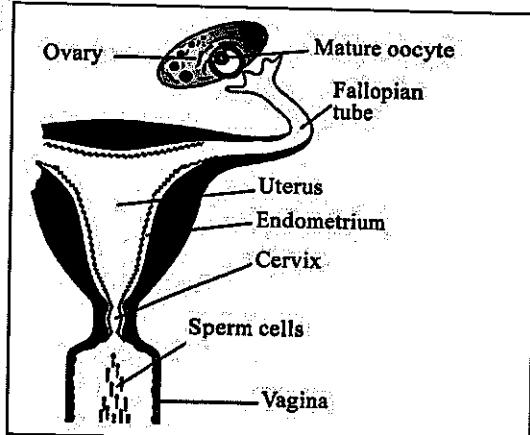
Document 1 shows a part of a female genital duct.

- 1- Indicate the site of fertilization and the role of the uterus.
- 2- Explain briefly the process of fertilization.

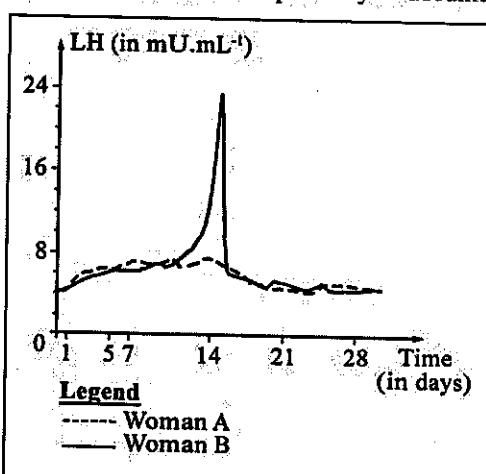
Two women, A and B, consult a gynecologist because of their infertility. In order to determine the origin of their infertility, the doctor prescribed the following tests:

- Measurement of the plasma concentration of LH hormone
- A radiologic exam of the genital duct after introducing an opaque liquid in the genital duct of each of the two women.

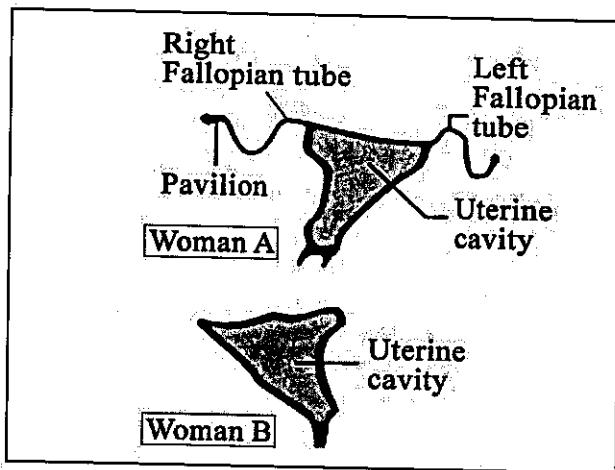
The results are shown respectively in documents 2 and 3.



Document 1



Document 2



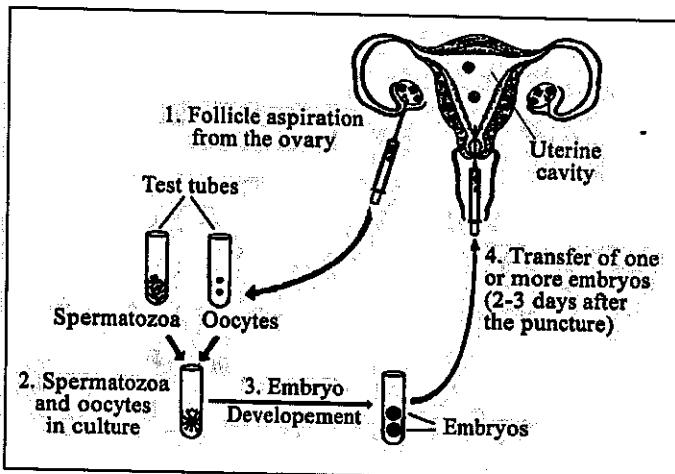
Document 3

- 3- Determine the cause of infertility of each of the women A and B.

After analyzing the results of the performed tests, the gynecologist decides to perform IVF and ET technique in order to solve the problem of one of the two infertile women.

Document 4 shows the different steps of this technique.

- 4- Describe, in a text, the IVF and ET technique.
- 5- Indicate which infertile woman A or B, the IVF and ET technique can solve her problem. Justify the answer.
- 6- Formulate a hypothesis explaining the probable origin of infertility in the second woman.



Document 4

Exercise 15 (5 pts) Infertility in a woman

Session 2016-2

Many factors lead to sterility in women. Most of them are irreversible, but some can sometimes be solved. Mrs. A consults her doctor for a sterility problem. He asks her to measure her body temperature for a certain period of time. The obtained results of Mrs. A as well as those of a normal woman are shown in document 1.

1- Determine the cause of sterility of Mrs. A.

The gynecologist supposes that the sterility of Mrs. A is due, either to a lack of stimulation of the ovaries by the pituitary gland (hypothesis 1) or to the insensitivity of the ovaries to the pituitary gland secretions (hypothesis 2).

2- Justify the two hypotheses that are formulated by the doctor.

The doctor requests Mrs. A to perform an echography accompanied by ovarian biopsies as well as hormonal measurements.

The echography reveals two ovaries of normal size while the multiple performed biopsies show only primary follicles.

The results of the hormonal measurements of Mrs. A concerning the pituitary hormones (LH and FSH) and the ovarian hormones (estradiol and progesterone), show concentrations that are obviously lower than that of a non-sterile woman during a normal cycle.

3- Show that the above obtained results are insufficient to validate hypothesis 2.

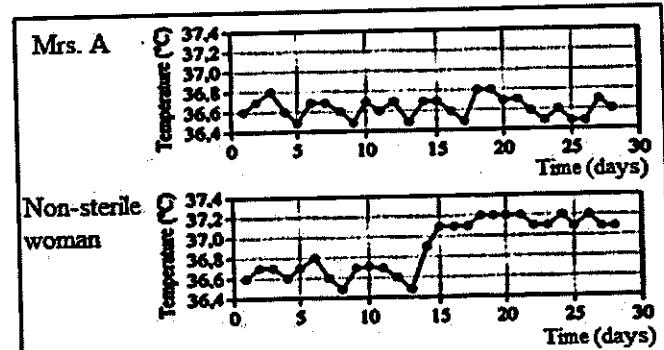
The doctor then performs a treatment that consists of injecting a mixture of LH and FSH followed by LH. Estradiol measurements are performed during cycle 1 before treatment and during cycle 2 with treatment. The obtained results are shown in document 2.

4- Specify which of the two hypotheses that are formulated by the doctor is validated by the above obtained results.

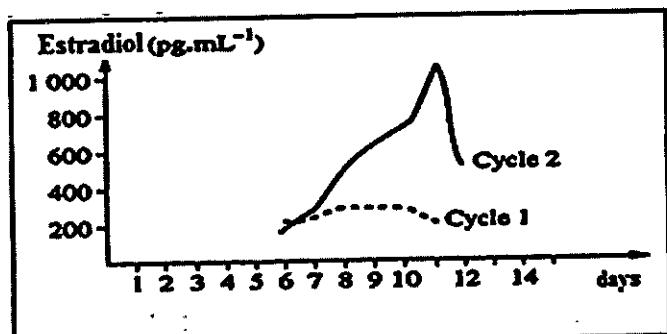
Following this treatment, the regular follow up of the development of the ovarian follicles gives the result presented in document 3.

5- Did this treatment solve the sterility problem of Mrs. A? Justify the answer.

The doctor announces to Mrs. A that she may have fraternal twins.

6- Justify this announcement concerning the possible birth of fraternal twins.

Document 1



Document 2



Document 3

Exercise 16 (5 pts) What determines the LH peak?

Session 2017-1

The secretion of the hormone LH by the pituitary gland varies in a cyclic manner. In a woman having a 28-days cycle, the LH peak on the 13th day of the cycle triggers the ovulation of the oocyte II blocked at metaphase II. Searching for the factors that determine the LH peak, different experiments are performed on female mammals.

Series of experiments 1: different treatments are performed on 4 lots of adult female rats, then the level of the secreted LH is measured.

Lot 1: the female rats are not subjected to any treatment. There is secretion of LH.

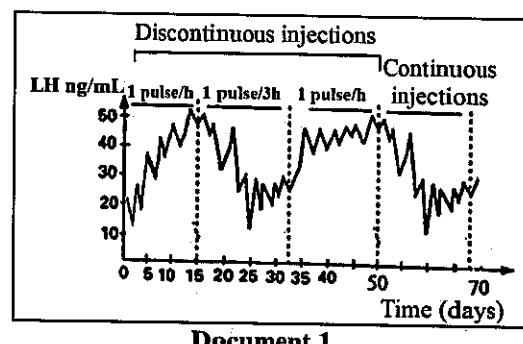
Lot 2: the female rats are subjected to the lesion of the hypothalamus. There is no secretion of LH.

Lot 3: the female rats are subjected to ablation of the pituitary gland followed by the graft of the pituitary gland in the anterior chamber of the eye. There is no secretion of LH.

Lot 4: the female rats are subjected to ablation of the pituitary gland followed by the graft of this gland in an area connected to the pituitary duct. There is secretion of LH.

- 1- Interpret the results of the series of experiments 1.

Experiment 2: In a female macaque, the arched nucleus of the hypothalamus has been destroyed and the secretions of FSH and especially of LH have dropped. This female is injected by GnRH (substance extracted from the hypothalamus) in a continuous manner and in a pulsatile manner at two different frequencies using an automatic micropump. The obtained results are represented in document 1.

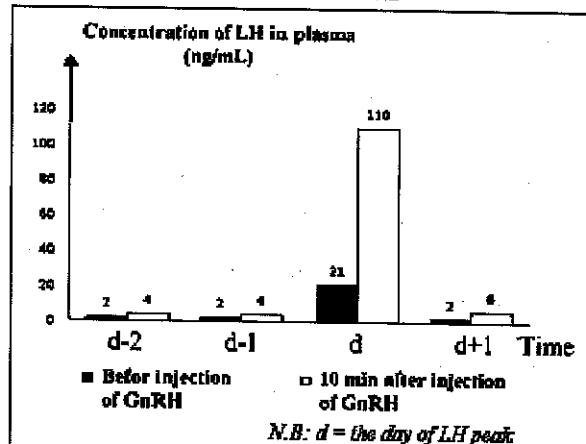


Document 1

- 2- Specify the mode of action of the hypothalamus on the pituitary gland as revealed in this experiment.

Experiment 3: female rats are injected on daily basis of the cycle at 16:00 o'clock with the same quantity of GnRH. The plasma level of LH is measured immediately before the injection and ten minutes after the injection of GnRH. The results are presented in document 2.

- 3- What can you deduce concerning the sensitivity of the pituitary gland to GnRH?



Document 2

Experiment 4: the same number of pituitary LH secreting cells extracted from female rats in the morning of day (d-1) is incubated in vitro. At the end of the incubation, the quantity of LH in the medium is measured. The experimental conditions as well as the results are presented in document 3.

Quantity of LH (μg)	Pituitary cells with estradiol	Pituitary cells without estradiol
	With GnRH	Without GnRH
	3.3	0.7
	< 0.2	< 0.2

Document 3

- 4- Name the structures that secrete estradiol during the sexual cycle.
 5- 5-1- Analyze the results of document 3.
 5-2- What can you conclude?
 6- Explain how the peak of LH is triggered.

Exercise 17 (5 pts) Stimulation of the ovulation**Session 2017-2**

The first phase of the menstrual cycle is marked by important development of the follicles. Out of these follicles, only one becomes mature and ready for ovulation. In order to better understand the factors and the mechanisms that cause ovulation to occur, the following studies are performed.

Study 1: the variation of the level of estradiol, an ovarian hormone, is monitored during a sexual cycle. The results are shown in document 1.

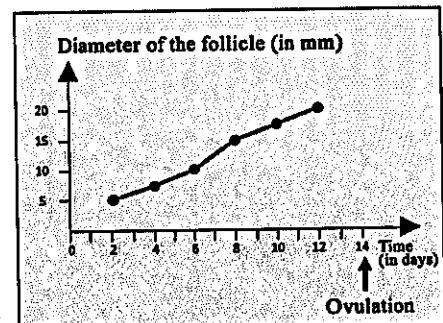
Time(days)	0	4	10	12	14	18	21	28
Level of estradiol (pg/mL)	60	75	150	240	75	100	150	60

Document 1

- 1- Draw the curve which represents the variation of the level of estradiol as a function of time.

Document 2 shows the variation of the diameter of a cavity follicle during maturation until ovulation. Note that the diameter of the follicle is proportional to the number of follicular cells.

- 2- Define ovulation
- 3- Explain how the transformation of the follicle (doc 2) leads to the variation of the level of estradiol during the follicular phase (doc 1).
- Document 3 shows a follicle at two different stages of the development in the ovary during a sexual cycle.
- 4- Name the follicle represented in each of the photos A and B.
Justify, by referring to document 2, the answer.

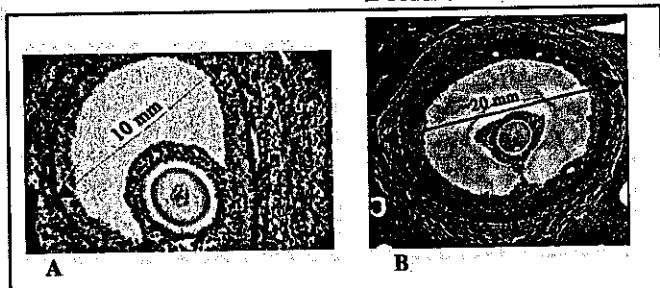


Document 2

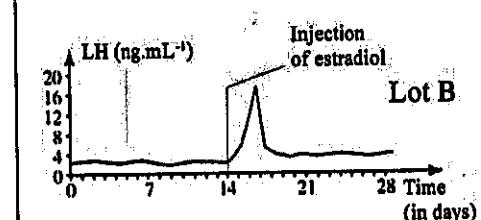
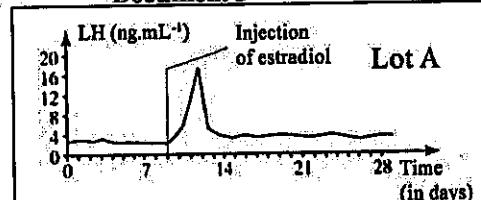
Study 2

Two lots A and B of female monkeys which are subjected to the ablation of their ovaries, receive a continuous injection of moderate level of estradiol, which keeps LH at a low level.

The monkeys of each lot receive later a unique injection of a high dose of estradiol, on a specific day. The variation of the LH level is monitored in these monkeys. The results are represented in document 4.



Document 3



Document 4

Regulation of female sex hormones

Official exercises answer key

Exercise 1 (4.5 pts) Role of progesterone

Session 2002-1

1. Variation of the concentrations of hormones in the two lots. (1 pt)

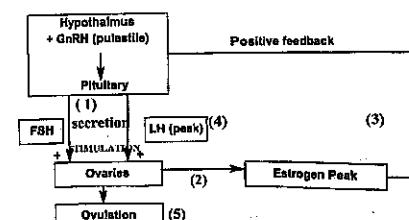
Day \ Hormone	Lot A		Lot B	
	Progesterone	LH	Progesterone	LH
0	2.5	0.5	2.5	0.5
1	0.5	5	2.5	0.5
2	0.1	5	2.5	0.5
3	0.1	5	2.5	0.5

2. Before the ablation of the corpus luteum, we observe in the two lots a progesterone concentration of (2.5 ng.mL⁻¹) and an LH concentration of 0.5 ng.mL⁻¹. After the ablation, we notice that the concentration of progesterone decreases from 2.5 ng.mL⁻¹ at day 0 to 0.5 g.mL⁻¹ after one day and remains almost constant (0.1 ng.mL⁻¹) two days later. The decrease in the quantity of progesterone is accompanied by an increase in the quantity of LH from 0.5 ng.mL⁻¹ at day 0 until 5 ng.mL⁻¹ at the end of the first day and fluctuate around 5 ng.mL⁻¹ during two days. This implies that the decrease in progesterone stimulates the secretion of LH. (1 pt 1/2)
 For lot B: The implantation of the capsule containing progesterone after the ablation of the corpus luteum maintains the concentration of progesterone (2.5 ng.mL⁻¹) during 3 days while the concentration of LH fluctuates around 0.5 ng.mL⁻¹ during 3 days. This reveals progesterone increases inhibits the secretion of LH. (1 pt 1/2)

Exercise 2 (6 pts) Organs of the sexual cycle and ovulation

Session 2002-2

1. Experiment 1 shows that without a pituitary (hypophysis), the ovarian cycle and the uterine cycle disappear. This implies the pituitary stimulates directly or indirectly the function of the ovaries and the uterus. (1/2 pt)
 Experiment 2 shows that the injection of pituitary extract permits the restoration of the cycles. Thus, the pituitary has an action on the ovaries and the uterus via chemical substance. (1/4 pt)
 Experiment 3 shows that in a female deprived of her ovaries, the injections do not restore the uterine cycles. Thus, the pituitary has no direct action on the uterus. (1/2 pt)
 Experiment 4 shows that the nutritive medium becomes enriched with FSH and LH which implies that the anterior cells of the pituitary secrete the hormones LH and FSH. (1/4 pt)
2. Experiment 5 shows that the destruction of certain cells of the hypothalamus results in the disappearance of the ovarian cycle and ovulation in the female monkey. Thus, the hypothalamus acts on the ovaries. (1/2 pt)
 Experiment 6 the graphs indicate that pulsatile injections of GnRH resulted in an increase in the secretion of FSH and LH respectively from 10 and 2.5 ng/ml to 100 and 15 ng/ml between 12.5 and 15 days, whereas after the continuous injections between 25 and 45 days, the amount of FSH and LH decrease back to their initial value, this means that the injection of GnRH in pulsatile manner stimulate the secretion of FSH and LH by the pituitary gland. (1/2 pt)
3. The hypothalamus stimulates the pituitary by pulsatile secretion of GnRH (part B). The pituitary increases the secretion of LH and FSH that stimulate the activity of the ovaries (part A). This indicates that the hypothalamus acts on the ovary only when the secretion is pulsatile. (1/2 pt)
4. At the 10th day, due to the growth of the follicle, the estrogen concentration peaks that results in the peaking of LH by positive feedback around 36 hours prior to ovulation. The hypothalamus-pituitary complex is then stimulated and LH peaks that appears to be responsible for ovulation. (1 pt 1/2)
5. The functional diagram of the mechanism of ovulation: (1½ pt)



Exercise 3 (4 pts) Regulation of LH secretion**Session 2004-2****1. (1pt)**

Time (in days)	Plasma concentration of LH (in ng/mL)	Graph of document 1	Graph of document 2
5	20	5	5
15	20	5	5
25	20	5	5
40	5	5	5
41	35	5	5
43	15	5	5
50	5	5	5

Variation of the plasma concentration of LH as a function of time

2. We observe that, 25 days after the castration, the amount of LH is constant and equal to 20ng/mL. After the introduction of estradiol implant at day 25, we observe a decrease in the amount of LH that reaches 5 ng/mL at day 40. After the injection of a large quantity (600pg/mL) we notice a peak of LH (35 ng/mL) within one day, while the amount decreases to 5 ng/mL at day 50.

This implies that a small amount of estradiol inhibits the secretion of LH (negative feedback); and in the case of large amount of estradiol there is an LH peak (positive feedback). (1 ½ pt)
On the other hand, we observe in graph B that as we inject estradiol (in small or in large amount) together with a large amount of progesterone, the amount of LH is weak and remains constant at 5 ng/mL.

This implies that progesterone prevents the increase in the amount of LH (negative feedback) whatever the amount of estradiol is. (1 ½ pt)

Exercise 4 (4 pt) Action of contraceptive pill**Session 2005-1**

1. The secretion of estradiol fluctuates between 5 pg/mL and 8 pg /mL all through the cycle in woman B while in woman A, estradiol shows a peak of 20 pg/mL on day 13 and another of 15 pg/mL on day 20 both higher than the value of estradiol in woman B.

In woman A, the secretion of progesterone by the ovaries is null from day 0 to day 14 similar to woman B. It starts increasing from day 14 to day 21 to reach 170 ng/mL in woman A, on the contrary, it decreases gradually until it becomes null on day 28. Oppositely, in woman B, the concentration of progesterone remains fluctuating near to a null value through the rest of the cycle. (1 ½ pts)

2. The pill attenuates the secretion of estradiol by the ovaries and blocks that of the progesterone. (1 pt)

3. In woman A, not taking the pill, a peak of estradiol is observed on day 13, which is followed by an LH peak on day 14. There is a correlation between the LH peak and the secretion of estradiol: the estradiol peak triggers, by positive feedback the peak of LH that provokes ovulation. This is not the case in woman B, who lacks an estradiol peak, which does not provoke LH peaking. The contraceptive pill attenuates by negative feedback the secretion of FSH and LH that do not allow the development of the follicles thus decreasing the secretion of estradiol. The absence of LH peak prevents ovulation. (1 ½ pt)

Exercise 5 (3 pts) Cause of the disappearance of follicles**Session 2005-2**

1. First hypothesis: The disappearance of the follicles is due to the aging of the ovary. (1/4 pt)
Second hypothesis: the disappearance of the follicles is due to the stoppage of the stimulation of the ovary by the pituitary. (1/4 pt)
2. The first hypothesis is the most probable; (1/4 pt) because document 1 reveals that the concentration of the blood pituitary hormones increased greatly at menopause, which reveals that the pituitary continues to secrete its hormones FSH and LH but the ovary does not respond to this stimulation. (3/4 pt)
3. At the beginning of the menopause we observe an increase of the concentrations of blood pituitary hormones, the LH from 1 to 2 a.u. and the FSH from 0.9 to 3 a.u. during about 8 years. This variation of the concentration of the pituitary hormones is due to the disappearance of negative feedback resulting from the stoppage of the ovarian activity and the fall of estrogen and progesterone hormones that exert a negative feedback and attenuate the secretions of the pituitary. (1/2 pt)
- 4.1. The consequences of estrogen deficit are: a decrease in the thickness of the skin which shrivels and a dryness of the genital mucosa, osteoporosis, arteriosclerosis which leads to cardiovascular troubles. (1 1/2 pt)
- 4.2. Remediation: Administration of estrogen in order to restore the previous hormonal levels. (1 1/2 pt)

Exercise 6 (6 pts) Birth control methods**Session 2007-1**

1. Variations of the concentration of progesterone as a function of time with or without the pill (1 1/2 pts)

Time in days	0	4	12	16	18	20	22	28
Concentration of progesterone without pill X (in ng/mL)	0	0	0	5	25	30	30	0
Concentration of progesterone with pill X (ng/mL)	0	0	0	0	5	10	5	0

2. Mrs. A with or without taking pill X, the concentration of progesterone is the same, almost null (0.2 ng/mL) from day 1 until day 12. Without pill X, this concentration in a cycle begins to increase earlier to reach 30 ng/mL (> 20 ng/mL) on day 20. On the other hand, in the cycle with the pill X, the concentration of progesterone increases less from 0 to 10 ng/mL (< 20 ng/mL) from day 14 to day 24 of the cycle. This concentration starts to decrease from day 24 in a cycle without the pill X and later from day 20 in a cycle with the pill to become null, in both cases, on day 28. (1 pt)
3. Hence, pill X has an effect of preventing ovulation. (1/2 pts)
4. The target organ is the uterus and the effect of this pill is to inhibit the development of the uterine lace, because in the presence of injections of estrogen and progesterone (lot 2) there is a thickening of the endometrium and a development of the uterine lace. On the other hand, the injection of estrogen alone (lot 1) there is only a thickening of the endometrium. This indicates that progesterone acts on the development of the uterine lace. When we add to the injections of estrogen and progesterone the intake of pill Y (lot 3), the uterine lace did not develop. This indicates that pill Y has blocked the action of progesterone on the development of the uterine lace. (1 1/2 pts)
5. Pill X corresponds to a contraceptive method because it intervenes before pregnancy. Pill Y corresponds to a contragestive method if it is used after the implantation to interrupt an early pregnancy. (1/2 pt)

Exercise 7 (7 pts) problems of sterility**Session 2008-2**

1. (2 pts)

Time (days)	0	4	10	12	14	18	21	28
Amount of estrogens (pg/mL)	60	75	150	240	75	150	200	60
Amount of LH (mIU/mL)	5	10	13	13	60	10	10	5

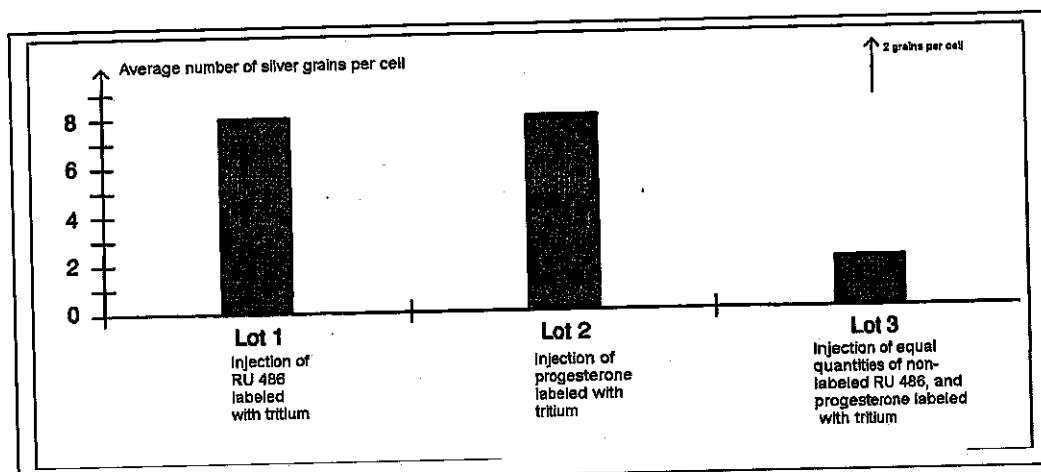
Variations of estrogens and LH amounts in the course of a cycle of a woman

2. The woman produces normal gametes, which shows a normal state of endocrine control and ovarian functioning. In fact, the results obtained indicate an increase in the amount of estrogens between days 0 and 10 that indicates a normal development of follicles. The estrogens peak on day 12 provokes a positive feedback on the hypothalamo-pituitary axis, which is explained by LH peak on day 14 that induces ovulation. Thus, the problem is at the level of the genital ducts. (1pt)
3. This woman must be subjected to IVF ET technique. (0.5pt)
4. During 28 days the levels of estrogens and LH in this woman show remain constant. The amount of estrogens fluctuates between 29.1 and 30.4 pg/mL and the amount of LH fluctuates between 5.3 and 7.3 mIU/mL through out the cycle. This implies that there are no cyclic variations for the amounts of estrogens and LH in this woman.(0.5pt)
5. The measurements of hormone levels done in this woman reveal the absence of cyclic variations of hormones, the release of estrogens at day 12 should trigger the peak of LH, which induces ovulation. Therefore, the probable cause of sterility in this woman is the a deficiency of estrogens that blocks LH peak which leads to ovulation. This signifies that this sterility is due to disturbances in the functioning of the hypothalamus, or pituitary, or ovaries. (0.5pt)
6. A hormonal treatment should be given to this woman. For example, a strong dose of estrogens can be administered in order to stimulate the occurrence of LH peak, which triggers ovulation.(0.5pt)

Exercise 8 (5 pts) Mode of action of RU486**Session 2009-2**

- 1- RU 486 has no effect on the secretion of progesterone because in the control and treated women we observe an increase in the amount of progesterone from almost null level to about 18 ng/mL of plasma from day 14 till day 22. Then, this amount of progesterone decreases progressively until it reaches the initial value(almost null) on day 26 and remains constant at this value until day 28.(½ pt)
 RU 486 leads to the early appearance of menses because we observe the appearance of menses on day 24 in the treated women 4 days before the appearance of menses in the control group which occurs on day 28.(½ pt).

2-



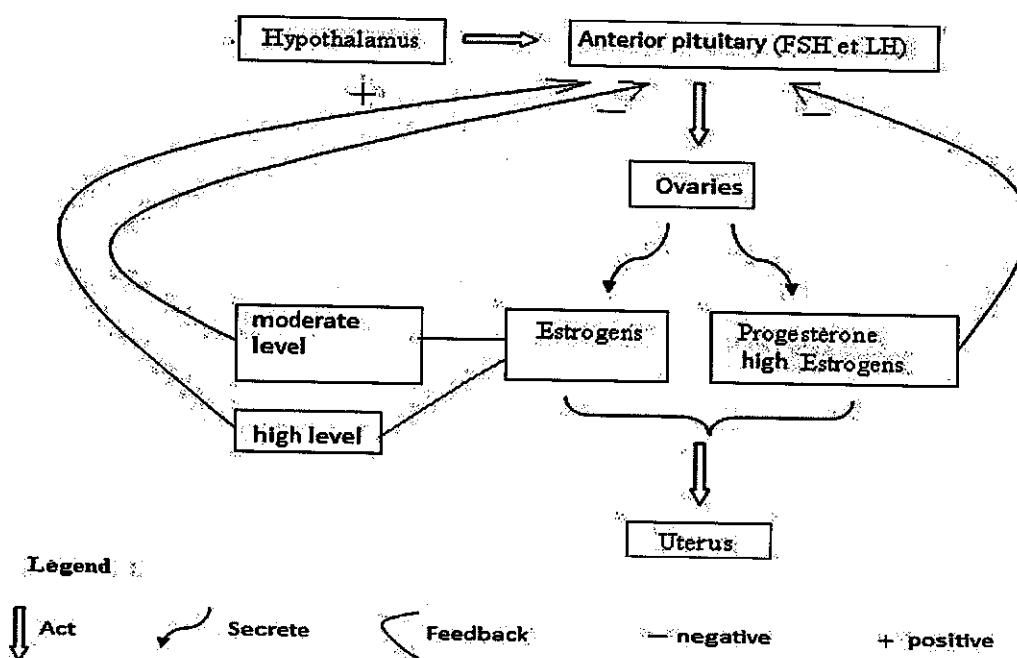
Histogram showing the average number of the silver grains per cell in function of the injections done in 3 lots (2 pts)

- 3- The average number of silver grains per cell is the same (8 grains/ cell) for the two lots 1 and 2 injected by RU 486 labeled with tritium and progesterone labeled with tritium respectively. This signifies that progesterone and RU 486 fix in the same manner at the level of the nucleus of the uterine mucosa cell. On the other hand, this number decreases to 2 grains/cell in lot 3 injected with non-labeled RU 486 and progesterone labeled with tritium. This indicates that RU 486 prevents the binding of a large quantity of progesterone (75%) in the nucleus of endometrial cells by binding on progesterone receptors with higher affinity. (1 pt)
- 4- The two molecules, progesterone and RU 486, have a similar structure at the level of sterol nucleus (doc .3). This allows RU 486 to fix on the progesterone nuclear receptors, and since RU 486 fixes more efficiently than progesterone by occupying almost 75% of the progesterone receptors , it prevents progesterone from performing its action.(Lot 3, doc. 2). This inhibits protein synthesis leading to the sloughing off of the surface layer of the endometrium and to the early appearance of menstruation (1 pt).

Exercise 9 (6½ pts) Regulation of the sexual cycle

Session 2010-2

- Atrophy of the ovaries and the uterus with a disappearance of the cycles are observed following the ablation of the anterior pituitary. This implies that the anterior pituitary gland is indispensable for the development of the ovaries and the uterus and for their cyclic activities. (1/2pt)
 The ovaries redevelop and the ovarian and uterine cycles are sometimes reestablished when anterior pituitary extracts are injected to animals submitted to the ablation of the pituitary gland. However the uterine cycle is never reestablished when the same injections are given to these animals after ablation of their ovaries. This implies that the anterior pituitary gland acts through blood directly on the ovaries and indirectly on the uterus. (1/2pt)
 The same effects as those of the ablation of the anterior pituitary are observed following the lesions of the posterior hypothalamus. This implies that the hypothalamus activates the pituitary gland. (1/2pt)
- Negative feedback: the moderate level of estrogens (70 pg/ml) alone or the high level of estrogens in presence of progesterone decreases the release of FSH and LH by the anterior pituitary gland (1pt).
 Positive feedback: the high level of estrogens alone (300pg/ml) increases the release of FSH and LH by the anterior pituitary gland (1pt).
- Functional diagram showing the relations between the different organs. (1½ pt)



Exercise 10 (5 pts) Relations between the pituitary gland and the testis

Session 2011-2

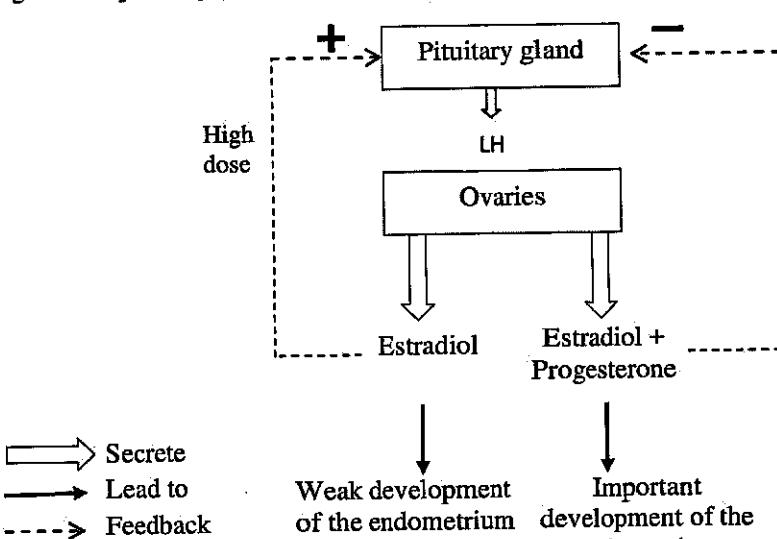
1	Spermatogonium: mother cell of male gametes.(0.25pt) Sertoli cell: nurturing role for germ cells.(0.25pt) Leydig cells : produce testosterone.(0.25pt)	0.75
2	2-1- Spermatogonia are only activated by FSH similarly Sertoli cells are only developed under the effect of FSH, however Leydig cells are not activated except by LH.(0. 5pt). 2-2- We can draw out that the target cells of LH are Leydig cells. (0.25pt) Whereas Spermatogonia cells and Sertoli cells are the target cells of FSH.(0.25pt)	1
3	The presence of LH in the culture of Leydig cells, in absence of TNF α , has strongly increased the production of testosterone, which passes from 2 a.u. (without LH) to 50 a.u. (with LH). Thus LH activates Leydig cells. However, the production of testosterone decreases 50 a.u. to 5 a.u. when TNF α is added to LH, thus the activation of Leydig cells is done by the fixation of LH to their free receptors.	1
4	The secretion of gonadotropins is 100% for FSH and LH in media 1 and 2 where the pituitary cells are alone or with kidney cells or spleen cells. However, only the level of LH decreases to 60% in the medium where the pituitary cells are with leydig cells. This shows that only Leydig cells are able to inhibit the activity of pituitary cells that secrete LH and have no effect on those that secrete FSH.	1.25
5	It is a negative feedback control.(0. 5pt) Because the level of LH (produced by pituitary cells) and the level of testosterone (produced by Leydig cells) vary in an opposite manner.(0. 5pt) Or When the level of testosterone , that is produced by Leydig cells, increases the level of LH decreases.	1

Exercise 11 (5 pts) Functional relationships between ovaries and uterus Session 2012-1

1	<p>Experiment 1: The average mass of the uterus of the ovariectomized mice without injection of estradiol is 12 mg. This mass increases from 20 mg to 100 mg when the dose of the injected estradiol increases from 0.005 mg to 0.1 mg. Thus, estradiol stimulates the development of the uterus. (3/4pt)</p> <p>Experiment 2: No significant changes were observed at the level of the uterus after the injection of progesterone only, however the development is more intense compared to document 1 (above 40 mg) after the injection 0.01 µg of estradiol followed by progesterone. This means that progesterone alone has no effect on the uterus, but it enhances the development of the uterus in the presence of estradiol in an ovariectomized female. (3/4pt)</p>	1 ½
2	We can draw out that estradiol has as targets the nuclei of uterine cells.	3/4
3	<p>After the injection of 0.2 µg of estradiol to ovariectomized rats, the increase of mRNA per uterine cell elevates from zero to 200%. (This corresponds to the phase of DNA transcription into mRNA), and the increase of proteins per uterine cell elevates from zero to 60% after a delay of 12 hours. (This corresponds to the translation phase which follows the phase transcription).</p> <p>So, estradiol stimulates the expression of certain genes for protein synthesis. (3/4pt)</p> <p>Also, this injection elevates the increase of DNA per uterine cell from 0 to 60% after a more delay of 24 h, indicating the replication of DNA molecules, which precedes cell division that corresponds to cellular proliferation. (3/4pt)</p>	1 ½
4	Document 4 shows an increase in progesterone receptors in thousands of sites per uterine cell from 6 to 50 between 0 to 1 day after the injection of estradiol. This shows that estradiol favors the production of progesterone receptors. Therefore, when injecting progesterone alone (experiment 2) there was few progesterone receptors in uterine cells and therefore this hormone has no effect on these cells, but after the injection of estradiol, the number of progesterone receptors increases and the fixation of progesterone on the receptors also increases thus modifying the cellular function and favoring protein synthesis which explains the increase in the mass of the uterus.	1 ¼

Exercise 12 (5 pts) Ovaries and sexual cycles

Session 2013-2

1	Ovaries are responsible for the development of the uterus and the cyclic variation of the uterine cycle. Ovaries act by secreting estradiol and progesterone in the blood in a variable or cyclic manner.	0.75
2	There is development in the endometrium of the uterus following the injection of estradiol for 6 days followed by an injection of progesterone for 4 days (lot A). However this development is less important following the injection of estradiol for 6 days alone (lot B). Thus estradiol stimulates the development of the endometrium and progesterone amplifies this action. While there is no development of the endometrium following the injection of progesterone alone from day 7 till day 10 (lot C). Hence, progesterone alone doesn't have any effect on the endometrium. On the other hand the endometrium shows a weak development less important than that in lot A following the injection of progesterone for 4 days followed by an injection of estradiol for 6days. Thus progesterone does not act on the endometrium unless it is preceded by estradiol.	2
3	In lot A, the injection of estradiol at the beginning of the cycle ensures slight development of the endometrium and increases the number of progesterone specific receptors. This increases the concentration of the progesterone bound to its receptors in the nucleus of target cells. this increases the synthesis of proteins and ensures the thickening of the endometrium, leading to the important development of the endometrium in lot A.	0.75
4	The amount of LH increases from 0.5ng/mL to 9ng/mL between the days 8 and 16 following the injection of estradiol alone on day8. This shows that estradiol exerts a positive feedback on the pituitary gland. On the contrary, the amount of LH decreases from 60ng/mL to 10ng/mL between day 12 and 28 after the injection of estradiol and progesterone at day 12. This shows that estradiol with progesterone exert a negative feedback on the pituitary gland. This shows that the activity of the pituitary gland is under the control of ovarian hormones.	0.75
5	Functional diagram showing the relations existing between the ovaries and the two other organs: the pituitary gland and the uterus  <p>Legend: → Secrete → Lead to → Feedback </p>	0.75

Exercise 13 (5 pts) Retarded puberty

Session 2014

1. The echography of Nadia's ovaries shows two ovaries with normal size, and document 1 reveals the presence of different types of follicles, (secondary, cavitary), in addition to primordial and primary follicles, but absence of mature Graafian follicles meaning that there is no problem at the level of the ovary structure and its components.
Also, her karyotype is normal with 44 autosomes and 2X chromosomes, meaning that she is not suffering from any chromosomal abnormality that might be at the origin of this problem. Therefore, the origin of Nadia's retarded puberty is limited to a deficiency in the follicular development.
2. LH and FSH: Anterior Pituitary gland
Estrogen: Ovary
3. Hypothesis 1: Lack in the production of GnRH by the hypothalamus.
Hypothesis 2: Secretion of abnormal GnRH by the hypothalamus.
4. The concentration of LH increases during the first 30 minutes from 33.1 U/L to reach its maximum 170 IU/L upon injection of 100 microgram of GnRH, while that of FSH remains constant at 0.6 IU/L, on the contrary, after 60 minutes, the amount of LH decreases to a value of 130 IU/L, the FSH increases slightly to 0.8 IU/L, on the other hand, ovulation is induced when the patient is injected by FSH, this means that GnRH stimulates the production of LH and fails in a remarkable stimulation of the secretion of FSH that, if injected, promotes ovulation.
5. Nadia is suffering from an inability to secrete FSH under the action of GnRH, the lack of FSH leads to a lack in the development of ovarian follicles into Graafian follicles that will be translated by a lack of estrogen, since estrogen is secreted by the follicles. And since estrogen is one of the hormones responsible for the secondary sexual characteristics and uterine cycle, then Nadia will suffer from absence of some signs of puberty like pubic hair and breast development, also, she will suffer from absence of menses.

Exercise 14 (5 pts) Female infertility**Session 2016-1**

1	Fertilization occurs at the level of the fallopian tubes. The uterus is the site of implantation of the embryo and the development of the fetus.	1/2
2	One of the spermatozoa that surround oocyte II blocked at metaphase II arrives to the zona pellucida. Pendunculated cells retract. The release acrosomal enzymes digest the zona pellucida. The head of the sperm binds to the oocyte membrane. Then, oocyte II gets activated and liberates the content of cortical granules thereby forming the fertilization membrane. Oocyte II continues the second division and releases the second polar body. The sperm is totally absorbed. Male and female pronuclei are formed and then they unite (karyogamy). The zygote is formed.	1
3	Document 2 shows that woman A has an amount of LH almost constant, fluctuating between 4 and 7 mU/mL without any peak on the 14 th day necessary for ovulation. However, document 3 shows that woman A has a uterus and 2 open tubes (oviducts) allowing opaque liquid to pass through. Thus this woman doesn't have any problem in her genital duct. Hence, Mrs A problem is the absence of ovulation due to the absence of LH peak. Document 2 shows that woman B has a normal variation of the amount of LH with a peak of 24 mU/mL in the middle of the cycle thereby provoking ovulation. On the contrary, document 3 shows a uterus without fallopian tubes. These tubes are invisible when radiology was performed, they didn't allow opaque liquid to pass through. Hence, the problem of woman B is blocked fallopian tubes and not hormonal.	1 1/2
4	Follicles are aspirated from ovaries; they are put in one test tube. Sperm cells are put in another one. Then, sperm cells and oocytes are cultured together. Embryos are obtained after embryo development. Two to three days after the puncture, one or more embryos are transferred to the uterine cavity.	1
5	Woman B can be treated by IVF and ET technique since this woman undergoes ovulation but her fallopian tubes are blocked, so sperm cells can't reach oocytes. This technique allows the sperm cells to fertilize the oocyte outside the woman's body.	1/2
6	Hypothesis : GnRH Receptors on pituitary cells are deficient. OR Amount of estradiol is not enough to exert a positive feedback on pituitary cells. OR Pituitary cells have a small number of estradiol receptor.	1/2

Exercise 15 (5 pts) Infertility in a woman

Session 2016-2

1	The temperature fluctuates in the 2 women around a value of 36.6°C, from day zero till day 14 of the cycle. This temperature increases abruptly on the 14 th day up to 37.1°C in the non-sterile woman indicating ovulation and remains high around 37.2°C for the rest of the cycle. On the contrary in Mrs. A, and throughout the whole cycle, the temperature undergoes variations which stay always slight around a value of 36.6 °C indicating the absence of ovulation in Mrs. A what causes her sterility.	1
2	The pituitary gland secretes two hormones FSH and LH: FSH triggers the follicle development and LH triggers ovulation. In case where one of these two hormones is deficient, there will be no ovulation, nor formation of corpus luteum and thus no secretion of progesterone which is responsible for the increase of temperature to above 37°C. This justifies the first hypothesis. Similarly, if the pituitary gland secretes hormones that cannot fix on the follicular cells due to the absence of receptors, we obtain the same results in the first case. This justifies the 2nd hypothesis.	1
3	The echography shows ovaries whose size is normal and containing primary follicles. Thus maybe these follicles can develop in the presence of pituitary hormones if they exist or maybe these follicles are not sensitive to these hormones. The results of hormone measurement show low concentrations of pituitary and ovarian hormones. Thus, maybe there's no control of the pituitary gland on the ovaries or maybe there is no positive feedback of ovarian hormones on the pituitary gland what maintains the low level of pituitary hormones.	3/4
4	Hypothesis 2 is validated by the results of document 2 since following the injections of FSH and LH followed by LH, the level of estradiol increases from 200 pg/ml to around 1000 pg/ml indicating follicular development. Thus the ovaries are sensitive to the pituitary secretions and hence it is the levels of FSH and LH in Mrs. A that are insufficient to stimulate the ovaries. What allows the rejection of hypothesis 2 and the validation of hypothesis 1.	1
5	Yes, the treatment has solved the problem of Mrs.A. Since the ovaries have, starting from the primary follicles, developed into two mature ovarian follicles that may undergo ovulation releasing two oocytes II blocked at metaphase II that have the possibility to be fertilized.	1/2
6	The birth of fraternal results from two different zygotes formed by the fertilization of two oocytes II issued from the two mature follicles presented in document 3 by two different sperm cells.	3/4

Exercise 16 (5 pts) What determines LH peak?

Session 2017-1

1	The secretion becomes null following a lesion of the hypothalamus (lot 2). Thus, the hypothalamus stimulates the secretion of LH by the pituitary gland. LH secretion is not resumed in the case of the graft of the pituitary gland at a location different from the original one (lot 3), whereas it is resumed when the graft is in contact with the hypothalamic-pituitary duct (Lot 4) or following the injections of GnRH which is a substance extracted from the hypothalamus. Thus, the hypothalamus acts via a substance, the GnRH, secreted in the blood of the capillaries of the hypothalamo-pituitary axis and this substance has no effect at a long distance.	1
2	The hypothalamus acts on the pituitary by secreting GnRH in a pulsatile manner, one pulse / hour. Because the LH level increases to 50 ng/mL following the discontinuous injections of 1 pulse/h between day 0 and day 15 and between day 33 and day 50. On the other hand, this rate decreases by fluctuating from 50 to 10 ng/mL following the continuous injections between day D-50 and D-73 and following discontinuous injections of 1pulse/3h between D-15 and D-33.	3/4
3	The concentration of LH increases with the injection of GnRH, regardless of the day of injection. Then the pituitary cells are always sensitive to GnRH. On the other hand, the LH level is 5 times higher (from 21.2 to 110.2) on D-Day, twice higher (from 2.1 to 4.2) than that of D-2 and it's even 4 times higher (from 2 to 5.8) that of the day D+1. Then the cells of the pituitary gland are the most sensitive to the action of GnRH on D-day.	3/4
4	Document 3 shows that the secretion of LH is less than 0.2 in the case where there is no estradiol or GnRH and also in the case where there is only estradiol. Thus oestradiol alone does not stimulate the secretion of LH. On the contrary, the secretion is 0.7 slightly greater than 0.2 (3 times) in the case where there is only GnRH but it is less than 3.3 (16.5 times) in the case where there is estradiol and GnRH at the same time. Then the LH peak requires the presence of both hormones GnRH and estradiol at the same time.	1
5	Follicular and internal theca cells during the follicular phase Luteal cells during the luteal phase	1/2
6	On day D-1, the estradiol that is secreted by the follicular cells of the graafian follicle at a high level, higher than 30 pg/mL, exerts a positive feedback on pituitary cells. The latter being the most sensitive to GnRH on day D, and under the action of this hormone (GnRH) secreted by the hypothalamus in a pulsatile manner, a pulse every hour, reacts strongly by increasing the secretion of LH. As a result, the LH peak is triggered.	1

Exercise 17 (5 pts) Stimulation of the ovulation

Session 2017-2

1	<p>Variation of the level of estradiol as a function of time</p>	11/2
2	Ovulation is the liberation or release of oocyte II from the mature ruptured graafian follicle into the pavilion duct.	1/4
3	The increase in the diameter of the follicle from 5mm at day 2 of the follicular phase into 20 mm at day 12 of the same phase is followed by an increase in the number of follicular cells. Knowing that these cells are responsible for estradiol secretion, as the number of these cells increases, the estradiol level increases from 60 pg/ml into 240 pg/ml between day 0 and 12 of the follicular phase (as shown in document 1)	3/4
4	<p>A= cavitary (tertiary) follicle. Since the diameter is 10 mm which corresponds to a follicle at day 6 of the cycle during its development.</p> <p>B= Graafian follicle. Since the diameter is 20 mm which corresponds to a follicle at day 12 of a follicular phase that's a mature follicle tends to ovulate.</p>	1
5	In both ovariectomized female monkeys of lot A and B a peak of LH of 16 ng/ml at day 12 and day 17 for the females that are subjected respectively at day 9 (lot A) and day 14 (lot B) to a unique injection of high dose of estradiol. However this level of LH is constantly maintained about 3 ng/ml following the injection of a continuous moderate level of estradiol. This shows that a high quantity of estradiol favors the peak of LH.	3/4
6	The ovary secretes a high concentration of estradiol (at the level of threshold) that stimulates by positive feedback the pituitary gland. Hence the peak of LH is responsible for ovulation. Moreover the follicle undergoing mature emits a stimulus, high dose of estradiol that favors its rapturing corresponding to ovulation.	3/4

