

---

This is a reproduction of a library book that was digitized by Google as part of an ongoing effort to preserve the information in books and make it universally accessible.

Google™ books

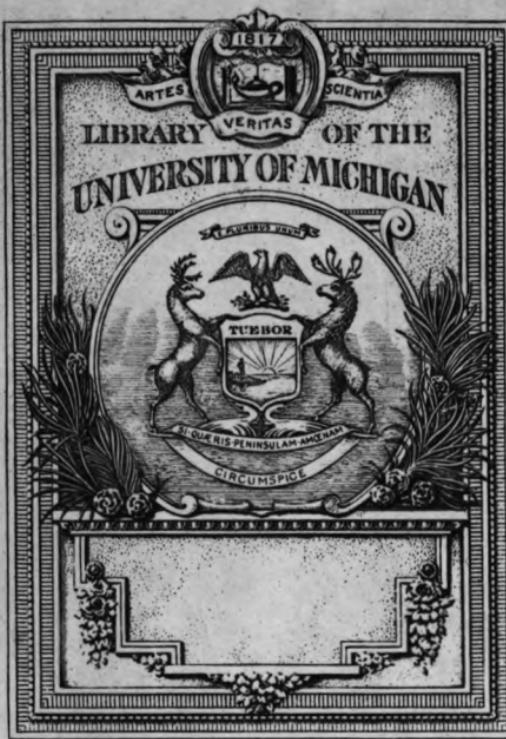
<https://books.google.com>



BUHR A



a39015 01801273 5b



Science Lib.  
QL  
961  
D29







AN INTRODUCTION TO  
EXPERIMENTAL  
EMBRYOLOGY

Oxford University Press  
*London Edinburgh Glasgow Copenhagen*  
*New York Toronto Melbourne Cape Town*  
*Bombay Calcutta Madras Shanghai*  
Humphrey Milford Publisher to the UNIVERSITY

# AN INTRODUCTION TO EXPERIMENTAL EMBRYOLOGY

BY

G. R. <sup>avirande</sup>  
R DE BEER

M.A., B.Sc., F.L.S.

Fellow of Merton College

Jenkinson Memorial Lecturer in  
Comparative and Experimental Embryology

OXFORD  
AT THE CLARENDON PRESS  
1926

*Printed in England*  
*At the OXFORD UNIVERSITY PRESS*  
*By John Johnson*  
*Printer to the University*

Sci. Lib.  
Zoology  
S. T. H.  
4-12-27  
14673

## P R E F A C E

THIS little book does not pretend to be an exhaustive treatment of the subject of Experimental Embryology. On the contrary, my difficulty has not been to find material for inclusion, but to discard from the voluminous literature and select only a few of those experiments which, taken together, may be said to throw a little light on the essentials of the problems of animal development. In spite of the comparatively short time that the experimental method has been in wide use in the service of Zoology, the amount of work which has been done is great. It is, however, scattered over the whole field, and being thus more or less incoherent it is difficult both to learn and to teach. The gaps in our knowledge are truly large, but it is now possible, since the recent work of Spemann and his school, to give a consistent formal account of the causal relations between some of the chief events in the development of at least one group of animals—amphibia.

I have consequently selected some hundred and eighty pieces of work by various experimenters, capable of being arranged in logical order, from fertilization to the assumption of the adult form. This is to be regarded as a skeleton upon which further knowledge may be built.

It is good to see how conclusions are drawn from the bare and hard facts of the results of experiments,

and in a few cases the reader will find the process of experimentation worked out so as to show exactly how the interpretation of the results is obtained. At the same time there is no reason to make the student tread the thorny path of the original experimenter without assistance.

A certain minimum of the facts of straightforward Comparative Embryology are assumed in what follows, for in a work of this size it is impossible to include these. The experimental cannot precede the comparative aspect of Embryology, or the student would be required to build and elaborate theories from materials which he neither knows nor understands. The best method is certainly to introduce the experimental facts immediately after the comparative, so as to correlate them. In this way Comparative Embryology ceases to be a dictionary of the existence or absence of organs at particular stages of development, and Experimental Embryology is no longer a chaotic jumble, but is capable of systematic treatment. The fact is that the two should not be separated; they are not different branches of science, but complementary methods of attacking one and the same subject.

A figure in brackets refers to a list at the end of the book containing the references to the original papers.

Since the writing of the last book in English on this subject (Jenkinson, *Lectures on Experimental Embryology*, Oxford, 1917), the important works of Lillie on fertilization, Child on axial gradients, Spe-

mann on differentiation, have appeared, as also the results of experiments on the hormone control of amphibian metamorphosis, tissue culture, and grafting. If for no other purpose this book may serve to draw attention to these results.

The development and determination of sexual characters has been purposely omitted, since excellent accounts of this subject are already available in English. (Goldschmidt, *The Mechanism and Physiology of Sex-determination*, Methuen, 1923; Crew, *Animal Genetics*, Oliver and Boyd, 1925.)

My thanks are due to the contributors, editors, and publishers of the following works and journals for permission to reproduce figures: *Proceedings and Philosophical Transactions of the Royal Society* (Harrison & Sons) for figs. 4 and 5; *British Journal of Experimental Biology* (Oliver & Boyd) for fig. 34; *Quarterly Journal of Microscopical Science* for figs. 48 and 49; *Journal of General Physiology* (Rockefeller Institute) for fig. 1; University of Chicago Press for figs. 26, 27, and 28 from *Individuality in Organisms*; the Wistar Institute for fig. 35 from *American Anatomical Memoirs*, fig. 36 from *American Journal of Anatomy*, figs. 17, 19, 22, 24, 32, 39, 42, 43, and 45 from *Journal of Experimental Zoology*; *Archives de Biologie* (Masson) for figs. 3 and 12; *Archives de Zoologie* (le Soudier) for fig. 20; *Archives de Morphologie* (Doin) for fig. 44; *Archiv für Entwicklungsmechanik* and *Gesammelte Abhandlungen* (Engelmann, and Springer) for figs. 2, 7, 16, 25, 31, 33, 50, and 51; *Zeitschrift für wissenschaftliche Zoologie* (Engelmann)

for fig. 37; *Anatomischer Anzeiger*, vol. xiii (Fischer), for fig. 46; fig. 30 from 'Mikrochirurgische Operations-technik' in Abderhalden's *Handbuch der biologischen Arbeitsmethoden* (Urban & Schwarzenberg). Figs. 40 and 41 are reproduced by permission from the *British Journal of Experimental Pathology*, 1923, vol. iv, No. 2; figs. 8, 29, and 38 are from *Archiv für mikroskopische Anatomie* (Cohen).

To the Oxford University Press I am most grateful for the care and skill shown in the preparation of this book.

Lastly, I wish to express my deep gratitude to Professor J. S. Huxley for reading the proofs of this book and for several helpful criticisms.

G. R. DE B.

*April 1926*

## C O N T E N T S

|        |   |      |
|--------|---|------|
| I.     | Introduction : The Experimental Method . . . . .                  | 7    |
| II.    | Fertilization . . . . .   | 13 — |
| III.   | Parthenogenesis and Activation . . . . .                          | 20 — |
| IV.    | Larval Hybrids . . . . .  | 27   |
| V.     | Relations between the Sizes of Nucleus and<br>Cytoplasm . . . . . | 30   |
| VI.    | The Value of the different Chromosomes . . . . .                  | 34   |
| VII.   | Cleavage . . . . .  | 36 — |
| VIII.  | Polarity and Symmetry . . . . .                                   | 38   |
| IX.    | Nuclear Division during Cleavage . . . . .                        | 42   |
| X.     | Cytoplasmic Division during Cleavage . . . . .                    | 44   |
| XI.    | The Factors of Development . . . . .                              | 51   |
| XII.   | External Factors and their Effect on<br>Development . . . . .     | 52   |
| XIII.  | Axial Gradients . . . . .   | 63 — |
| XIV.   | Plasticity and Chemo-differentiation . . . . .                    | 71   |
| XV.    | Visible (early) Differentiation . . . . .                         | 75   |
| XVI.   | Functional (late) Differentiation . . . . .                       | 85   |
| XVII.  | Regeneration . . . . .  | 88   |
| XVIII. | Tissue Culture . . . . .  | 93   |
| XIX.   | Hormones and Development : Amphibian<br>Metamorphosis . . . . .   | 100  |

|  |     |
|--|-----|
| XX. Nerves and their Relation to Muscle during Development . . . . . | 108 |
| XXI. The Blastopore and the Primitive Streak: Concrescence . . . . . | 112 |
| XXII. Dedifferentiation and Reduction . . . . .                      | 115 |
| XXIII. Regulation . . . . .  | 121 |
| XXIV. Review of Development . . . . .                                | 131 |
| List of Experiments described . . . . .                              | 136 |
| Index . . . . .  | 145 |

# I

## INTRODUCTION: THE EXPERIMENTAL METHOD

ALL sciences must begin by being purely descriptive. Accurate observation is made of the phenomena presented in nature, and the information gained thereby is sorted into subjects. As many facts of a similar kind as possible are included under general headings forming hypotheses and theories.

For example, in the development of most animals there is found an arrangement of layers of cells, usually three in number, from which the various regions of the embryos arise in an orderly and regular way. This germ-layer theory, as it is called, is a good instance of a conclusion derived from pure observation. As to its significance it will be shown that experiment has a good deal more to say.

Because in development a particular organ always arises from one and the same germ-layer, does it mean that this organ cannot be formed from the other layers? Comparative embryology cannot answer this question, but experiments can.

Descriptive anatomy reveals complicated structures, wonderfully co-ordinated and interdependent, and often marvellously adapted to the functions which they perform. Embryology brings to light an extraordinary sequence of structural changes, one stage following upon another until the adult structure described by the anatomist is reached. The arising of certain organs and the relation

of the events to one another is left unanswered. Would the things which one sees at any stage be as they are if certain other things at a previous stage had not been as they were? And if so, what are these necessary antecedents?

The descriptive branches of zoology, while giving a consistent account of isolated structures carved out of organisms, are continually setting problems as to the mutual relations of these structures when they are considered together and not isolated. These problems can only be attacked by the experimental method.

The egg of the frog is spherical and contains a certain amount of yolk. Yolk is heavier than the other constituents of the egg, so that it occupies the lower pole of the egg and is absent from the upper pole. Now the upper pole of the egg can be observed to become the anterior end and head of the animal, the lower pole with the yolk the posterior end. Since yolk is affected by gravity, and the region where there is no yolk in the egg becomes the head, it might easily be supposed that it was gravity which determined which region of the egg developed into the head.

This supposition can be tested experimentally. If gravity is really necessary to determine an axis in the egg by attracting the yolk to one pole, then if the action of gravity be eliminated, the egg will have no axis, no regions will be determined to become either anterior or posterior, and the egg will not develop. This is the working hypothesis. (44)

The action of gravity can be eliminated by placing the eggs in a clinostat revolving slowly about a horizontal axis, so that the eggs are constantly rolling over in all directions and never present the same region for any length of time to the centre of the earth. This constitutes the experiment.

The result is that eggs treated in this way develop normally. The interpretation of this result is, of course, that the egg had an axis, since the head did develop at a particular pole and the tail at the opposite pole; but this axis cannot be determined by gravity, since this force was not working. It is, therefore, another cause which determines the egg axis, and therefore which pole of the egg contains yolk. But yolk happens to be heavy, and therefore what gravity does is to make the egg-axis vertical in a normal egg.

This example will show how rash it was to infer from observation alone to what the determination of the egg-axis was due.

It is worth while analysing the experiment itself, to see in what it consists. In its working hypothesis it singled out two of the facts presented to it by observations, i. e. the force of gravity and the localization of yolk at the future tail end of the animal. It tested the relation between them and found the latter not to depend on the former.

Again, a meal of thyroid gland substance is fed to frog tadpoles; they metamorphose almost at once and become little frogs. In this case the relation between two things (thyroid substance and the processes of metamorphosis) are tested and it is found that the one depends on the other. Two important points are to be observed here.

First, the fact that a single substance (thyroid) should produce metamorphosis as its result does *not* mean that it is the *sole* cause of metamorphosis. Neither does the movement of an electric-light switch by itself cause the light. The light is dependent on a number of *conditions*, such as dynamos, batteries, leads, and switches, all of which must be present before the light can result.

In a similar way, when it is shown that a relation exists between two phenomena in the development of an

organism and that one will only arise in the presence of the other, it must not be thought that such phenomena are due to single causes. All that one can say is that a particular phenomenon is one of the necessary conditions for a certain event.

The second point concerns the identification of the particular condition which is really responsible. How can one be sure that it is the thyroid substance which was the condition necessary for metamorphosis, and not some other food, or possibly an effect of temperature or light or pressure? Only by comparing the results of the experiment with those of another in which the conditions are identical save for one, which is that which is being tested.

So it is necessary to compare the results of the thyroid-fed tadpoles with those of another lot of identical tadpoles from the same batch of eggs (to ensure the identity of genetic conditions), of the same age, at the same temperature and pressure, in the same light and water, and fed on the same food *except* that it contains no thyroid. In that way only can the results noted in the experiment be attributed to thyroid substance. This second checking experiment is usually called the control and is absolutely essential for the interpretation of any result. The control is often normal development, as in the case of the eggs withdrawn from the influence of gravity. The ecologist working out the relations of organisms and environment often has to rely on natural experiments and natural controls.

As a last word on the subject, let us consider the hypotheses underlying the theory of tumours, the importance of the clarity of the concepts involved, and the ful-

To begin with, some of the facts relating to the problem are as follows :

- (i) Tumours are growths and multiplications of cells of particular tissues.
- (ii) Tumours can be passed on from one animal to another by implanting tumour cells, but :
- (iii) The implant must be to an animal of the same species, often of the same strain, and :
- (iv) The implant must be to the same tissue.
- (v) One kind of tumour is known which is capable of being propagated by injection of an extract or filtrate of the tumour quite free from cells. (The Rous fowl tumour.)
- (vi) The cell-free filtrate of the Rous tumour remains infective for about two days under ordinary conditions, and for seven days when kept free from oxygen.
- (vii) Fresh cell-free filtrate can be made non-infective in a very short time by treatment with chloroform.

Now, the first hypothesis is that the loss of infectivity with time and with chloroform treatment is not due to the same cause. This implies that infectivity requires two conditions, of which one is destroyed in time by exposure to oxygen, the other is destroyed at once by chloroform. If this is so, then by mixing 'time-expired' filtrate with fresh filtrate treated with chloroform, an infective mixture should be obtained from non-infective constituents. Experiments can be brought in here to test the hypothesis, and they show that the mixture is infective while its two components separately are not. Infectivity must therefore be due to two agents whose co-presence is necessary.

The next step is to determine the nature of the difference between these two agents. If one of them is a chemical substance in solution, and the other consists of very small solid particles, it should be possible to separate them by centrifuging a tube of filtrate very rapidly. The

solid agent should thereby be accumulated at the bottom of the tube while the dissolved one should still be equally distributed.

As a result when this was tried the material at the bottom of the tube was found to be infective, that at the top non-infective unless 'time-expired' filtrate was added to it.

The two agents may therefore be regarded as the one dissolved, the other particulate.

This particulate agent may be alive. If so it should grow and multiply when cultured in suitable medium, and from this culture others should be obtainable. It was found that substance derived from the fifth sub-culture, when mixed with chloroform-treated filtrate, was infective. As the chances are infinitesimal of the fifth sub-culture containing the actual particles of the original culture, the particulate agent must be capable of growth and multiplication and is probably alive, or a virus.

Chloroform treatment of the filtrate therefore kills the virus.

Now, returning to the fact that a rigorous specificity of tissue and of species is necessary for the artificial propagation of a tumour, it is improbable that this specificity resides in the virus, or there would have to be an incredible number of kinds of virus to account for all the known kinds of tumours. The dissolved agent is therefore suspected of being the cause of the specificity, and this can be experimentally proved by showing the virus to be non-specific. As it has been possible to produce a tumour in a hen by mixing chloroform-treated (virus-free) filtrate of hen tumour with fresh extracts of tumours of mouse, rat, and man, the experiment has verified the hypothesis.

This excursion has led into the realms of experimental Biology and Pathology, but it should serve to show the working of the experimental method.

## II

### FERTILIZATION

**FERTILIZATION** is the union of a sperm with an egg, and is regarded as the first event in the life-history of an organism. Under this one word, however, many processes are included, and it is necessary to distinguish at least three results of the process of fertilization. Briefly they are :

- (i) the stimulation of the egg to activity, cleavage, and development ;
- (ii) the transmission of hereditary characteristics from the father as well as the mother ;
- (iii) in some forms, the determination of a plane of bilateral symmetry in the egg.

The penetration of the egg by the sperm may take place at very different times : (i) in some (e. g. *Dinophilus*) in the oogonium stage, or (ii) just before maturation (e. g. *Nereis*), in others (iii) after the prophase of the first maturation division (e. g. *Chaetopterus*), or (iv) after completion of the first maturation division and extrusion of the first polar body (e. g. Vertebrates), or in others again (v) after extrusion of the second polar body (e. g. *Echinoidea*).

In all these cases, however, the sperm nucleus only fuses with the egg nucleus after maturation has been completed.

Surrounding the unfertilized ripe Echinoid egg is a jelly-like substance, and round the edge of this the sperms swarm. The egg shoots out a protoplasmic filament towards the first sperm to come into contact with the jelly. The filament is presumably directed to the sperm by a substance diffused from the latter. At all events, it engulfs the sperm and retracts dragging it recalcitrant

and struggling into the egg. By lashing its tail it sometimes frees itself, and the wriggling sperm escapes. (2)

A very fine membrane encloses the unfertilized Echinoid egg, and immediately after the entry of the sperm it becomes lifted off from the surface of the egg and

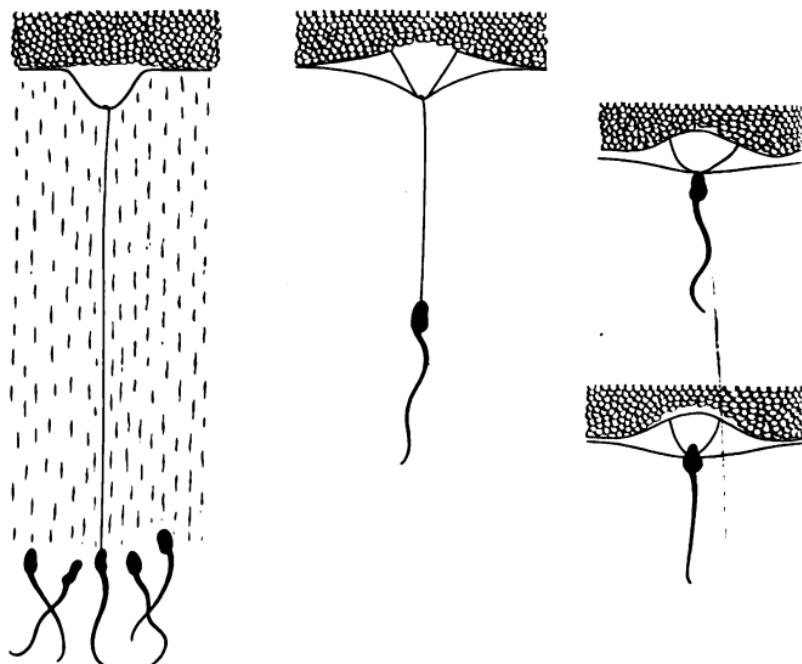


FIG. 1. Stages in the fertilization of the egg of the starfish, showing the filament which is extended from the egg and engulfs the sperm. (From Chambers.)

separated from it by the interposition of fluid. This is the so-called fertilization membrane. The extrusion of substances from the surface of the egg appears to be a common sequel to fertilization. Jelly-like substances are secreted in other forms such as *Nereis* and *Ascaris*.

*connexion with  
Nereis, only the  
piece being left  
arising de novo*

in connexion with the sperm nucleus, and need not be the same as the one in the middle-piece.

When the egg is ripe its nuclear membrane disappears and fusion of the two nuclei takes place.

Usually only one sperm enters an egg ; in some forms, however, polyspermy occurs (Vertebrates, Insects, and Polyzoa) but only one sperm fuses with the egg nucleus and the others may assist in the absorption of yolk, and degenerate.

Cells of no other tissues can fertilize or be fertilized, and normally it is difficult for fertilization to take place between members of different species. Once an egg is fertilized it can never be returned to the unfertilized condition. As a whole, therefore, fertilization can be said to be an irreversible, tissue-specific, and species-specific reaction.

The experimental study of fertilization necessitates an inquiry into the conditions necessary for the reaction to take place.

As regards sperm, it must be mobile. In the testis it is motionless, but it acquires the power of movement when extruded. Now, it has been found that the total amount of CO<sub>2</sub> given out by a sperm is the same whether it has had a short and active or a long and sluggish existence. CO<sub>2</sub> renders sperms inactive, and therefore crowded sperms become inactivated by the concentration of their own CO<sub>2</sub> output, and their existence prolonged. On the other hand dilute sperm suspensions do not produce a sufficient concentration of CO<sub>2</sub> to curb the activity of the sperms, which soon exhaust their stock of energy.

Nevertheless, fertilizing power is lost earlier than mobility, which suggests that a substance carried by sperm diffuses into the water and is lost. (3)

With regard to eggs, before they can be fertilized it is necessary that they should have completed maturation and

that the nuclear membrane should have ruptured. The egg need not be intact, for small portions of egg, even without a nucleus, can be fertilized. It is necessary, however, that some portion of the rind of the egg or cortex be present. If the protoplasm from the central region of the egg be isolated it will form a sphere without any cortex, and a sperm entering it will remain quiescent without activating it in any way. (4) Even a small portion of cortex enables the sperm to activate it.

Certain eggs secrete substances which have peculiar properties. (3) Water in which eggs (of certain sea-urchins, starfish, worms, and molluscs) have been standing, or 'egg-water', has a peculiar action on sperms, causing them to stick to one another, or agglutinate. But while egg-water of a particular species only produces a reversible reaction on sperm of its own species, the sperms becoming free again, on sperms of a different species the action is irreversible. The substance which acts on sperm of the same species is not the same as that which agglutinates foreign sperm. One of these substances can be used up by treating the egg-water with excess of sperm of one kind, which leaves the other constituent of the egg-water free to act on the other kind of sperm. They persist for different lengths of time, and whereas agglutination of sperm of the same species is only produced by an egg secretion, foreign sperm can be agglutinated by other substances such as blood serum.

It is the substance producing the reaction in sperm of the same species ('iso-agglutinin') which is of particular interest here. It is not produced until the egg is ripe and the nuclear membrane has gone, it is not produced after the egg has been fertilized. In fact, it is only produced by eggs which can be fertilized. Those which give no agglutinin cannot be fertilized, those which for some reason or other will not fertilize give no agglutinin, and

if the agglutinin is removed from an egg by repeated washing, the egg will not fertilize.

There is, therefore, good reason for believing that iso-agglutinin is identical with a hypothetical substance termed 'fertilizin' which is necessary for fertilization. The agglutination of sperms to one another probably has no significance in fertilization, but it shows that the sperms undergo some chemical change, and that the reaction is strongly species-specific. For the purpose of experiments, the iso-agglutinating reaction is used as an indicator of the presence of fertilizin.

The hypothesis as to the course of events in fertilization is therefore as follows :—

The rupturing of the nuclear membrane releases a substance in the egg which becomes localized in the cortex, and capable of diffusion out of the egg. This substance, fertilizin, is acted upon in some way by a substance carried by the sperm, and as a result its activating effect is released. At the same time a sperm must be acted upon by fertilizin or it will not produce a sperm aster. The release of the activating reaction of fertilizin may be effected by agencies other than sperm, in which case natural and artificial parthenogenesis will result.

By way of experimental evidence for this hypothesis, the answers to certain questions will now be considered.

(i) A sperm is inert in an immature egg, or in a mature egg devoid of cortex. In the immature egg the nuclear membrane may be regarded as withholding the fertilizin, and the lack of cortex likewise involves lack of fertilizin.

(ii) Fertilization is tissue-specific, which is well accounted for by the fact that eggs and only eggs produce fertilizin.

(iii) Fertilization is species-specific as a rule, which must be based on the specificity of fertilizin which is evident in the iso-agglutinating reaction.

(iv) Fertilized eggs cannot be refertilized. The fertilization reaction is complete and irreversible; all the fertilizin is bound and no more is produced. At the same time it is possible to produce a 'partial activation' by artificial parthenogenesis, and in this case a certain amount of fertilizin remains free, which enables fertilization to be superim-

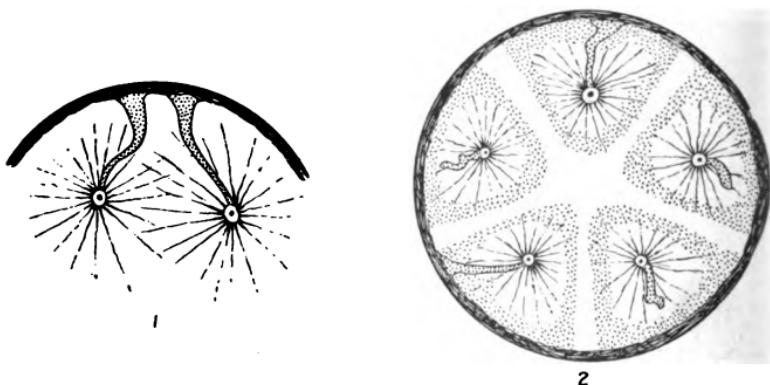


FIG. 2. 1. The mutual repulsion of two sperm asters which have entered a frog's egg. 2. The cytoplasm of a frog's egg which has been penetrated by several sperms. The cytoplasm is divided into as many regions as there are sperm asters. (From Brachet.)

posed. Fertilization after complete activation by artificial parthenogenesis is impossible.

(v) An aster forms in connexion with the sperm nucleus. On the other hand, if a sperm enters an egg devoid of cortex no aster is formed. The reaction of sperm and fertilizin is therefore reciprocal, and the sperm has itself to be 'fertilized' before its functions can be completely performed.

Normally one sperm enters an egg. The raising of the fertilization membrane helps to keep off other sperms, but it cannot be the sole cause as the following experiment shows. If the membrane is removed from an egg into which a sperm has penetrated, other sperms will still not

enter it, or any fragments of it, while they readily enter fragments of eggs which have not been already penetrated by another sperm.

Some change must therefore take place in the protoplasm of the egg and spread as a wave round the circumference from the point of entry of the sperm. In a large egg containing much yolk it will take a longer time for this change to spread round, and in these eggs normal polyspermy occurs.

This change appears to be related to an action of the aster. By using a large quantity of sperms, more than one can be made to enter a frog's egg at the same time. Each forms an aster, and each aster repels every other aster. (5) The cytoplasm is divided up into as many regions as there are asters, and in none of these regions is there more than one aster. Under normal conditions in monospermic eggs, when the concentration of sperms is not so high, the successful sperm will have elaborated an aster which, taking command of the whole of the egg, renders it impenetrable to other sperms.

The theory of fertilization outlined above (Lillie's) is not to be regarded as complete, especially as it leaves the physical (electric and surface-tension) processes out of account, but it does form a consistent working hypothesis

### III

## PARTHENOGENESIS AND ACTIVATION

PARTHENOGENESIS, or the development of an egg without the stimulus of fertilization by a sperm, is a natural phenomenon in many groups of the Animal Kingdom. Without attempting to be exhaustive, the following list of groups in which it occurs will give an idea of its prevalence.

Crustacea : Apus, Artemia, Daphnia, Cypris.

Insecta : Aphidae and Coccidae, a few Lepidoptera, a Trichopteran, a Coleopteran, a few Diptera, nearly all Hymenoptera.

Arachnida : Syringobia (Acarina).

Annelida : Dinophilus.

Mollusca : Paludestrina.

Rotifera.

In some cases, as in the honey-bee, any egg when mature may be fertilized or may not, but in either case it develops, producing females or males respectively. This means to say that the activation which is given by the sperm can also be given by other agencies.

The course of events in parthenogenetically activated and in fertilized eggs is so similar that the same mechanism must be at work. Since the activation can take place with or without the sperm, the action of the sperm cannot be exerted directly on the egg, but through some intermediate substance. This substance is present in the egg, and in the absence of sperm can be otherwise stimulated. In this way a mechanism can be imagined which will account for the cases of parthenogenetic and sperm activation. Meanwhile it is interesting to note that the fertilization which was dealt with in the last chapter answers well to

the requirements of this substance intermediate between the sperm and the processes of activation in the egg.

A large number of agencies have been found by experiment to exert an action on the unfertilized egg. To start with, if a sperm is allowed to come into contact with the surface of an egg of *Nereis*, and is then forcibly removed by centrifuging, the egg behaves as if it had been fertilized, in that it completes its maturation and the nucleus prepares to fuse with the sperm nucleus which is of course not there. (3) No cleavage takes place and after a time the egg dies. This experiment shows that important effects can be produced in an egg by stimulation of the cortex only, and it has already been seen that the cortex is important from other points of view.

Treatment of sea-urchin eggs with hypertonic sea-water (i.e. in which the concentration of salts in solution is higher than normal) results in the formation of an aster and attempts at cleavage, which are, however, not very successful. (6)

In this last case no 'fertilization' membrane was formed, but if the egg is first treated with substances like butyric acid, such a membrane, identical with a normal fertilization membrane, results. Later on, however, such an egg undergoes decomposition and cytolysis unless treated with hypertonic sea-water. This double treatment, first with butyric acid and then with hypertonic sea-water, produces perfect development of the sea-urchin egg, even up to the adult condition.

These experiments have led to the notion that the sperm introduces a cytolysing substance into the egg which, after extruding the fertilization membrane, would itself undergo cytolysis unless prevented by a second 'corrective' substance likewise brought in by the sperm. It is doubtful, however, if this view is correct. It is more likely that the butyric acid affects the cortex of the egg in a way

comparable to that produced by contact with a sperm (see above), and that the hypertonic sea-water causes the formation of an aster. The egg then has all the conditions requisite for development. (170)

Another successful method of producing artificial parthenogenesis rests on the idea that the colloid substance of the egg during activation undergoes alternative coagulation and liquefaction. The formation of the fertilization membrane, the aster, and the spindle are regarded as coagulations; the disappearance of the nuclear membrane and the division of the chromosomes as liquefactions. Now acids have the property of coagulating, and alkalis of liquefying, colloids. Be that as it may, experiment has shown that treatment with tannic acid and ammonia is highly successful in inducing parthenogenesis. (7)

The experiments are of great interest, but it is still open to doubt whether the results are to be attributed to specific properties of the agents used. On the contrary, it is highly probable that they work by releasing an activating mechanism in the egg: that is to say, that they do not induce parthenogenesis by artificial means, but that they cause the egg to become 'autoparthenogenetic'. They are, therefore, of interest in connexion with the nature of stimuli to which eggs respond, but they do not throw much light on the internal processes of activation.

Following upon the observation that attempts at activation are followed by mere presence of sperms at the surface of the egg, it was found that pricking the frog's egg with a needle resulted in many changes characteristic of the beginning of activation. (8) The perivitelline fluid is expelled and an aster appears, and even divides, but the egg does not cleave. On the other hand, if the needle carries other objects such as blood or lymph cells into the egg, perfect development follows and adult frogs can be produced.

Why such a change should be produced by the entrance of lymph or blood cells it is difficult to understand, but an ingenious explanation has been offered, based on other experimental work.

In a frog's egg which has been penetrated by several

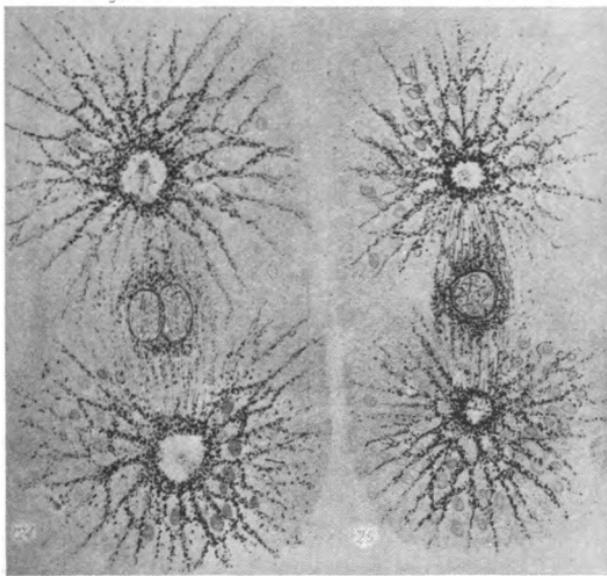


FIG. 3. Nuclear spindles in a frog's egg which has been penetrated by several sperms. One spindle is related to the combined pronuclei of the egg and one sperm (1), the other is related to one sperm pronucleus only (2); the length of the latter spindle is regularly four-fifths that of the former. (From Herlant.)

sperms, as described in the last chapter, each sperm nucleus and aster occupies a separate territory in the egg, and one fuses with the egg nucleus. At the same time all the asters divide and the nuclei arrange themselves on the spindles so formed. One spindle carries the double nucleus of egg and sperm; all the others have single sperm nuclei. It is found that a relation exists between the length of the spindle and the size of the nucleus on

it. (9) The spindles carrying the single sperm nuclei are consistently one-fifth shorter than that concerned with the double nucleus of egg and sperm together.

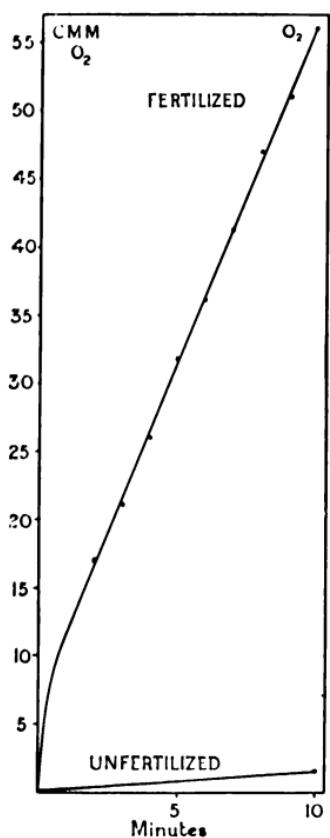


FIG. 4. Graph showing the difference in consumption of oxygen by eggs of *Echinus microtuberculatus* (sea-urchin) which have and which have not been fertilized.  
(From Shearer.)

- (i) increasing the activity of the asters,
- (ii) increasing the amount of nuclear material (by normal fertilization),
- (iii) decreasing the distance between the spindle and

the surface, and the amount of cytoplasm to be worked upon.

Incredible as it may at first sight appear, there is evidence that the presence of the lymph or blood cells bring about this last condition. It has already been shown that asters in an egg repel one another, and occupy territories of their own into which the influence of the

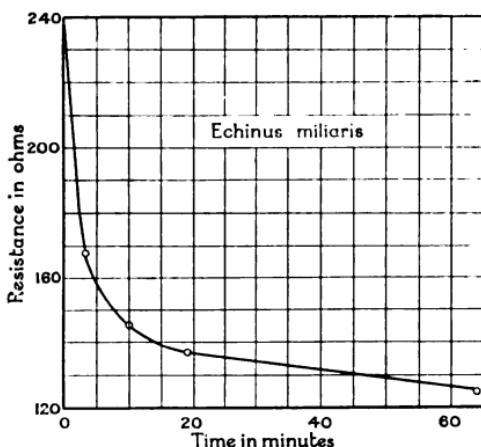


FIG. 5. Graph showing the decrease in resistance and increase in electrical conductivity of eggs of *Echinus* consequent upon fertilization. (From Gray.)

other asters does not extend. Now asters form in the frog's egg around the lymph or blood cells introduced by the needle. The amount of cytoplasm on which the real egg aster can work is accordingly diminished and the spindle is able to bring about cell division, which, keeping time with nuclear division, results in proper cleavage of the egg.

Whatever the processes of activation and parthenogenesis may be, it is obvious from the experiments that have already been described that the cortex of the egg is the site of important changes. Without going into details it may be mentioned that immediately after activation,

whether by fertilization or by parthenogenesis, the electrical conductivity of the egg increases and its resistance is reduced. (12) This means that a change has taken place at the surface of the egg in the direction of increasing the permeability to electrolytes. The same thing is shown by the fact that for a few minutes after activation the egg is much more sensitive to hypertonic solutions.

The consumption of oxygen by a fertilized egg increases immediately, and in ten minutes is thirty-seven times as great as that of the unfertilized egg. (13) It might be thought that this increased oxygen consumption is correlated with the activity of the egg in cleavage and cell division; but this is not the case because cleavage can be prevented by the addition of phenylurethane, nevertheless the oxygen consumption is the same as if cell division were going on. (14)

On the whole, it is unlikely that the changes in permeability, &c., are to be regarded as the cause of activation, but rather as some of its effects. One is reduced in the present state of incomplete knowledge to conclude that activation, whether by fertilization or parthenogenesis, is a 'specific reaction of the egg', carried out by the intermediary of fertilizin, and capable of being started by a variety of stimuli.

## IV

### LARVAL HYBRIDS

THE addition of sperm of one species to an egg of another gives results from which conclusions of importance can be drawn for two different aspects of experimental embryology. On the one hand they bear on the problem of activation, on the other they contribute to the knowledge of the relative values of nucleus and cytoplasm as regards the determination of the type of development which will ensue.

When the parent species are not too distantly related (e.g. the sea-urchins *Sphaerechinus* and *Strongylocentrotus*) the hybrids are more or less intermediate. (15) Artificial means have to be used to assist the fertilization, such as narcotics which reduce the resistance of the egg, or by using large quantities of sperm. The plutei obtained from such a cross exhibit characters in various structures, such as the skeleton, some of which are maternal and some paternal, showing that activation has been normally produced and that the sperm nucleus has been equivalent to the egg nucleus in influencing the course of development. This case does not differ much from that of a wide cross between members of the same species.

In wider crosses, however, the course of development is purely maternal, and the sperm, although it has activated the egg, has not contributed any hereditary characters. It is interesting to note that this form of pseudo-parthenogenesis is the rule in *Rhabditis aberrans*, where the sperm nucleus never plays any part in development at all.

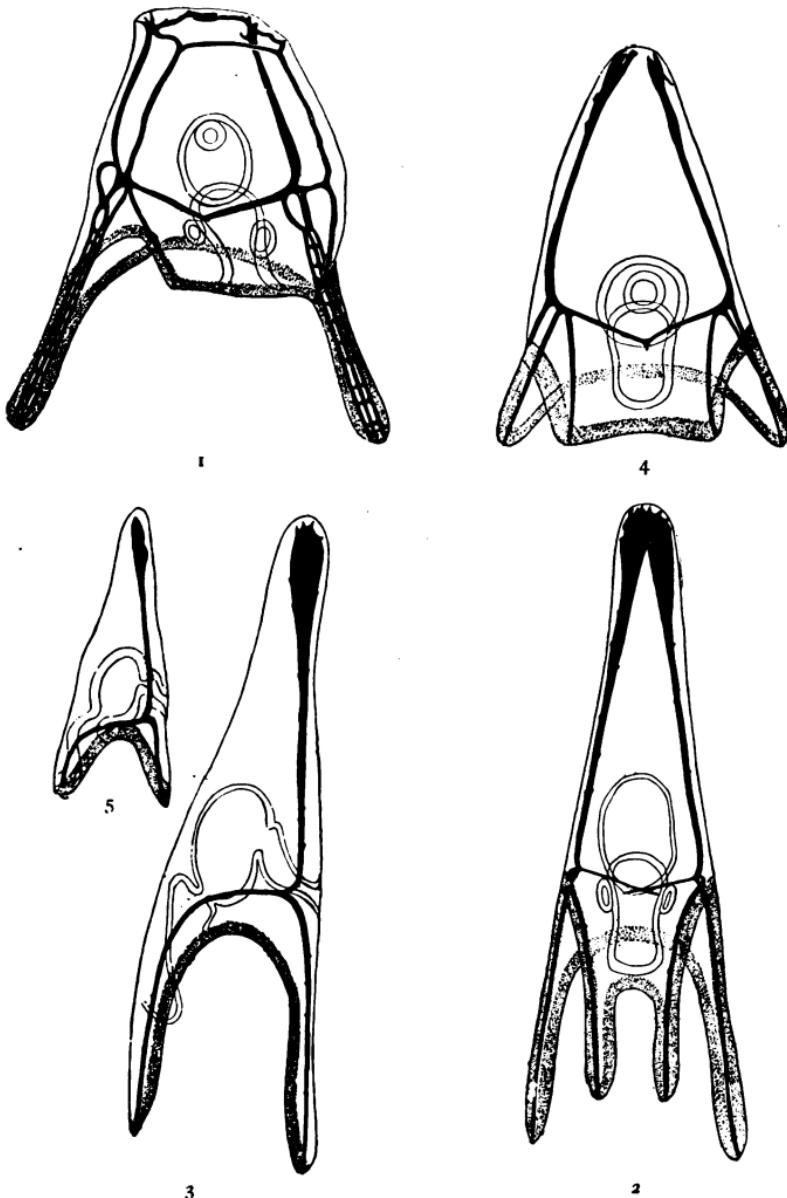
Fragments of eggs can be fertilized even if they do not contain the egg nucleus. An enucleated fragment of an

egg of *Sphaerechinus* can be fertilized by a sperm of *Echinus*, and the resulting larva is of pure paternal type, as regards its skeleton at least. (16) The sperm nucleus is therefore able to influence the strange but closely related cytoplasm.

On the other hand, if the enucleated fragment of *Echinus* be fertilized by a sperm of *Antedon*, which belongs to a different order, the embryo shows a purely maternal type of development as far as it goes. (17) The processes of cleavage and gastrulation are typical of Echinoids and not of Crinoids. The sperm nucleus is therefore impotent to draw the egg cytoplasm out of the course of development characteristic of its order.

The evidence from experiments on larval hybridization therefore shows that

- (i) the activating effect of the sperm can be separated from its hereditary effect;
- (ii) some factors for the characters of the phylum, class, and order are transmitted through the cytoplasm of the egg;
- (iii) some factors for the characters of the genus, species, variety, and individual are transmitted through the nucleus, and are therefore capable of being inherited from both parents.



**FIG. 6.** 1. Normal pluteus larva of *Sphaerechinus granularis*. 2 and 3. Normal pluteus larva of *Echinus microtuberculatus*. 4. Hybrid larva produced from an egg of *Sphaerechinus* fertilized by a sperm of *Echinus*. 5. Dwarf larva produced from an enucleated egg of *Sphaerechinus* fertilized by a sperm of *Echinus*, and showing paternal characteristics. (*From Boveri.*)

## V

### RELATIONS BETWEEN THE SIZES OF NUCLEUS AND CYTOPLASM

It was mentioned above that the size of the nuclear spindle and the distance apart of the asters was influenced by the quantity of nuclear material present to be divided. (9) This was deduced from the proportions of the spindles to the single sperm nuclei and the double sperm-plus-egg nucleus in the frog's egg, into which several sperms had penetrated by artificial means.

Further, it was seen that the length of the spindle also depended on the size of the cell, which could be observed during the cleavage of the egg (of Crepidula) where the blastomeres continually get smaller. (10)

Lastly, as a result of experiments on sea-urchin eggs, it appeared that the formation of a furrow dividing a cell into two depended on the length of the spindle and its distance from the surface. (11)

From these facts it follows that a relation exists between the size of the nucleus, the length of the spindle, the size of the cell, and the ability of the cell to divide.

By other experiments it is possible to bring out clearly the relation between cell size and nuclear size.

If of two sea-urchin eggs one is fertilized normally and the other is fertilized after removal of its nucleus, both will develop into larvae. It is to be noticed that the former has twice as much nuclear material and twice as many chromosomes as the latter. It is found that in equal areas of the two larvae the nuclei will be twice as numerous in the latter as in the former. (18) As each nucleus corresponds to a cell, in the latter the cells are half the volume.

The same result is obtained by comparison of the larvae obtained from a normally fertilized and a parthenogenetic egg ; the latter, of course, having half the nuclear material of the former.

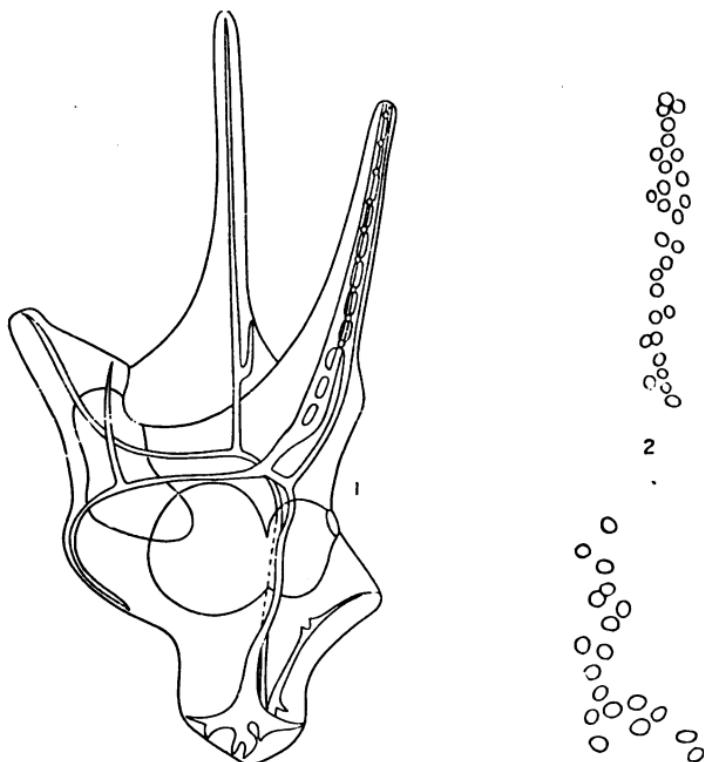


FIG. 7. 1. A partial hybrid larva produced from an egg of *Sphaerechinus* activated by artificial parthenogenesis. One of the blastomeres at the 2-cell stage has in addition received a sperm of *Strongylocentrotus*. One side of the larva is a hybrid, and the cells on that side are twice as large and half as numerous as on the other. 2. Relative sizes of the nuclei of the cells on the maternal (haploid) and hybrid (diploid) sides of the larva. (From Herbst.)

If an egg of *Strongylocentrotus* is stimulated to artificial parthenogenesis, and fertilized at the same time, the sperm nucleus will fuse with the nucleus of one of the two blastomeres. One half of the resulting larva then has

twice as much nuclear material as the other, and on that side the nuclei are half as numerous. (19)

The number of cells in equal volumes of similar tissue is then inversely proportional to the amount of nuclear material present, and to the number of chromosomes.

The nuclear material of a sperm can be destroyed by exposure to radium, without impairing the capacity of the sperm to activate the egg. If an egg of a toad be fertilized by such a radiated sperm, it can be seen that the nuclei have half the surface-area of those of normal larvae. (20) The surface-areas of the nuclei of fertilized (enucleate) egg fragments of sea-urchins are likewise half those of the controls.

Since the number of chromosomes is proportional to the amount of nuclear material present, it follows that the surface-area of a nucleus is proportional to the number of chromosomes which it contains. From the previous experiments it is known that the size of the cell depends on the number of chromosomes, since in equal areas of tissue the number of cells is inversely proportional to the number of chromosomes.

It follows, therefore, that a relation exists between the volume of the cell, the surface-area of its nucleus, and the number of chromosomes which it contains.

In mosses, it is possible to obtain plants with two, three, or four times the normal quantity of nuclear material. In these cases, however, the volume of the cells is not directly proportional to the amount of nuclear material. For each additional quantity of the nuclear material ( $n$ ) in excess of the normal amount ( $n$ ) the volume of the cell is multiplied by a constant which is peculiar to the species. In other words, the cell-volume increase takes place in geometrical, not in arithmetical progression. (176)

During cleavage, when the amount of nuclear material increases (by synthesis), but the cytoplasm is being split

up into smaller and smaller blastomeres, the ratio between nucleus and cytoplasm changes in favour of the nucleus. At the same stage, however, in different eggs, corresponding blastomeres have the same nucleo-cytoplasmic ratio (10), and in some cases it can be observed to hold good within

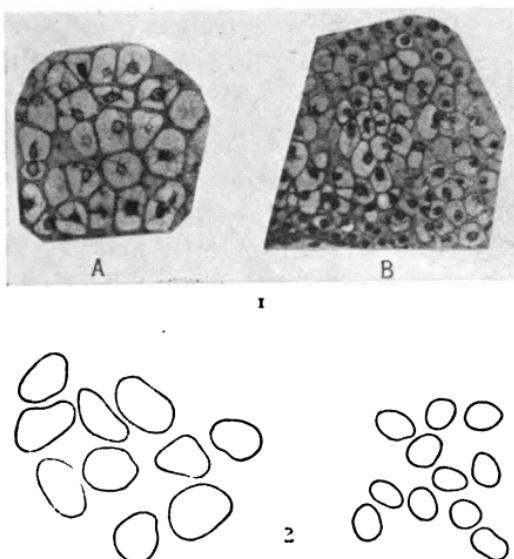


FIG. 8. 1. Cartilage cells of a normal (diploid A) and parthenogenetic (haploid B) toad showing the difference in volume. 2. The nuclei of such cells drawn to scale. The surface-area of the diploid nucleus is twice that of the haploid. (*From Hertwig.*)

the same tissue, as for example in the cells of the pancreas, or nerve-cells and heart-fibres of the rat. (21)

During development the ratios change. It is suggested that, during cleavage, the ratio of nucleus to cytoplasm increases until a stage is reached at which the hereditary factors in the nucleus can exert their effect on the cytoplasm, and from that point the moulding of the future embryo and differentiation begin.

## VI

### THE VALUE OF THE DIFFERENT CHROMOSOMES

QUITE apart from breeding experiments, it is known from experiments of fertilizing enucleate egg fragments that the nucleus plays an important part in transmitting the internal factors which regulate the development of an organism.

Genetical experiments have further indicated the chromosomes as the bearers of hereditary factors, and each chromosome of the sets brought in either by the sperm nucleus or the egg nucleus has a particular and essential part to play. It is interesting to note that evidence for this hypothesis can be derived from experimental embryology.

When two sperms enter a sea-urchin's egg, each brings in an aster which divides, with the result that there are four, and between them a quadripolar spindle forms. (22) There are three nuclei in such an egg, and if the number of chromosomes in each nucleus is designated  $n$ , there will be  $3n$  chromosomes spread over the four spindles. Each chromosome divides producing  $6n$  in all, to be distributed between four cells, for the egg divides at once into four blastomeres. There will be an average of  $\frac{6n}{4}$  or  $\frac{3n}{2}$  chromosomes to each cell.

Since in parthenogenesis  $n$  chromosomes are sufficient for satisfactory development, if all the chromosomes are equivalent, any cell that receives at least  $n$  should develop normally.

As a matter of fact, such eggs do not develop normally. If, however, it is assumed that each chromosome of the  $n$  number (brought in by each nucleus) is functionally different, and that when absent altogether its place cannot be filled by another chromosome of the same set, although

any particular chromosome of one  $n$  set can be replaced by the corresponding one of another set, then knowing the number of chromosomes in each nucleus it is possible to calculate the chances of any one blastomere of the four containing at least one complete  $n$  set. The blastomeres can be separated, and in a normal egg will develop normally. In this case, however, the proportion of separated

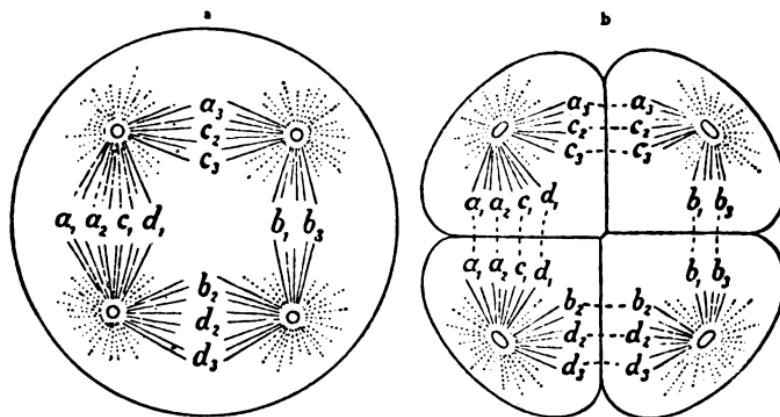


FIG. 9. Diagrammatic representation of a dispermic egg with a quadripolar spindle. Four chromosomes  $a$ ,  $b$ ,  $c$ ,  $d$  represent the haploid set. In spite of there being six representatives of each chromosome to be divided among four cells, the chances are small that one cell will contain at least one representative of each kind. Only such cells will develop.  $a$ , before division, when there are three representatives of each chromosome;  $b$ , after division, when each chromosome has six representatives. (From Boveri.)

blastomeres which develop normally agrees with the calculation made on the theory of probabilities.

There is, therefore, evidence that the different chromosomes of an  $n$  set are not all equivalent, but that at least one specimen of each kind is necessary for successful development.

This evidence is corroborated by the fact that in those cases where a tripolar spindle forms, and the egg divides into three blastomeres, the proportion of these which develop normally also agrees with the mathematical probability.

## VII

### CLEAVAGE

**CLEAVAGE** is a process of cell division whereby the egg is split up into a number of smaller blastomeres. It is to be regarded simply as a fractionating or 'cellularization' of the egg. As will be shown below, in several cases the form of cleavage can be completely altered without interfering with development; in some cases, as in the egg of *Chaeopterus*, cleavage can be prevented entirely, and yet certain features of development nevertheless take place, such as the development of cilia and the rearrangement of internal materials. (23)

The nucleus occupies the centre of activity of the cell, and normally it can be said to lie roughly in the centre of the cytoplasm. But when the egg contains a large amount of yolk, the protoplasmic contents of the egg are displaced, as is the nucleus.

By compressing eggs between glass plates it can be shown that the spindle elongates in the line of the long axis of the protoplasm, and that the furrow dividing the two cells is at right angles to this axis. (24) It will be remembered that the spindle plays an important part in the formation of this furrow. The division of the cell appears to be due to a diminution of the surface tension of the egg along a line farthest removed from the two poles of the spindle. This line will run round the circumference of the egg and coincide with the plane of the equatorial plate of the spindle.

Cleavage is modified by the presence of yolk, which

opposes resistance to the separation of the cells. When scarcely any yolk is present (Echinoderm eggs) the cleavage is complete or holoblastic, and all the blastomeres are of the same size. In the frog's egg there is a certain quantity of yolk, but cleavage is still complete although the blastomeres containing yolk divide more slowly than the others and are therefore larger. When enormous quantities of yolk are present, as in the eggs of birds, cleavage is restricted to the surface and results in the formation of a disk of cells, the blastoderm, resting like a cap on the top of the undivided yolk (meroblastic cleavage).

That these modifications are due to the density of the yolk is shown by the fact that if a frog's egg is strongly centrifuged, the yolk which it contains is rammed together, and the cleavage which follows results in the formation of a blastoderm. (25)

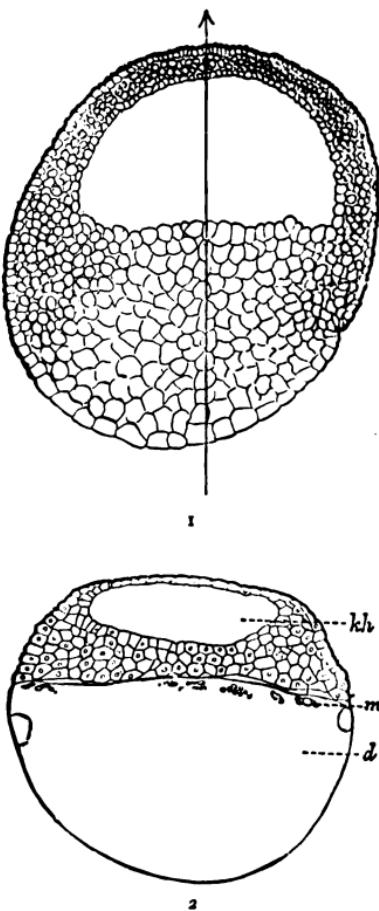


FIG. 10. 1. Normal cleavage of a frog's egg. 2. Cleavage of a frog's egg which has been centrifuged. The yolk has thereby been compressed and cleavage is meroblastic, resulting in the formation of a blastoderm over undivided yolk. *d*, yolk; *kh*, blastocoel; *m*, yolk-nuclei. (From Herwig.)

## VIII

### POLARITY AND SYMMETRY

Most eggs have an axis, and possess two poles even when they are spherical. One pole is characterized by the near presence of the nucleus, the point of extrusion of the polar body, and the absence of yolk. This is the animal pole, which ultimately becomes the anterior end and head of the organism. The other, or vegetative pole, contains mere yolk when yolk is present. In sea-urchin eggs at least, this axis is determined in the ovary by the orientation of the oocyte with regard to the follicle wall. (26)

The possession of this axis makes the egg radially symmetrical.

Some eggs, like those of insects or squids, are visibly bilaterally symmetrical. Other eggs being spherical or cylindrical either have an invisible bilaterality or no bilaterality determined at all.

In the frog's egg, at the point diametrically opposite the entrance of the sperm the grey crescent forms. This is usually the site of the future dorsal lip of the blastopore, and marks the dorsal side of the embryo. (27) The egg at this stage is therefore bilaterally symmetrical, and the sperm has determined by its point of entrance which shall be the dorsal and ventral surfaces, the right and left sides of the future embryo.

The appearance of the grey crescent is due to the retreat of water and pigment into the interior of the egg, and its localization appears to be due to an impulse which travels through the egg from the point of entry of the sperm and acts at the antipodal point. If two sperms enter an egg at the same time, wide apart or close together, the grey

crescent forms at the antipode of the line bisecting the angle between the points of entry of the two sperms. (9) No demonstration could show more decisively the dependence of the bilateral symmetry of the frog's egg on the point of entrance of the sperm.

It must be remembered, however, that under certain circumstances it may be affected by other conditions, such as light and gravity for instance. (28)

Further, these experiments do not disprove the possibility of there being a very vague and feeble determination of bilaterality before the entrance of the sperm. In the cases of frog's eggs induced to develop by a prick from a needle, a plane of bilateral symmetry does appear, although it has no relation whatever to the point of pricking. In such an egg it cannot be pure chance which meridian becomes the plane of bilaterality, one must have a predisposition towards it, however slight, and the nature of this tendency will be described in the section on axial gradients. This ephemeral determination is completely overridden and obliterated by the powerful stimulus given by a sperm.

There is one more point to be considered in connexion with the bilateral symmetry of the egg, and that relates to the plane of the first furrow of cleavage. Now this first furrow often coincides with the plane of bilaterality, but it may equally well and does arise at any angle to that plane. This means that it is quite immaterial whether the first cleavage separates the right and left halves of the embryo, or the back and front, or oblique halves. At the same time, it becomes of interest to inquire into the causes which determine it.

As the sperm nucleus advances into the egg, the aster divides forming a spindle at right angles to the sperm path. If the path is quite straight from the point of entrance to the centre, then the spindle will be at right

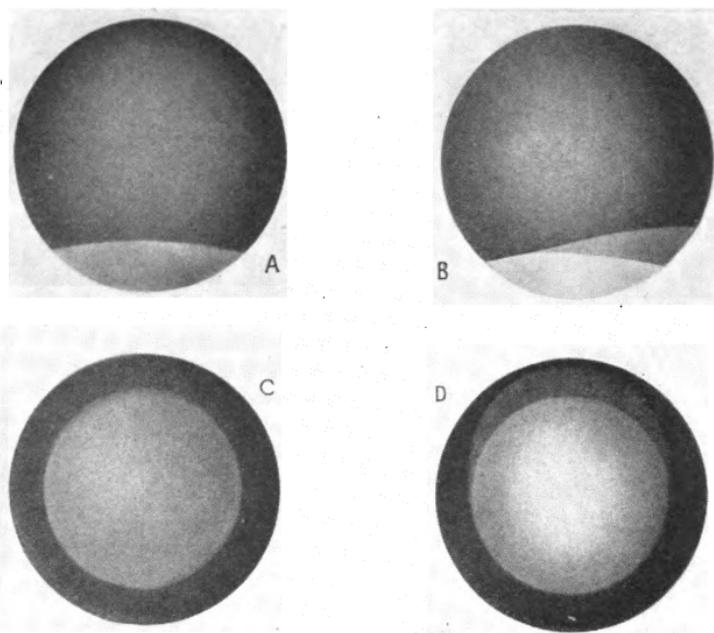


FIG. 11. The egg of the frog (*Rana temporaria*) before (A and c) and after fertilization (B and D) showing the dark animal and the light vegetative poles. The grey crescent appears after fertilization and marks the site of the dorsal lip of the blastopore and the dorsal side of the future embryo. A and B side views, C and D aspects of vegetative pole. (From Jenkinson.)

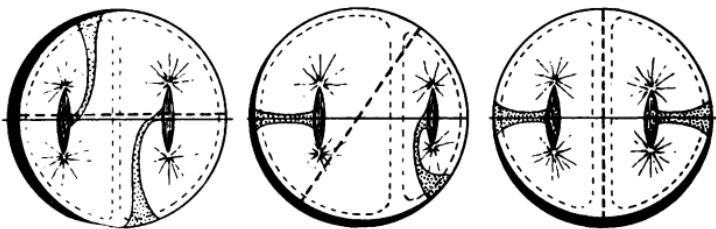


FIG. 12. Diagrams of frog's eggs into which two sperms have entered, projected on to the equatorial plane. The side opposite the grey crescent is indicated by the thick black line, and bisects the angle subtended at the centre by the entrance points of the two sperms, whatever their distance apart. The thick broken line is the plane of bilateral symmetry. (From Herlant.)

angles to the plane of bilaterality, and the first furrow will coincide with that plane. (28)

On the other hand it often happens that the path of the sperm is not straight. In this case the first furrow will occur at right angles to the later portion of the sperm's

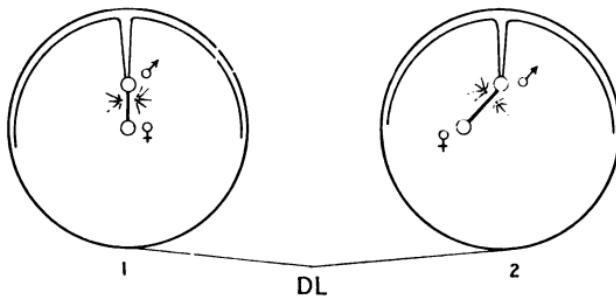


FIG. 13. Diagrams showing the relations between the plane of bilateral symmetry, the plane of the first furrow of cleavage, and the path of the sperm in the frog's egg DL; the dorsal lip of the blastopore and the point of entry of the sperm ( $\sigma^{\sigma}$ ) mark the plane of symmetry. The sperm asters lie in a plane at right angles to the copulation path of the sperm and to the cleavage furrow. When the entire path of the sperm is straight (1) the furrow and the plane of symmetry coincide. When the egg-nucleus ( $\Omega$ ) is eccentric and the copulation path of the sperm makes an angle with the penetration path (2), the planes do not coincide. (From Jenkinson.)

path, termed the copulation path. This may make any angle with the first portion or penetration path of the sperm, and from this it is easy to see that the plane of the first furrow may make any angle with the plane of bilaterality.

## IX

### NUCLEAR DIVISION DURING CLEAVAGE

As a result of cleavage the egg is split up into a number of blastomeres which will in time give rise to the different and differentiated regions of the embryo. It is important to know whether the first step in this differentiation is to be found in qualitatively unequal division of the nuclei of the different blastomeres and unequal distribution of nuclear material. If this could be proved to be the case, there would be a ready explanation of the origin of the differentiation of parts.

It has already been mentioned that the direction of the spindles can be controlled by producing a long axis of protoplasm in a cell. (24) If a fertilized frog's egg be made to undergo cleavage between horizontal glass plates, the first two furrows are as in the normal egg at right angles to one another and in the vertical plane, the third, instead of being horizontal, is vertical, and the fourth is horizontal instead of vertical. The result is a sixteen-celled morula in which four nuclei which normally belong to cells at the animal pole come to be situated at the vegetative pole, and four of the vegetative pole nuclei find themselves in animal pole cells. Nevertheless normal embryos are produced by these eggs, which would be inexplicable if nuclear division were qualitatively unequal. It does not matter which nucleus any cell contains.

Similar experiments with sea-urchin's eggs under pressure produce plates of sixteen cells all in one plane, and the normal distribution of nuclei is completely upset without interfering with normal development. (29)

In the same way, the egg of *Nereis*, which normally

produces twelve small (micromeres) and four large (macromeres) at the 16-cell stage, can by subjection to pressure be made to give rise to eight of each kind. (30) The small cells give rise to ectoderm and the large ones to endoderm. Normal development follows although four

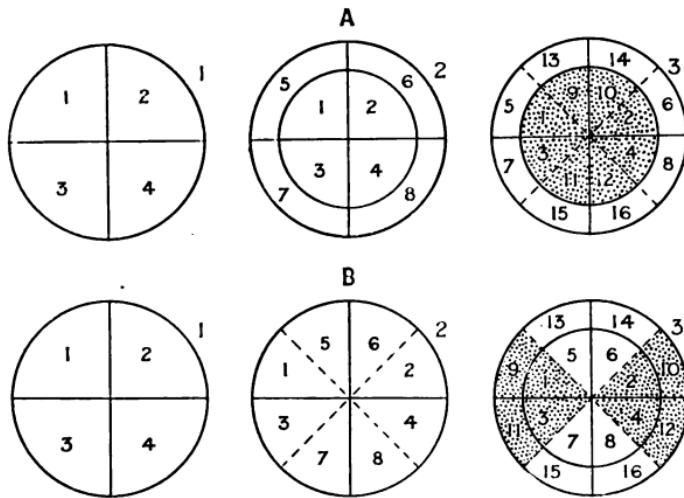


FIG. 14. Diagram of the cleavage of the frog's egg under normal conditions (A) and under pressure (B). The cells which normally form part of the animal pole are stippled. The nuclei belonging to cells 9, 10, 11, and 12 are normally in the animal pole hemisphere; 5, 6, 7, and 8 in the vegetative. Their positions are reversed under pressure. (From Hertwig.)

nuclei which would normally have been in ectodermal cells are situated in the endoderm.

There is, therefore, decisive proof that the nuclei of the blastomeres during cleavage are qualitatively identical, and that the cause of differentiation is not to be found in the division of the nucleus.

## X

### CYTOPLASMIC DIVISION DURING CLEAVAGE

If differentiation is not due to nuclear division, it is possible that a cause for it may be found in unequal division of the cytoplasm. This may be tested in various ways.

If the blastomeres of the egg or the sea-urchin are separated at the 2-cell stage, each nevertheless gives rise to a perfect larva of half the normal size. (31) A blastomere from the 4-cell stage ( $\frac{1}{4}$ ) will form a proper diminutive gastrula of quarter normal size. Blastomeres from the 8-, 16-, and 32-cell stages do not gastrulate very well; they do not gastrulate at all if they come from the animal pole, except a few  $\frac{1}{8}$ ; and a blastomere from the 64-cell stage will not produce a gastrula. But inability to gastrulate does not depend on lack of specific enteron-forming substance, because although a  $\frac{1}{16}$  blastomere from the animal pole will not gastrulate,  $\frac{4}{16}$  blastomeres from the animal pole will produce a perfect pluteus larva. The inability must therefore be due to insufficiency of material and too small size.

It is worth noticing that in these larvae, whose size compared with the normal is the fraction which the isolated blastomere represented in relation to the total number of blastomeres at the stage when it was isolated, the number of cells they contain is also proportional to this fraction ('germinal value'). The size of the cells themselves is therefore the same as in normal larvae, which from what has been already seen is to be expected, since the nuclei and the number of chromosomes which they contain are the same. An embryo from a blastomere of the 64-cell

stage will therefore have sixty-four times fewer cells than a normal embryo at the corresponding stage. It is therefore easy to see how insufficiency of material will hamper further development.

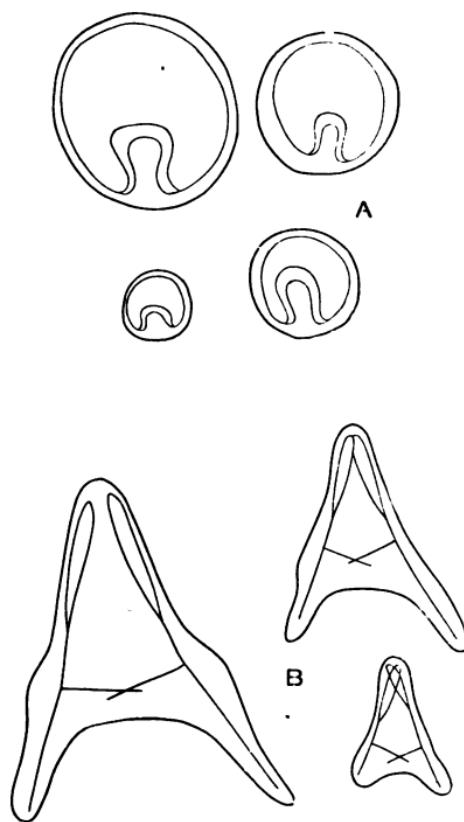


FIG. 15. Relative sizes of gastrulae (A) and plutei (B) derived from  $\frac{1}{8}$ ,  $\frac{1}{4}$ ,  $\frac{1}{2}$ , and  $\frac{1}{1}$  eggs of Echinus. (From Driesch.)

The blastomeres of the sea-urchin's eggs, then, have the same potency as the ovum, which is expressed by saying that they are totipotent at least up to the 4-cell stage. There can therefore be no unequal distribution of specific

cytoplasmic material in these forms. It is also to be noticed that the proportions of the parts in these embryos and larvae is correct, and that any given cell in one of these may occupy a position or be situated in an organ very different from what would have been the case if the blastomere had not been separated but continued to form part of the original egg. The embryos are 'equipotential systems' in which the destiny of any nucleus or blastomere is independent of cleavage and of the position it occupied before separation, but is determined by the position that it actually does occupy in the developing embryo.

Coelenterates may have blastomeres which are totipotent up to and including the 4-cell stage. (32) In some forms the blastomeres normally separate during development and reunite later, and the rearrangement cannot be always the same.

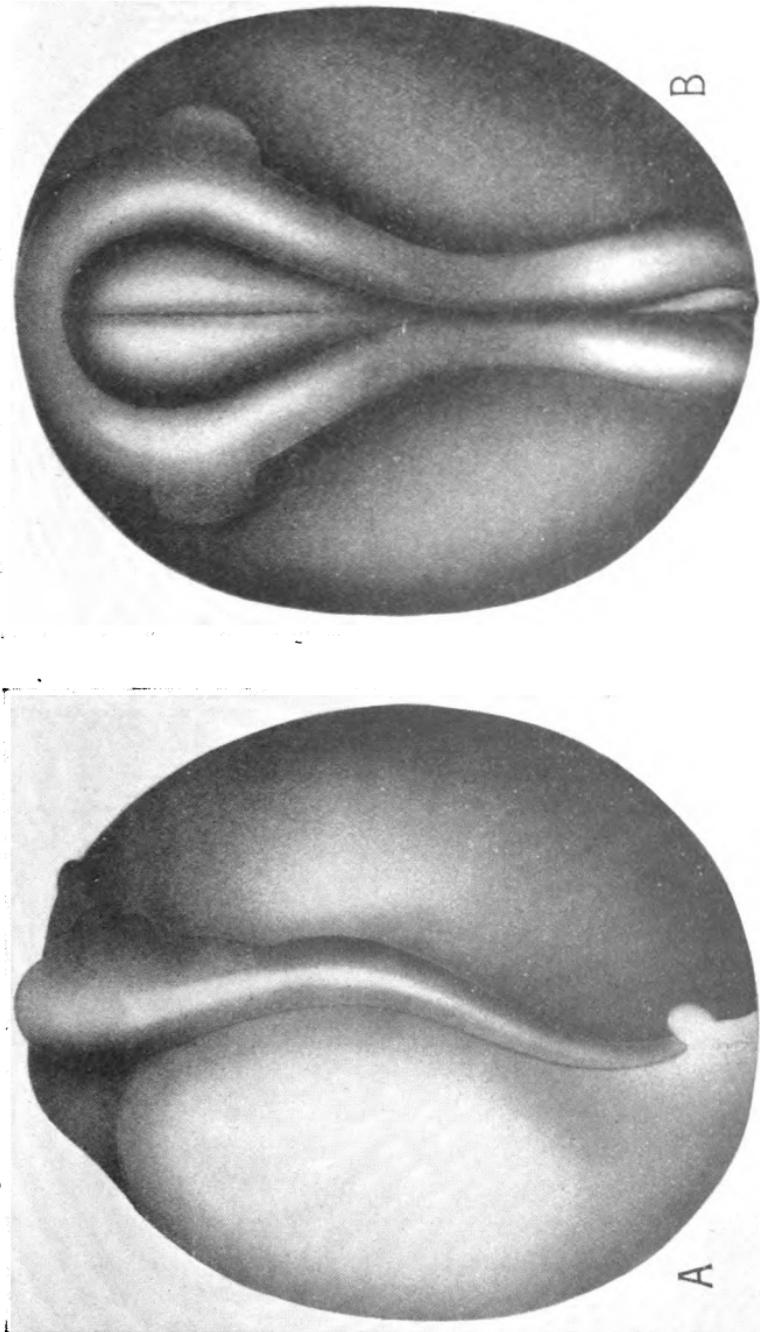
In Nemertines,  $\frac{1}{4}$  blastomeres are not quite totipotent, for the resulting Pilidium larvae lack a few structures, such as the apical organ and lappets, and the gut is solid (*Cerebratulus*). (33)

Amphioxus blastomeres of the 2-cell stage will produce normal embryos; those of lower germinal value, however, are impeded by lack of material. (34)

In Amphibia,  $\frac{1}{2}$  blastomeres can be totipotent provided that they contain some of the region (of the grey crescent) which will give rise to the dorsal lip of the blastopore. (35) If the first furrow does not lie in the plane of bilateral symmetry one blastomere will not contain any of the dorsal lip zone, and it will not develop. If on the other hand one blastomere at the 2-cell stage is killed instead of being removed, the other may develop into a half-embryo only. A whole embryo may be formed later by a kind of regeneration.

From these experiments on frogs it is obvious that one region, the dorsal lip of the blastopore, is necessary for the

FIG. 16. A half-embryo of a frog obtained by killing one of the blastomeres at the 2-cell stage (A), compared with a normal embryo (B) (*From Roux.*)



blastomere to develop, so that the blastomeres are not all necessarily totipotent. The fact that a different result is obtained according as to whether a blastomere is isolated, or allowed to remain in contact with its partners, shows that the destiny of the part is a function of its position in the whole. When the blastomere is isolated, it becomes a whole itself, at least in several forms.

The Ctenophora are a group in which the blastomeres are not totipotent, and a distribution of essential cytoplasmic substances takes place during cleavage. The normal Ctenophore has eight combs, but larvae from blastomeres isolated at the 2-cell stage have only four combs, those from the 4-cell stage only two, and from the 8-cell stage only one. (36) If the vegetative pole is removed from the egg the resulting larva has no combs and no sense organ. The Ctenophore egg therefore possesses something which is distributed among the cytoplasm of the blastomeres during cleavage. At the same time all these larvae have a complete gut and other organs, so that the isolated blastomeres have given rise to more than they would have if left in contact with the others, except for the combs.

In the mollusc *Ilyanassa* a mass of cytoplasm protrudes after fertilization and is eventually absorbed into one of the blastomeres. If this lobe is cut off, the larva resulting from this egg has no mesoderm. (37)

*Dentalium* also has a protruding lobe, removal of which produces a larva deficient in apical organ, and in the region behind the ring of cilia. (38)

Similarly in *Myzostoma* a lobe is present, absence of which prevents the formation of mesoderm. (39)

In Nematodes isolated blastomeres only produce those structures which arise from them in normal eggs (40), and in Ascidians necessary organ-forming substances are distributed to the blastomeres. (41)

As a result of these experiments it appears that in some groups the blastomeres are able to regulate their further development and produce a normal larva. In others the power of regulation is not so great and more or less imperfect larvae result. To the former class the term 'regulation-egg' is applied, to the latter, 'mosaic-egg', for their development resembles a mosaic in that the different

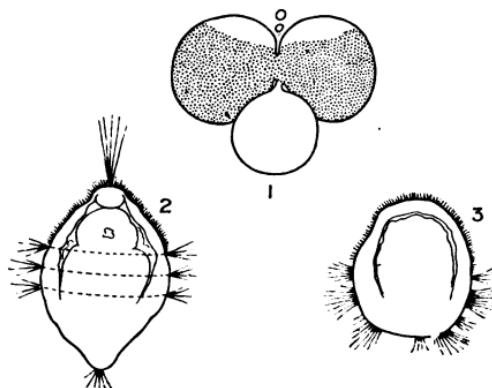


FIG. 17. 1. Early stage of cleavage of the egg of *Dentalium*, showing the two blastomeres and the 'polar lobe'. 2. Normal larva of *Dentalium*. 3. Larva without apical organ and with diminished hind region obtained after removal of the polar lobe. (From Wilson.)

regions develop independently of one another, and the loss of one piece out of the mosaic spoils the pattern.

There is, therefore, evidence that in some groups cytoplasmic raw materials are qualitatively sorted out to the different blastomeres, whereas in others all the blastomeres have equivalent cytoplasm. There are, however, all stages of transition between the perfectly regulative sea-urchin's egg and the definitely mosaic egg of Nematodes, and, in fact, an animal falls into one group or the other, according as to whether the loss of totipotence takes place late or soon, sometimes even before fertilization.

The cytoplasmic raw materials of mosaic eggs are organ-forming substances. Sometimes they are visible, as in the case of the Ascidian *Styela* where a yellow substance is necessary for the formation of muscle-fibres. (41) Others are invisible. On the other hand eggs are not wanting in which certain visible substances are present, and which in normal development are always distributed to certain cells. Yet when these eggs are centrifuged and the visible substance in question is completely disarranged, normal larvae are nevertheless produced. (42) The experiment of centrifuging shows that these substances are not localized organ-forming materials. On the other hand, in the case of *Styela*, centrifuging upsets the development.

## XI

### THE FACTORS OF DEVELOPMENT

A NUMBER of factors are transmitted from the parents to the developing egg. These are the internal factors.

It must be understood that these factors work in conjunction with others situated in the environment, and to which attention will be paid in the next section. At present, however, it is necessary to inquire whether all the internal factors operating during various stages of development were actually present in the fertilized egg.

The answer is no. If in the 2-cell stage of the frog's egg one blastomere be killed with a hot needle, the other will develop mosaically as a half. (43) On the other hand, if the dead blastomere were removed, the remaining one would become spherical, regulate itself, and develop as a whole. (24) Therefore one of the factors which in normal development ensures that a blastomere of the 2-cell stage will produce half the embryo, is the presence of the other blastomere. But the presence of this other blastomere is not a factor which exists as such in the egg.

Not only are there factors in the egg, then, but there are others which partake of the nature of mutual relationships, positions, and interactions of parts, factors which were not present in the egg, but are indirectly due to those in the egg. These are of increasingly great importance in the later stages of development. Although internal to the organism, they are often more accessible to influence by external conditions.

## XII

### EXTERNAL FACTORS AND THEIR EFFECT ON DEVELOPMENT

WHEN the internal factors of development in an organism are considered, the environment is taken as normal and constant. As they only exert their effects in conjunction with the external factors, however, the latter have an important bearing, and some at least of them must be considered by way of illustration.

#### *I. Gravity.*

In the Introduction it was shown how gravity does not determine the axis of the frog's egg, this being pre-determined in the ovary by factors such as the proximity of the blood-vessels. (44) Once determined, however, yolk is restricted to the vegetative pole, and, being heavier than the remainder of the contents of the egg, sinks. In this way gravity ensures the vertical position of the axis of the egg of *Rana fusca*.

Whatever be the relation of the first cleavage furrow to the plane of bilateral symmetry of the egg, it is important to note that it always passes through the vertical axis. It looks therefore as if gravity had something to do with the first cleavage.

An egg can be made to take up and maintain a position with its axis at any desired angle to the vertical. The jelly surrounding the egg sticks to it and prevents it rotating if only very little water is used. (45) It was found in these inverted eggs that the first cleavage was vertical, and that it bore no relation to the original egg-axis. This original egg-axis is externally visible since the vegetative

pole yolk cells are white and the animal pole cells are dark in colour. But although the surface of the egg can be maintained in an inverted position, the fluid interior cannot be displaced in this way, for most of it streams back into a position with the mass of heavy yolk at the bottom. (46) This internal streaming movement is largely invisible from the outside. As in the normal egg the stratification of yolk and protoplasm is horizontal, and the spindle being horizontal also necessarily causes the cleavage furrow to lie in a vertical plane.

The relation of the planes of the cleavage furrows to gravity is therefore indirect.

In these forcibly inverted or tilted eggs, the plane of bilateral symmetry is often that which includes both the original egg-axis and the new vertical axis; i. e. it is the 'streaming meridian', (28) along which the substances at the original poles of the egg move under the influence of gravity.

## *2. Temperature.*

Temperature is an important factor in development. As a rule, increase in temperature accelerates, and decrease retards the developmental processes, within a certain range of course. For the various processes there is therefore a temperature coefficient, and this is often different for different processes. It is possible to find a temperature at which some processes go on well while others are almost if not quite impeded, and by this means remarkable differential results can be obtained. If the blastoderm of a hen's egg is kept at a lower temperature than normal, growth and cell division will continue, but no primitive streak will form. In other words, growth is permitted but differentiation is inhibited at this temperature. (47)

In a similar way, a raised temperature in which sea-urchin eggs have been put to develop will allow divisions of the nucleus to take place, but will stop divisions of

the cytoplasm. The result is therefore multinuclear masses. (48)

In the development of any animal the rate of growth can be obtained by measuring the amount of the increase in volume or in weight in a given time. This rate is dependent

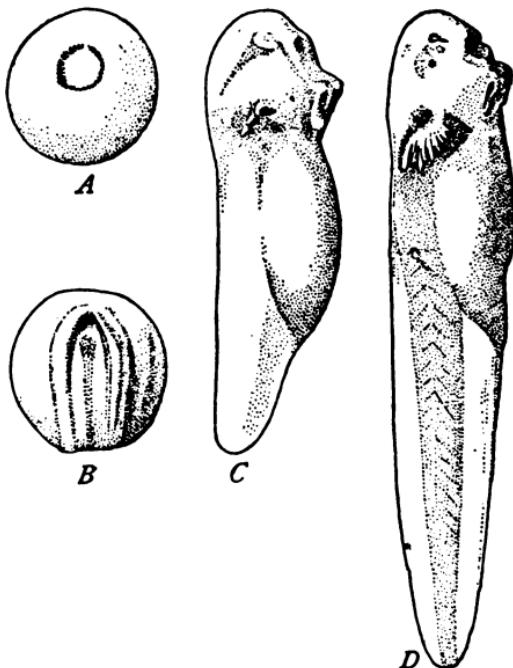


FIG. 18. Effect of temperature on the rate of development of the frog's egg. A two days old, B three days old; at 14.5°-15°C. C three days old, D four days old; at 20°C. (From Hertwig.)

on temperature to a large extent, and it has been found that in certain grasshoppers the increase in rate of growth is directly proportional to the rise in temperature. (49)

When different organs have different temperature coefficients, actual differences in proportions between the various organs can be obtained, which is of importance, especially in experiments on amphibian metamorphosis.

## 3. Electricity.

The electric current has been shown to have an effect in determining polarity in certain cases, and this is not

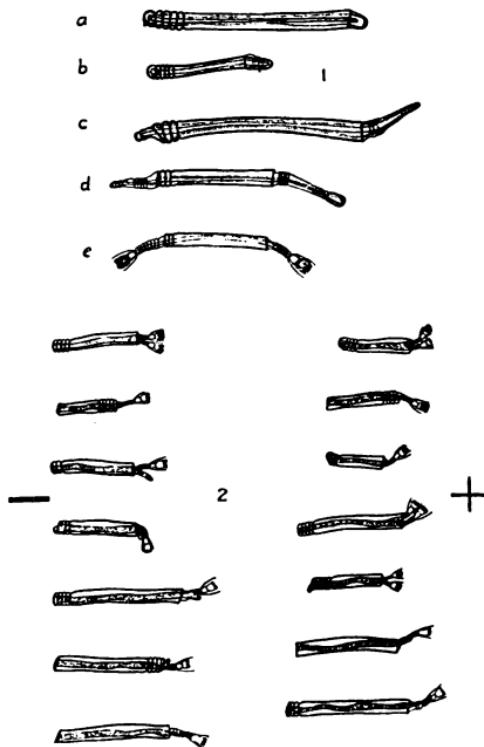


FIG. 19. 1. Normal regeneration of polyps in *Obelia*. The basal end of the piece is marked by the corrugations in the protective case. A polyp regenerates first at the apical end. a-e, progressive stages in the regeneration; at the stage a polyp is formed at the base also. 2. Regeneration of *Obelia* electric current. Regardless of polarity, polyps regenerate only at the nearest to the anode. (From Luna

surprising since one of the features of is a difference of electric potential along If internodes of the stalks of *Obelia* are

in various ways to electric currents, it is found that the current influences the regeneration of the hydranth, which appears at the end of the stalk nearest to the anode and not at the other. (50) Without the current, hydranths usually are regenerated from the originally distal end of the stalks; so that this experiment shows the polarizing effect of the current.

The electric current has a marked effect in determining the direction of outgrowth of nerve-fibres from cells artificially cultured *in vitro*; the description of this experiment and its results will be deferred until later.

#### 4. *Osmotic pressure.*

Variations in the concentrations of salts in media in which organisms are developing are of great importance, for the cell membrane is semi-permeable, and passage of water one way or the other causes swelling or shrinking. A good deal of water is absorbed during the later stages of development, and osmosis can be demonstrated by subjecting eggs to high (hypertonic) and low (hypotonic) concentrations of salts. In these cases, however, it is difficult to estimate the effects of the difference of concentration of the salts because the cell membrane of the egg itself probably changes its degree of permeability.

A striking though simple example of the action of osmotic forces is given in the behaviour of isolated collar cells of sponges. By various methods the collar cells from a sponge can be separated from the other cell elements. As soon as these cells come together, they arrange themselves in the form of a sphere with the collars and flagella directed outwards. In appearance these artificial spheres, which never exist as such in nature, are not unlike a colony of Volvox. Now although these cells were completely isolated, when banded together in the sphere they immediately form a closed semi-permeable membrane round it. (51)

If one of these spheres is subjected to hypotonic conditions it swells. Without going in any detail into the causes of osmotic pressure, the explanation of this swelling is briefly as follows:—There is a lower concentration of substances in the water outside the sphere than there is inside; the pressure exerted on the inner surface of the

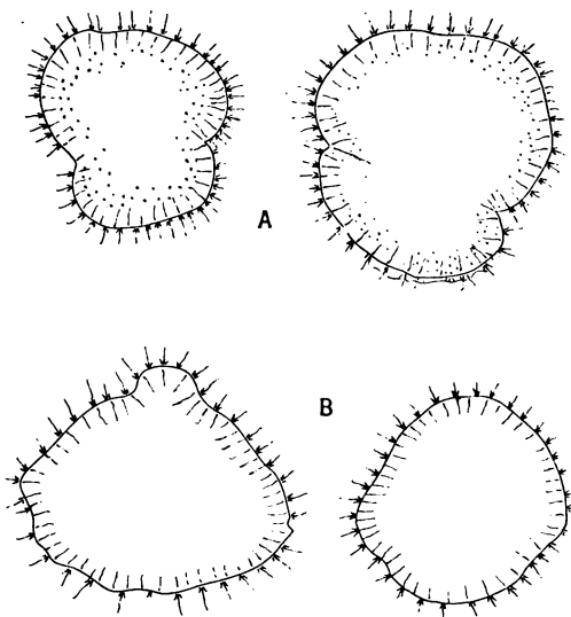


FIG. 20. Effect of osmotic pressure on spheres of collar cells of the sponge *Sycon*. A. Swelling in hypotonic sea-water. B. Shrinkage in hypertonic sea-water. (From de Beer.)

membrane and the number of bombardments of molecules on this inner surface is therefore greater than that on the outer; consequently the membrane is pushed outwards and the contained space expands and is filled with water which passes in through the membrane. The reverse process takes place when the sphere is placed in a hypertonic solution.

*5. Chemical composition of the medium.*

The action of external factors on development is very well shown by the effects which certain chemical substances have on the development of various organisms. For instance, the notochord in the frog arises from the topmost layer of cells of the roof of the primitive gut. In a solution of cane sugar, however, the notochord is formed from the whole thickness of the roof of the gut; and it is interesting to note that this is the normal method of its formation in lampreys and newts. (52)

Normally, a process of vacuolation of the cells of the notochord takes place, giving its characteristic appearance, and distinguishing it sharply from all other tissues. In a solution of urea, however, this vacuolation also affects the cells of the nerve-cord.

The nerve-cord in the frog arises as a groove which becomes closed over to form a hollow tube: but in potassium chloride solution it forms as a solid ingrowth (the normal method of its development in bony fish). Magnesium salts cause the neural folds of the frog to close over as in *Amphioxus*; lithium chloride prevents the folds from closing over at all. (52)

Magnesium chloride produces a remarkable effect on embryos of the fish *Fundulus*, for in this solution one median 'cyclopic' eye develops instead of the normal paired and separated two. (53) This effect is, however, not specific to magnesium chloride, for dilute solutions of alcohol, chloroform, and ether will also produce it. (88)

Potassium salts added to sea-water prevent sea-urchin larvae from developing the skeleton and arms typical of the pluteus stage. In lithium salts solutions the enteron fails to invaginate at all, resulting in hour-glass shaped larvae termed 'exogastrulae'. (54) The original outer wall of the larvae may be smaller than that of the evaginated gut, and this disparity is increased with higher

concentrations of lithium until the larva is practically all gut, with a tiny knob of ciliated cells representing the original gastrula wall. It is important to notice the

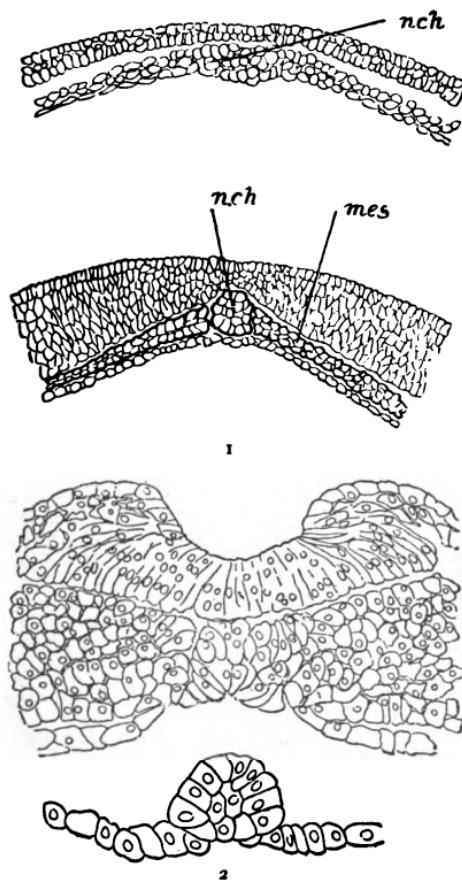


FIG. 21. 1. Normal method of formation of the notochord in the frog, from the cells of the upper layer of the roof of the gut. *nch*, notochord; *mes*, mesoderm. 2. Formation of the notochord from the whole thickness of the roof, in a frog developing in a solution of cane sugar. (From Jenkinson.)

differential action of the salts on ectodermal and endodermal material, suppressing the latter at the expense of the former.

These effects of various substances when artificially introduced into the environment lead to the conclusion that 'normal' development cannot take place without a 'normal environment'. This conclusion is entirely confirmed by experiments on sea-water, from which the various constituents were omitted one by one. The effects of these 'artificial sea-waters' were then tested on sea-urchin development.

In the absence of the constituent to be tested, the concentration of the others was proportionately raised so that

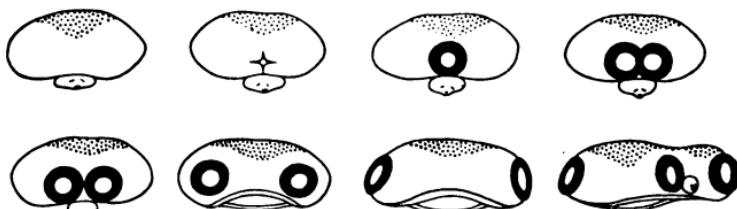


FIG. 22. Effect of magnesium chloride on the development of the eyes of the fish *Fundulus*. Views of embryos from in front showing various degrees of fusion of the two eyes. (From Stockard.)

the solution should be isotonic with sea-water, and thus eliminate any possible effects due to osmotic phenomena.

The sulph-ion is found to be necessary for the proper development of the gut. Chlorine is essential, or no cleavage will take place; absence of potassium brings about serious disturbances and early death. Perhaps the most remarkable of all is the necessity of calcium, for without it the blastomeres are unable to stick together, but become separated.

These experiments show not only that the normal processes of development are related to the presence of certain chemical substances, but also that these substances must be present in that concentration which occurs in nature and which is regarded as normal.

The external factors are therefore of great impor-

tance. A few inorganic ones have been considered in this chapter, which will be concluded with a consideration of the effects produced by an abnormal organic environment.

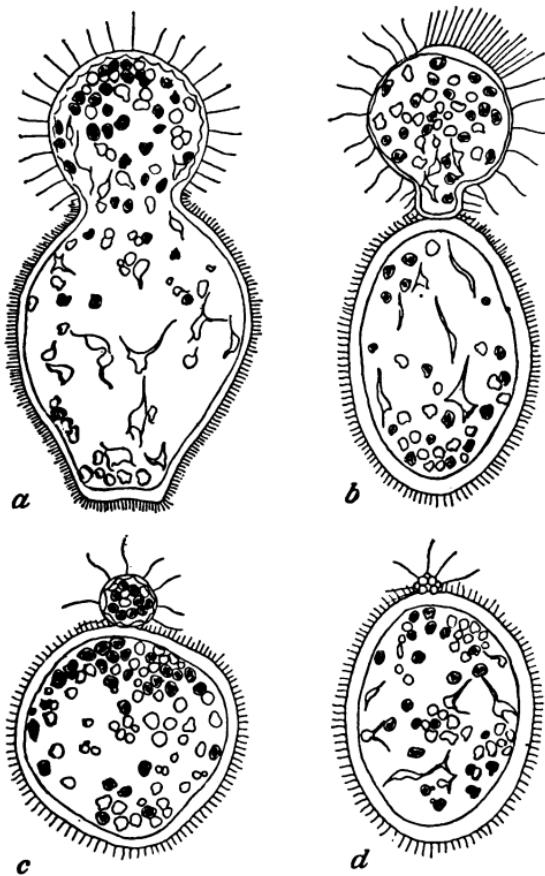


FIG. 23. Sea-urchin larvae developed in solutions containing lithium. The enteron fails to invaginate, giving rise to an 'exogastrula'. The ectodermal gastrula wall diminishes with increasing concentration of lithium (*a-d*). (From Herbst.)

If a fertilized frog's egg is removed from the water in which it is developing and planted inside the body cavity of another frog, curious results are obtained which vary with

the exact spot of the implantation and the age of the egg.(55) In no case is there normal development from such an egg. It may try and form a very imperfect embryo, or the blastomeres may separate into a number of isolated groups, which remain undifferentiated and are said actually to invade the tissues of the hostlike tumours. The altered environment has completely upset the regulative powers of the egg, and a mode of life which may be described as parasitic causes it to behave in a manner similar to that characteristic of some parasites.

## XIII AXIAL GRADIENTS

THE rate of 'metabolism' of the tissues is not the same all over a developing organism. There is a region of high rate of protoplasmic activity, which coincides with the anterior end of the animal, the animal pole of the egg, and the growing point of the plant. From this region the rate decreases progressively towards the posterior end so that there is a gradient. Further, this gradient coincides with the axis of the animal, so that it may be called an axial gradient. (56)

Gradients can be demonstrated experimentally by several methods.

Potassium permanganate is a substance which the oxidizing activities of protoplasm reduce, with the formation of a brown coloration due to manganese dioxide being deposited. It is found that there is a gradation of colour along the axis of organisms which reveals the state of activity of the oxidizing processes in the various regions, when placed in dilute solutions of potassium permanganate.

The rate of oxidation can be measured by the amount of carbon dioxide given off, and this can be estimated

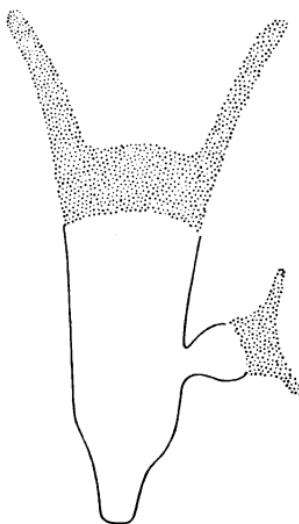


FIG. 24. Demonstration of an axial gradient in *Hydra* by means of ultra-violet light. The apical regions (hypostome and tentacles) are the first to be affected. (From Hinrichs.)

with accuracy by the Tashiro biometer, the principle of which is the observation of the formation of a precipitate in a drop of a solution of barium hydroxide. (57) One ten-millionth of a gramme of carbon dioxide can be detected

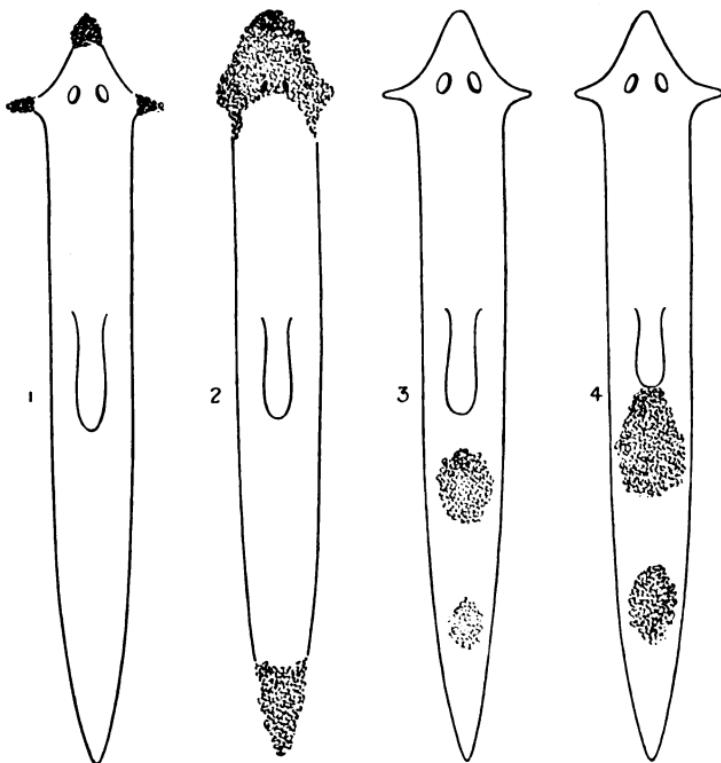


FIG. 25. Demonstration of an axial gradient in Planaria. In this animal there is a high rate at the head and also at the hind end in connexion with the zone which will give rise to other zooids. These are the first regions to be affected by the direct susceptibility method (1 and 2). By the indirect susceptibility (acclimatization) method (3 and 4) these regions are the last to be affected. (From Child.)

by this method, and it also reveals an axial gradient of 'metabolic' rate in organisms.

Protoplasmic activities also involve electric phenomena, and a gradient of electric potential has been demonstrated

along the axis of organisms. (58) The regions of high rate are electro-negative to the others in the external circuit.

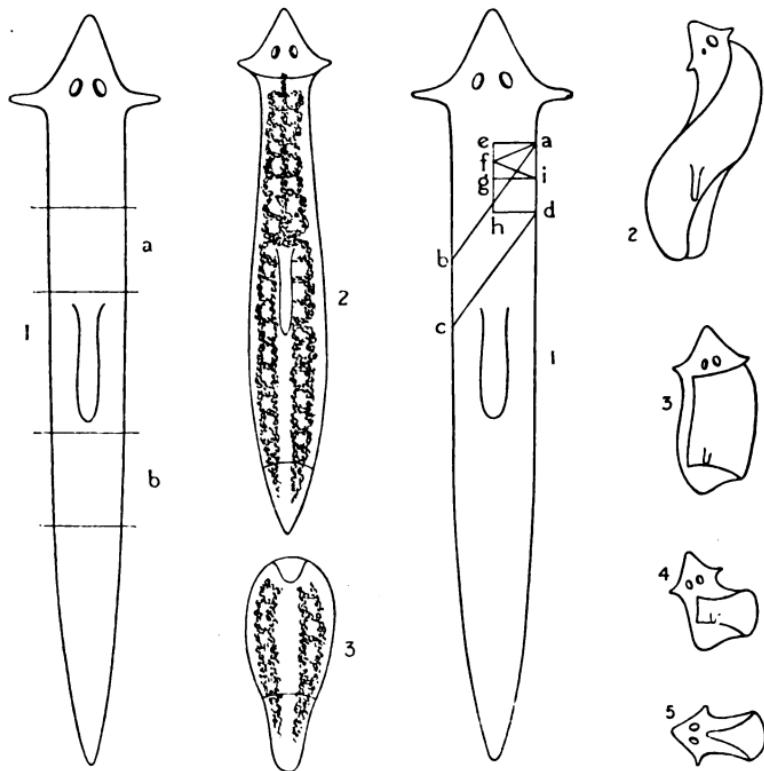
The susceptibility of different regions to poisons reveals the existence of axial gradients; the degree of the susceptibility depends on the intensity of protoplasmic activity in a particular part. (56) In high concentrations of poisons (various reagents can be used, such as cyanides, alcohol, acids, for the effect is not specific to them but to the 'metabolic' processes, as is well shown by the fact that ultra-violet light produces the same effects (169)) the region of highest rate succumbs first, followed by the others in succession down the gradient. Thus the animal pole of the egg, the hydranth of *Obelia*, the head of *Planaria* are affected first.

To very dilute solutions of poisons animals have the power of acclimatizing themselves, but in this case the region of highest rate has the largest power of acclimatization. By this method, therefore, the results are the opposite of those obtained by the previous method, and the regions of highest rate are affected last.

The next step is to establish a relation between the demonstrated axial gradient in an organism, and the arrangement and position of its various structures. In the trochosphere larva of a worm the head end starts by being the region of highest rate. Subjection to poisons at this stage produces larvae with abnormally small heads. Later on, the region of the blastopore, where the most active cell division is going on, becomes that of high rate, and exposure at this stage produces worms with abnormally large heads. There is, therefore, experimental evidence that interference with the gradient alters the structure of the organism. (175)

As well as a major axis extending along the middle line from head to tail there are minor axes extending from the middle line to the side. If a piece is cut out from the

body of a Planarian, such that it contains a length of the major axis, this will become the major axis of the



**FIG. 26.** Head regeneration in Planaria. 1. Worm showing the regions *a* and *b*. 2. Regenerated piece (from *a*) with head and pharynx. 3. Regenerated piece (from *b*) without head or pharynx. (From Child.)

**FIG. 27.** 1. Planaria showing regions from which pieces are cut. 2. Piece *abcd* regenerated. 3. Piece *aehd* regenerated. 4. Piece *aegi* regenerated. 5. Piece *afi* regenerated. Notice that the new head forms from the region of highest rate on the gradient of activity which runs down the middle line of the worm from head to tail. (From Child.)

organism regenerated from this piece. On the other hand, if the piece is wedge-shaped, with the apex of the wedge touching the major axis, then in that piece the apex

will have a higher metabolic rate than every other part, and it will regenerate a head.

Transversely cut isolated pieces from the posterior portion of a Planarian regenerate a head if the rate of the anterior cut edge of the piece is high enough, relatively to the rest of the piece. If not, a tail is regenerated instead. The frequency of head formation can be controlled experimentally by altering the protoplasmic activity of the piece by poisons. For a head to develop there must be a sufficient 'potential difference' of rate between the anterior cut surface and the rest of the piece. The stimulus of cutting raises protoplasmic activities. Pieces cut from the hinder portion of the worm *Lumbricus* so as to include the hind end will regenerate a head fairly often. On the other hand, if these same pieces are made smaller by cutting off the hind end of the worm, the frequency with which heads regenerate is much lower. This is because the gradient in the smaller pieces is not 'steep' enough, and the rate of the front cut edges is not high enough relatively to that of the rest of the pieces for a head to be formed. (178) The quality of the structure which regenerates is therefore determined by the metabolic rate of the tissues which are regenerating. One must therefore look to quantitative differences in intensity of activity to explain the differentiation of qualitatively different regions of an organism in development.

In the egg (that of the frog, for example) there is an axial gradient due to some previous external directional stimulus in the ovary, such as proximity of blood-vessels. As a result of this gradient, yolk which is easily oxidizable, is confined to the region of lowest rate, and marks the vegetative pole. The opposite is, of course, the animal pole.

Apart from the fact that the animal pole will produce the head, there is as yet no determination or differentia-

tion of region-forming substances, since the embryo is ready to form in any direction.

Normally the sperm arrives and fixes the 'Greenwich meridian' which becomes the plane of bilateral symmetry, and in which lies the grey crescent which marks the position of the future dorsal lip of the blastopore. This is another region of high rate, and it sets up a gradient. There is

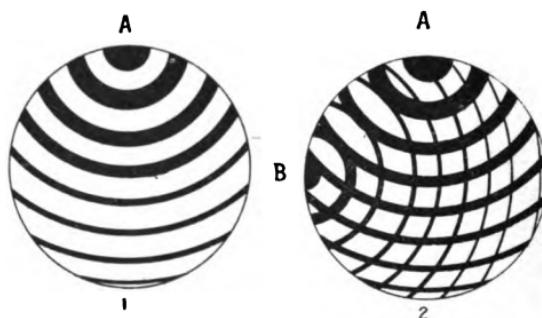


FIG. 28. Diagrammatic representations of axial gradients in a spherical egg. 1. A single gradient with highest rate at A; comparable to the radially symmetrical frog's egg with its apico-basal axis. 2. Two gradients with highest rates at A and B respectively; comparable to the frog's egg with an animal pole A, and a dorsal lip of the blastopore B. (From Child.)

also a gradient from the surface to the interior. There are now three gradients in the egg, and any spot can be given a value in terms of co-ordinates with reference to these gradients. The egg is now 'set', and the rate of activity at any particular spot will determine what develops there. In the case of the egg activated by a prick, any region which has a rate higher than the others presumably determines the 'plane of bilateral symmetry'.

The different organs are determined to occupy certain definite levels with regard to the axial gradients. For example, the lateral line is a system of sense organs in fish and amphibia which grows back from the region of the

neck on each side, down the body to the tail. Now it is possible to cut two embryos across transversely and fit the head end of one on to the tail end of the other and vice versa. If this is done with the dark head end of *Rana sylvatica* and the light coloured tail end of *Rana palustris*, the dark lateral line can be observed to grow back along the sides of the light coloured trunk at a definite level on the side. (59)

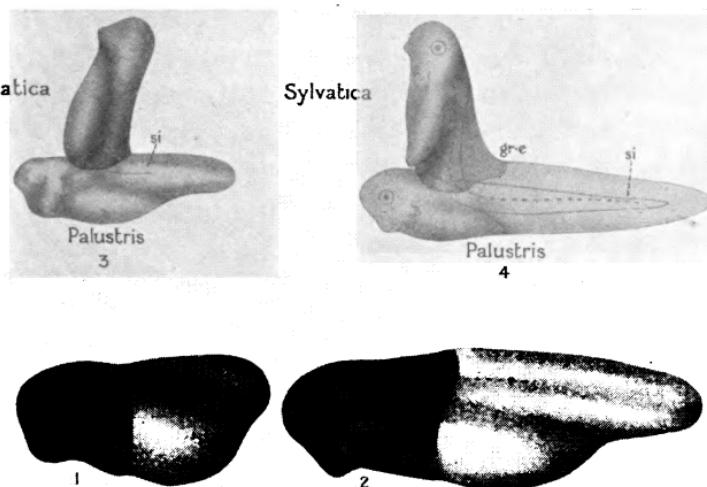


FIG. 29. 1. The anterior region of an embryo of *Rana sylvatica* (dark) grafted on to the hind region of an embryo of *Rana palustris* (light). 2. The lateral line grows back from the dark anterior portion. 3 and 4. The anterior half of an embryo of *Rana sylvatica* grafted into the back of an embryo of *Rana palustris*. The lateral line grows down and back at the proper level in the latter. *si*, lateral line; *gr-e*, limit of the *sylvatica* tissue. (From Harrison.)

The fact that the lateral line must occupy one particular level and no other is still better shown by a slight variation of the last experiment. If the front half of a dark embryo is planted in the middle of the back of a light coloured one with its axis at right angles to that of the latter, the lateral line will grow down from the cut edge of the dark implant on to the side of the other embryo, and continue growing

ventrally downwards until it reaches the proper level on this embryo. It then turns back at right angles and grows back along this level.

There will be occasion to refer to axial gradients and the levels at which the different organs arise in the chapter on Regulation. Meanwhile it may be noticed that the existence of an axial gradient explains certain phenomena in normal development, such as the manner in which certain organs appear before others. For instance, in vertebrates, such as the chick, differentiation progresses from the front backwards along the mid dorsal line, resulting in the formation of nerve-cord, somites, and notochord; and from the middle line outwards. In other words, the major and minor axes of gradients of metabolic rate are reflected in gradients of development and differentiation.

Similarly, in invertebrates, such as Planarians, the antero-posterior axis is well marked in development, as is also the ventro-dorsal. In most higher invertebrates the major axis runs along the mid ventral line, in vertebrates the mid dorsal; agreeing in fact with the positions of the nerve-cords in the two groups respectively.

As to what the activity which decreases along the gradient actually is, and how it produces its effect, it is difficult to say. It appears to be a transmission of excitation from the region of highest metabolic rate perhaps comparable to the transmission of a nervous impulse.

Further consideration of axial gradients must be relegated to the chapter of Regulation.

## XIV

### PLASTICITY AND CHEMO-DIFFERENTIATION

IN various animals it has been shown that blastomeres may be totipotent. This property may persist into later stages (such as the blastula and early gastrula) and show itself by the capacity of any particular piece of tissue to give rise to structures which it would in the ordinary course of events never have formed. This indetermination is called plasticity.

In amphibian embryos before gastrulation, a piece of epidermis which would normally have been folded in to form the nerve-cord can be exchanged for a piece of ordinary epidermis, with the result that 'presumptive' nerve-cord will become ordinary skin, and presumptive skin will become nerve-cord. (60) In other words, the fate of these regions had not been irrevocably determined at the time of transplantation; they were still plastic. The experiment can be made very demonstrative by exchanging portions of the dark tissue of *Triton taeniatus* and the lighter coloured tissue of *Triton cristatus*. By this means the transplanted portions can be recognized for a long time.

If, however, the exchange is made after gastrulation has been completed, the pieces are no longer able to take one another's places. Although no differentiation is visible, it is obvious that their fate has been determined. This loss



FIG. 30. 1. A dark embryo of *Triton taeniatus* with a small portion of epidermis tissue of *Triton cristatus* (light) grafted in. 2. The graft differentiates according to its position and is recognizable between x x forming part of the neural tube. (From Spemann.)

of plasticity must rest on chemical alterations of the tissue, so that the term 'chemo-differentiation' has been applied to this invisible determination. (61)

It has been shown that before the nerve-cord has been

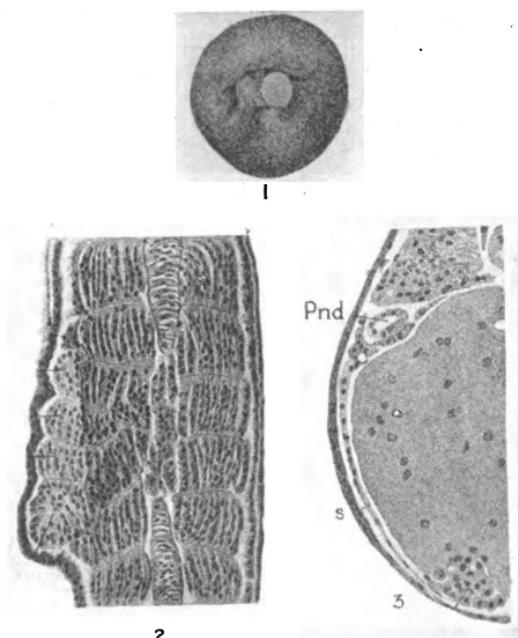


FIG. 31. 1. A graft of 'presumptive' epidermal tissue (light) implanted beneath the dorsal lip of the blastopore of a developing embryo of Triton. 2. The graft has contributed to the formation of the mesodermal somites (recognizable by lighter colour). 3. The graft has contributed to the formation of the pro-nephric duct (*Pnd*) and the lateral plate mesoderm (*s*). (From Mangold.)

formed definite regions are predetermined to produce the eyeballs. Not only that, but the different portions of the eyeball, i.e. stalk, retina, and pigment layers, are invisibly determined. (160) This determination is not yet a spatial preformation in the sense that the exact shape of the predetermined organs is mapped out. The regions are

mapped out as centres, and from these points the determination becomes more and more indefinite towards the edge of the regions.

In the plastic stage tissues can be exchanged even with others which would normally belong to different germ

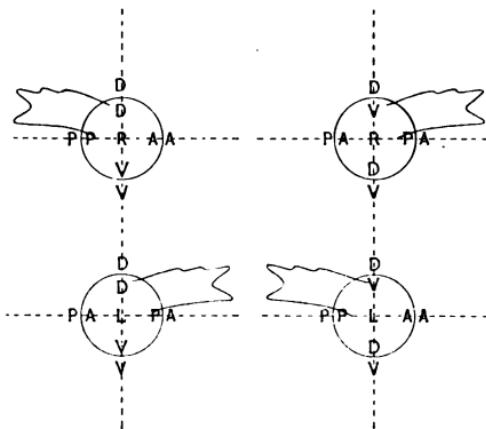


FIG. 32. Polarity of limb buds in newts. The axes of the embryo are shown by the letters A and P (anterior and posterior), D and V (dorsal and ventral), outside the circle. The polarity of the limb bud (represented by the circle) is shown by the letters within the circle. If the antero-posterior axis of the bud is reversed, as by planting a left bud the right way up on the right side, the limb points forwards instead of backwards. On the other hand, the dorso-ventral axis of the bud can be reversed without affecting the normality of the limb provided that the orientation of the antero-posterior axis is correct. R, right bud; L, left bud. (From Harrison.)

layers. A piece of presumptive ectoderm (i.e. tissue which would normally become ectoderm) can be planted just beneath the dorsal lip of the blastopore, and gets carried in with the invagination in the process of gastrulation. Later on, this piece of tissue can be recognized as forming part of either notochord, centrum of vertebra, pronephric tubule, myotome, lateral plate musculature, gut, gut wall or body wall, according to the position in which it finds

itself. Similarly pieces of presumptive mesoderm or endoderm can give rise to epidermis. (62)

These experiments show that tissues normally destined for different germ layers can be exchanged before chemo-differentiation, which only sets in later.

When disks of the outer wall of the body, representing the buds of the future limbs, are transplanted at a certain stage, it is found that the original anterior edge of the bud always produces the preaxial part of the limb. So a limb bud of the left side, planted the right way up on the right side of the embryo, will develop into a limb with the elbow pointing forwards instead of backwards, if it is a forelimb. On the other hand, if the disk is rotated so that the original dorsal edge of the bud is ventral, the original anterior edge will be anterior again, and a normal limb develops. Therefore the dorso-ventral axis can be inverted, but not so the antero-posterior axis, without producing derangements. (63) The medio-lateral axis can also be inverted without affecting the result. (168) This means that the dorso-ventral and medio-lateral axes are still plastic, while the antero-posterior axis is predetermined. This has an interesting bearing on axial gradients, for the major antero-posterior gradient is stronger than the secondary ones; so that it is to be expected that the former axis of the limb bud would be polarized before the latter.

It may be mentioned with regard to the organ-forming substances considered in a previous section as occurring in *Dentalium*, *Styela*, &c., that they are merely cases of precocious chemo-differentiation, without any visible spatial preformation.

## XV

### VISIBLE (EARLY) DIFFERENTIATION

As the dorsal lip of the blastopore travels backwards from the equator of the blastula to the vegetative pole, the axial structures of the back of the organism, viz. nerve-cord, notochord, and somites, appear in the same meridian as the dorsal lip of the blastopore. If, before these axial structures arise, the upper hemisphere of the blastula be cut off, rotated through 90°, and stuck on to the lower hemisphere again, the axial structures form in the meridian of the dorsal lip of the blastopore (which is in the lower hemisphere), and not in the original position on the rotated hemisphere. In other words, the dorsal lip determines the position of the axial structures. (60)

But it does more than that, for if the dorsal lip of the blastopore of one embryo be grafted into the flank of another, it will induce the formation there of axial organs which would otherwise never have arisen. (64) These may consist of nerve-cord and brain, ear vesicles, notochord, somites, pronephric tubules and sometimes eyes, and they are formed partly from the actual tissues of the host embryo. The axial structures are therefore dependent on the dorsal lip of the blastopore in their differentiation, and since they represent the main part of the embryo, the dorsal lip which 'organizes' them has been called the 'organizer'. (65)

More remarkable still is the fact that the rudiments of an embryo can be formed in *Triton* by organizers of other species, such as *Amblystoma* and *Bombinator*, (118) belonging to other genera, families, or even sub-classes. There is, therefore, something in the region of the dorsal lip which exerts an extraordinary influence on the neigh-

bouring tissues. This influence is not a peculiar property of the substance of the tissue which composes the dorsal lip of the blastopore, because strange tissue can be grafted into it, and can then be used as an organizer. It is, so to speak, 'infected'. The determination of the organizer therefore forcibly suggests the effect of gradients established with regard to the form of the whole organism, i. e. the relations of the whole egg, the egg-axis, and the point of

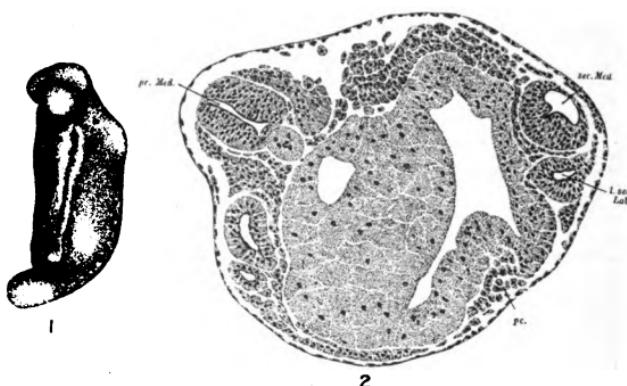


FIG. 33. 1. Secondary embryo produced on the flank of a normal embryo of Triton by the implantation of an organizer. 2. Transverse section through 1. *pr. Med.* neural tube of main embryo. *sec. Med.* neural tube, *l. sec. Lab.* left auditory vesicle, of the secondary embryo. *pc.* pericardium. (From Mangold and Spemann.)

entrance of the sperm, and regardless of the actual substance at any place.

The organizer requires contact for the spread of its influence. If a cut is made, no differentiation takes place on the other side of the cut. (66) At an early stage the epidermis close to the organizer is determined to produce the nerve-cord, while farther away it is still indifferent. The spread of the influence therefore takes time. (156) From the importance of the effects of the organizer it is not surprising that some of the grey crescent must be

present in one of the 2-cell stage isolated blastomeres if an embryo is to be produced. The gastrula of the newt can also be divided by tying a hair round it and pulling it tighter, but only that half which contains the organizer will develop. The other half remains a mere ball of cells. (157)

Once determined, the future chemical and histological

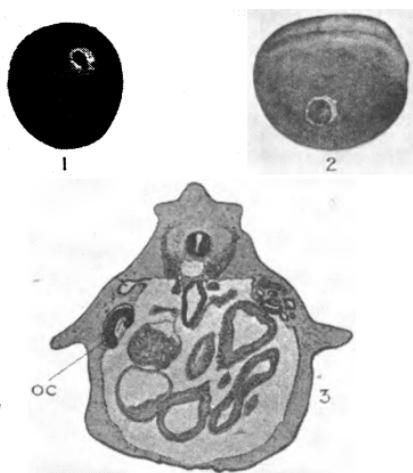


FIG. 34. 1. The rudiment of the eye-ball is removed from an embryo, and, 2, planted in the flank of another. 3. Self-differentiation of the eye-ball (oc) in the flank of an embryo. (From Spemann.)

differentiation of the axial structures goes on regardless of the surroundings. If a piece of the mid dorsal region of a newt embryo be cut out, rotated through  $180^\circ$ , and planted back again, it will continue differentiating in the reversed position, and the relations of the parts which it forms will be reversed with regard to the rest of the organism. So the eye-cups, instead of being in front, will develop far back. The behaviour of the brain and eye-cups in this case is described by saying that they are self-differentiating, which means that the necessary factors

for further differentiation are contained inside the piece of tissue in question. Now the piece of tissue which was rotated did not include the ventral portion of the gut, or the heart. Nevertheless these latter structures, which are normally asymmetrical one way, now develop with the opposite asymmetry. The liver is on the left instead of the right, the stomach is on the right instead of the left, the duodenum on the left instead of the right, and the heart is also inverted. The heart and viscera are the mirror images of normal organs, i. e. the embryos show *situs inversus viscerum et cordis*. These organs, therefore, show dependent differentiation with regard to the axial structures, just as the latter did with regard to the organizer. All that this means is that in dependent differentiation all the factors are not present in the piece in question, but some are in neighbouring structures. With regard to the viscera, therefore, the axial structures are organizers of the second degree. (67)

The eye-cup is self-differentiating in amphibia, as is strikingly shown by the fact that it can be transplanted, even to very incongruous situations such as the body wall on the ventral side of the abdomen, and, nevertheless, it differentiates into a typical optic cup. (68)

The lens of the eye, however, is different. In normal development it arises from the ectoderm covering over the eye-cup, that is to say, it has a separate origin from the latter. Now in most species of amphibia the lens's differentiation is dependent on the presence of the eye-cup. So in the case of the eye-cup removed from its normal position and planted in the body wall of the trunk, no lens develops in the normal position, whereas a lens does appear from the ectoderm covering the eye-cup in the trunk. (69) On the other hand, if a piece of 'presumptive' lens epithelium is exchanged with a piece of ordinary epithelium, a lens is formed opposite the eye-cup from the

strange epithelium, and no lens arises from the piece transplanted elsewhere. It is possible to demonstrate conclusively, therefore, that in certain amphibia (*Rana fusca*, *temporaria*, *palustris*, and *sylvatica*, *Hyla arborea* (161), *Bufo vulgaris* (162), *Ambystoma punctatum* (163)) the lens arises by dependent differentiation.

The influence of the presence of the eye-cup is necessary for its differentiation, and, further, it must not be too far distant. If tadpoles develop in certain solutions ( $\text{NaCl}$ ,  $\text{NaBr}$ , or  $\text{NaNO}_3$ ) the eye-cups remain deep beneath the surface of the skin, and in these cases no lens is formed. (52)

On the other hand, in other forms such as *Rana esculenta* (*Salmo* and *Fundulus* also) the lens is self-differentiating. It develops whether the eye-cup is present or not, and the eye-cup is powerless to induce the formation of a lens from strange epithelium of this species, although it can do so from strange epithelium of a species like *Bufo vulgaris*. (166, 70) *Bombinator* occupies an intermediate position, for on removal of the optic cup a small lens arises by self-differentiation, and a lens can be formed from any piece of epidermis of the head, but not of the trunk. (68) It is at first sight perplexing that such a structure as the lens of the eye should develop by different means in two such closely related species as *Rana fusca* and *Rana esculenta*. Too much importance must, however, not be given to the difference between self- and dependent-differentiation. The method of analysis is still crude, and all that the experimental evidence for self-differentiation allows one to say is that in a certain organ, *at a given stage*, no relation can be found with other organs, and the necessary factors for differentiation are self-contained. It may well be that there is a time factor here and that at earlier stages the lens of *Rana esculenta* is dependent on something else for its differentiation, just as

the axial structures are in the first place dependent on the dorsal lip of the blastopore, and later on become self-differentiating.

As will have already appeared, the method of transplantation and grafting is valuable in giving experimental evidence as to the manner of development of the various organs. A few more cases will be considered so as to complete the picture.

The differentiation of the cornea is dependent on the eye-cup, for it does not become transparent in the absence of the latter. (71)

The differentiation of the ear in amphibia is of interest. It arises normally from a vesicle formed by the sinking in of the skin, just to the side of the brain. This vesicle gives rise to the membranous labyrinth which becomes surrounded and protected by the cartilaginous auditory capsule.

If the vesicle is removed, no new vesicle forms: if replaced upside down it develops with the ductus endolymphaticus pointing down instead of up: (72) if the vesicle of the left side is grafted into the right side, the lagena points forwards instead of back. (73) These experiments show that the membranous labyrinth is self-differentiating.

Now when the vesicle is extirpated, no cartilaginous auditory capsule is developed. The latter is therefore 'dependent-differentiating', which is supported by the fact that a vesicle implanted between the normal position of the ear and the eye becomes surrounded by a cartilaginous capsule. (74) Comparable experiments show that the rudiments of liver, pancreas, gut-wall, and heart are self-differentiating in the toad *Bombinator*. (167)

Limb buds are self-differentiating in amphibia, and can be transplanted to different regions of the body. (75) Even in absurd situations such as the side of the head, they differentiate normally, and may become innervated by

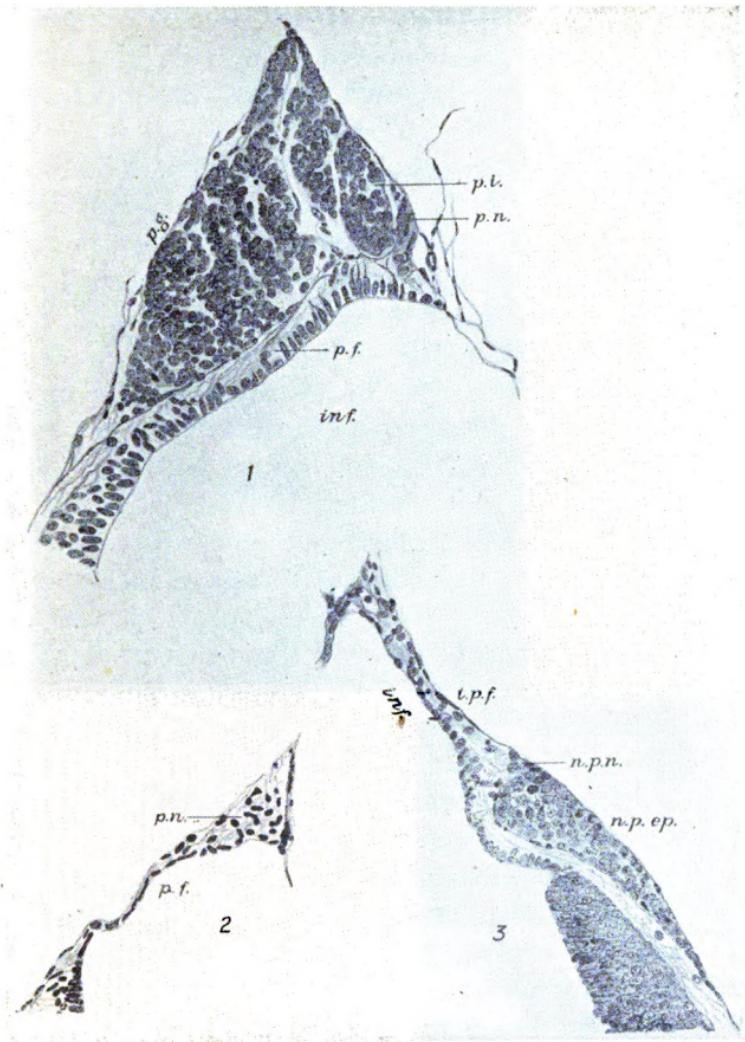


FIG. 35. 1. Longitudinal section through the normal pituitary body of a frog tadpole. 2. Pituitary of a tadpole from which the hypophysis was removed, showing the undeveloped state of the infundibular portion. 3. Abnormal position of a portion of the hypophysial constituent of the pituitary body, showing dependent differentiation of the infundibular tissues in its vicinity. *p.a.* pars anterior, *p.f.* infundibular floor, *p.i.* pars intermedia, *p.n.* pars nervosa, *n.p.n.* new neural lobe, *i.p.f.* thickened portion of infundibular floor, *n.p.ep.* hypophysial portion in abnormal position. (From Smith.)

a branch of the trigeminus nerve. (76) This is of interest since normally the trigeminus never forms the nerve supply to the limb.

Normally in the frog tadpole the right forelimb as it develops pierces a hole in the operculum which covers over the gill slits. If the limb bud is extirpated, however, the hole in the operculum forms just the same. (77) The presence of certain other tissue appears to play a part in the differentiation of this hole. (164)

In tadpoles it is possible to destroy the whole or part of the rudiment of the hypophysis while it is still superficial, and before it comes into contact with the infundibulum to produce the pituitary body. (78) Where the hypophysis is completely removed, the pars nervosa is only half its normal size, and the wall of the infundibular sac remains thin, without any development of the high columnar and other layers. If the extirpation of the hypophysial rudiment is incomplete, that portion of it which is left may come to occupy an abnormal position, attached to the floor of the infundibular sac. In these cases the tissue of the infundibulum is thickened and differentiated in the region of the hypophysis. The complete differentiation of the infundibular constituents of the pituitary body is therefore dependent on the presence of the hypophysis.

The cases hitherto considered have all referred to amphibian material. It is now necessary to mention a few experiments on birds, using a new and special method. Surrounding the embryo as it develops inside the shell, there are the chorio-allantoic membranes, which contain blood-vessels by means of which the yolk is conveyed into the embryo. It has been found possible to graft portions of chick embryos on to this membrane where they are nourished by the blood-vessels, and to observe their differentiation in a situation in which they are deprived of the influence of structures which normally surround them.

Portions of chick blastoderm will develop properly, provided that the blastoderm is old enough. (79)

In these cases of differentiation of *portions* of a blastoderm on the chorio-allantoic membrane, the degree of differentiation reached increases with the age of the blastoderm from which the portion was taken. In the case of the eye, a piece cut out of a blastoderm after four hours' incubation (provided it is the correct region) will produce an eye with pigment cells only. After six hours' incubation a piece will produce pigment and retinal cells; after eight hours the stratification of the retina will appear; and complete self-differentiation of the eye is obtained from a piece cut out of a blastoderm which has reached the stage when somites are differentiated. (119)

The anterior portion of the blastoderm will differentiate into a proper head with brain, eyes, and pituitary. (80)

Isolated rudiments of the eye, the nose, and the ear of the chick differentiate independently on the chorio-allantoic membrane. (81) The same is true of rudiments of pronephros, mesonephros, neural crest, liver, pancreas, intestine. Myotomes also will undergo primary differentiation in the solitude of the membrane, without receiving any nerves from the spinal cord. (82)

The rudiment of the metanephros undergoes differentiation in the same way. (83)

A portion of the limb bud of the chick, representing the left thigh region, will differentiate into a typical femur. (84) It shows the proper asymmetry of a left femur; its cartilaginous axis is surrounded by bone. The proximal end has a typical head and trochanter, while the distal end begins to form condyles.

These examples will suffice to show that in the early stages of development, after chemo-differentiation has determined the fate of the different regions, visible differentiation sets in without function playing any part in its



formation. This is in contrast with the later stages of development.

It is also worth noticing that visible differentiation may be morphological (change of shape, e.g. growth in length, formation of limb buds) and histological (change of substance, e.g. specialization of retina layer in the eye, or tubules in the pronephros).

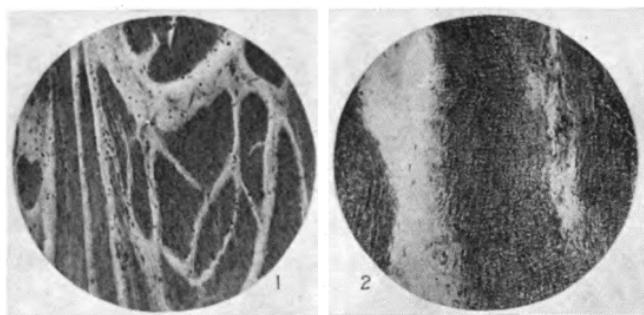
## XVI

### FUNCTIONAL (LATE) DIFFERENTIATION

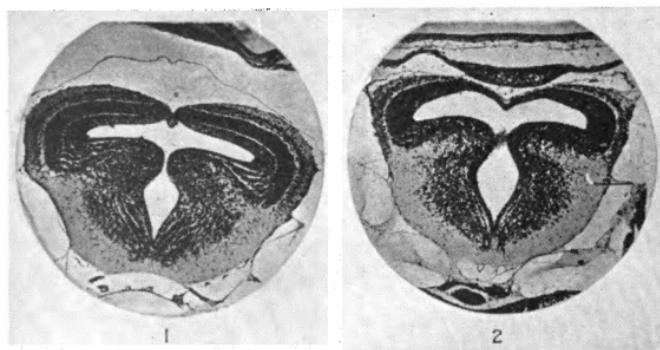
A TIME comes in the formation of various organs and structures when the stimuli produced by function are necessary for further differentiation. One of the best examples of this is to be found in the case of blood-vessels. The first appearance of the main trunks of blood-vessels takes place without involving function. (85) Later on, however, the detailed connexions, the area in cross-section of the vessel, and the thickness of its walls are conditioned by the functions which the vessel performs in conveying blood to the neighbouring tissues and organs. A section of vein can be transplanted into the course of an artery, where it will take on the characteristic arterial structure. The connective tissue increases and the circular muscles are more than doubled in thickness. At the same time the lumen is diminished by contraction. (177)

The effect of function in producing structural modifications is well shown in the following experiment. The bladder of a dog normally evacuates about 250 c.c. of fluid in twenty-four hours. Its walls are composed of smooth muscle cells, and are about 0.5 mm. thick. A tube connected with the bladder enables a neutral fluid to be passed into it, and the quantity of fluid passed was raised to 50,000 c.c. in twenty-four hours. (86) As a result of this increased function it was found that not only had the wall thickened to 5 mm., but that the tissue of which it was composed had developed striations very similar to those characteristic of heart-muscle. Further, the bladder pulsated rhythmically 200 times a minute.

This experiment throws a flood of light on the manner of origin of the heart, the heart-musculature, and the heart-beat, which by analogy can be ascribed to the pressure of contained fluid.



**FIG. 36.** 1. Normal smooth muscle of a dog's bladder. 2. Muscle of a dog's bladder, with striations artificially produced by pressure. (*From Carey.*)



**FIG. 37.** 1. Section through the brain of a normal frog. 2. Corresponding section through the brain of a frog without hind legs, showing underdevelopment. (*From Dürken.*)

Bone is not solid throughout, but is made up of a number of spicules and splinters. It is found that the orientation and direction of these spicules coincides with the lines of stress produced by the pressure of the weight supported by the bone. If the lines of stress are altered, owing to

a bone being broken and reset slightly differently, the spicules of bone are altered also, in conformity with them. (87) In fact the formation of bone spicules is dependent on function in the form of pressure.

The function of the nervous system is the conveyance of impulses to and from the brain and spinal cord. If power of function is denied to certain nerves by removal of the part which they normally supply, the corresponding region in the brain will fail to differentiate properly. For instance, if the hind-leg buds of a frog are removed, a stunting is observed in the hind brain. (89) Whole regions of centres fail to develop, and the difference in appearance as contrasted with the normal is very striking.

If an eye is removed at a very early stage in a frog, the centres in the brain known as the optic lobes do not develop, owing to lack of function and impulses from the optic nerve, which does not leave the brain case. (90) At same time, the optic foramen in the wall of the skull and the eye-muscles are present though small, showing that they have arisen by self-differentiation.

A good example of functional adaptation is to be found in the intestines of frog tadpoles which have been fed on different diets. A vegetarian diet results in tadpoles with longer intestines than those in whose diet meat is included. The absorptive area is twice as large in the former as in the latter. (91)

It is only a short step from the cases last described to the effects of use and disuse in modifying structures.

It is seen, therefore, that following on the period of early differentiation, when self-differentiation of parts is the rule, and there is no necessity a period of functional differentiation determines t particular tissue will perform, in the on the tissue and perfects it.

## XVII

### REGENERATION

REGENERATION is the capacity for replacing parts which have been amputated or otherwise lost. Its study is of great interest in itself, as one of the most important properties of living matter. Here, however, it will be considered from the point of view of the light which it throws on problems of development and differentiation.

As a rule it may be taken that the lower on the evolutionary scale and the younger an animal is, the greater are its powers of regeneration. At the same time it is most important to notice that at the stage in development where the various regions have been determined and the embryo is a mosaic of self-differentiating organs, no regeneration is possible. If the limb bud of a chick be removed at this stage, the chick will not regenerate it. (92)

The histological processes of regeneration are of interest. The wound is healed over and a bud of undifferentiated embryonic tissue is formed at the tip of the stump. Of the muscles of the stump, the nuclei in the outer fibres divide amitotically at first. (93) In the frog embryo the muscle of the regenerating tail arises from embryonic protoplasm derived from dedifferentiation of muscle. (94)

Some lizards have the power of regenerating their tails, which are sometimes provided with joints at which breakage more readily takes place (autotomy joints). Whether the breakage takes place there or elsewhere makes no difference ; regeneration sets in all the same. (95) Haemorrhage is prevented by constriction of the open ends of the blood-vessels, and the wound is covered over by fresh skin. A bud is formed, composed of undifferentiated cells

derived from the connective tissue. This proceeds to grow and differentiate into the new tail, and gives rise in so doing to cartilage, fat, muscles, blood-vessels, and the sheath of the spinal cord. No nerves arise from the regenerated spinal cord; all the nerves of the new tail are derived from the original stump, the ganglia of which become enlarged. The regenerated tail differs considerably from the original one.

In regeneration, therefore, the processes are not necessarily the same as in development. The regenerated tissues do not always arise from similar tissue, but may be produced from cells belonging to another germ layer.

A good example of the difference between development and regeneration is to be found in the case of the lens of the eye of Triton. Instead of being re-formed from the superficial epithelium, the new lens is formed from the edge of the iris. (96) A small vesicle develops, its cavity being originally in communication with the space between the outer and inner layers of the eye-cups. This case is remarkable not only for the method of regeneration which it involves, but also for the apparently purposive nature of the process of restoring the visual function of the eye.

A small piece of the nemertine *Lineus*, cut off from the front of the head in front of the mouth, naturally contains no endoderm. Nevertheless this little piece undergoes a process of regulation and remodelling, which results in a small worm with a well-formed gut. The latter is derived from mesenchyme cells. (97)

If the head ganglia of the worm *Allolobophora* are removed, new ganglia are regenerated. The material for these may be derived to a small extent from the remaining nervous system; but the epithelium of the dorsal part of the wall of the pharynx undergoes dedifferentiation and gives rise to a mass of embryonic cells which is continuous with the rudiment of the new ganglia. (98). Cells which

were in the endoderm may therefore contribute to the regeneration of the nervous system in worms.

Some cases of regeneration are instructive in showing dependent differentiation.

After amputation of a limb of a newt and severance of the nerve supplying it, regeneration will not continue until

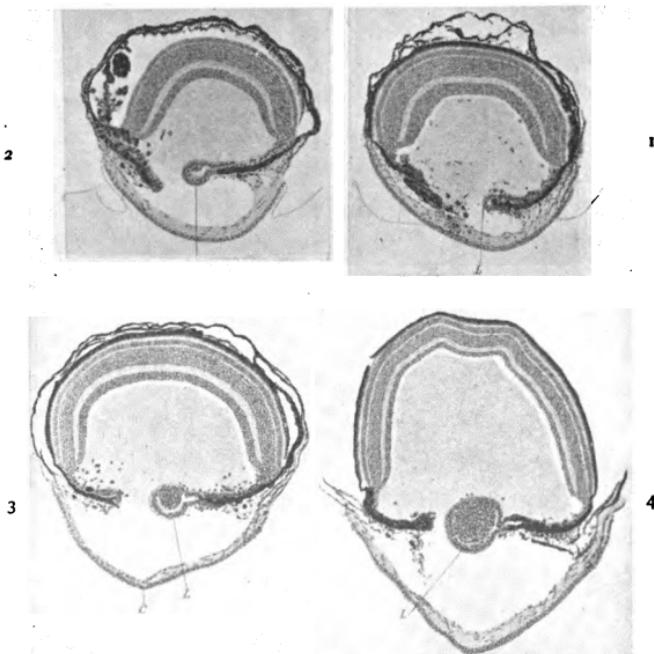


FIG. 38. Stages of the regeneration of the lens of the eye of Triton from the edge of the optic cup. L lens, C cornea. (From Müller.)

the nerve has grown again and innervated the limb. The formation of the new limb is, therefore, dependent on the nerve. (99) Further, it has been proved that it is the sympathetic nervous innervation which is responsible. (165)

In the snail Tachea eyes are regenerated whether the corresponding cerebral ganglion is present or not. However, if the nerve to this region is cut, regeneration takes

place more slowly. In this form therefore the effect of the nervous system is not *qualitative* but *quantitative*. (171)

In catfish the barbels surrounding the mouth bear taste-buds which are innervated by the facial nerve. Cutting of the nerve results in rapid degeneration of the taste-buds. After three weeks the nerve regenerates and grows down the barbel at the rate of about 1 mm. per day. (100) Concurrently with the arrival of the nerve the taste-buds are regenerated. Here the presence of the nerve is necessary for regeneration and for maintenance.

In a similar way, the presence of the nerve-cord is necessary in worms for regeneration to take place from the cut surface. (101) If the head end of a worm be removed and the nerve-cord also extirpated for a short distance behind the cut surface, a head will regenerate at the end of the nerve-cord, but not from the original cut surface.

In the formation of a new tail after amputation in the frog, the notochord must come right up to the cut surface. If a piece of the notochord is cut out so that it does not reach to this surface, no regeneration of the tail takes place until the notochord itself has regenerated and reached that surface. (102)

Perhaps the most instructive case of dependent regeneration is that of the eye of the rock-lobster. If the stalked eye is removed, while leaving the optic nerve ganglion intact at the base of the stump, an eye will be regenerated. On the other hand, if the nerve ganglion is removed also, the organ which regenerates is not an eye at all, but a little feeler or antenna. The regeneration of the eye is therefore dependent on the optic nerve ganglion. (103) Another case of heteromorphosis is known in stick-insects, where a limb appears instead of an antenna.

Remembering the fact that no regeneration takes place during the period of mosaic self-differentiation of inde-

pendent regions, most cases of regeneration fall into the subsequent period of functional differentiation, and co-ordination and interrelation of the separate parts. It is not surprising, therefore, that most cases of regeneration are dependent on other structures and functions, and that the regenerated material bears a definite relation to the whole organism. Since, as a rule and under normal conditions, that which is regenerated is equivalent to that which is lost, that amount can only be gauged by referring to the whole organism. That the processes of regeneration of a given organ should differ from those of the development of that organ from the egg, is what is to be expected owing to the difference of internal conditions.

Regeneration is not an adaptation, since the ability to regenerate is not as a rule correlated with the liability to injury, but it is one of the manifestations of the self-regulative powers of organisms. However, in certain cases as in that of the lizard's tail, this faculty has been retained, and is probably of advantage to the animals.

## XVIII

### TISSUE CULTURE

It is possible to take tissues out of animals, keep them sterile in small glass vessels, feed them on serum, plasma, or other suitable substances, and keep them alive indefinitely. This method of tissue culture, growth *in vitro* or explantation as it is called, is of great value in testing the separate tissues as to their powers of histological differentiation and of cell division or growth. The method is really an extension of that of transplantation ; it results, however, in a more rigorous elimination of factors which are external to the piece of tissue in question.

Freed from the various influences of the whole organism, some tissues have astounding powers of survival. Fibroblasts from the heart of the chick have been cultured and the strain kept alive for over ten years. (104) The piece doubles itself in two days and is then transferred to fresh medium, an operation called *sub-culturing*. In the case of these fibroblasts, there were nearly two thousand generations of sub-cultures in ten years, and the tissues are as healthy as ever. Tissues can, therefore, live *in vitro* for longer periods than the lives of the organisms of which they formed part.

Some tissues, like the heart of the chick, maintain their differentiation indefinitely, and pieces of heart tissue actually pulsate regularly *in vitro*. (105) The testis of moths will proceed a long way with the process of sperm formation *in vitro*. (106)

Nerve-cells are of particular interest. *In vitro* they will produce axons which grow out into the surrounding medium. (107) The maximum speed observed was 56  $\mu$

in an hour, and the longest axon so obtained was 1.15 mm. long, having taken 53 hours to grow. The direction of the outgrowth of axons can be controlled by the electric current. By applying a very weak current (two billionths of an ampere) to a culture *in vitro* it is found that the fibres grow out along the lines of the field of force. If a conductor goes through the culture and a current passes through it, the fibres all grow out perpendicular to the conductor. (108).

The significance of these experiments on the behaviour of nerve-cells *in vitro* will be discussed again below in Chapter XX.

Other tissues such as kidney or cartilage ordinarily lose their characteristics *in vitro*, and grow as sheets of undifferentiated cells. (109) Growth and cell division take place in cultures of some tissues, such as nerve and smooth muscle which in the adult animal do not grow.

Epithelium and connective tissue in pure cultures by themselves undergo dedifferentiation and revert to an embryonic condition. When both kinds of tissue are present together in the same culture, differentiation is maintained. (110) This shows the effects of the tissues on each other in maintaining differentiation, as does the fact that if the connective tissue in such a culture dies, the epithelium no longer maintains its differentiation.

Cultures of kidney tissue of the mouse, and of carcinomatous tumours of the mammary gland, grow as sheets of undifferentiated cells. They can, however, be made to redifferentiate by the addition of some connective tissue-cells to the cultures. The kidney cells produce tubules, and the tumour cells give rise to structures strongly resembling the normal mammary gland. (111)

That a tissue should fail to differentiate in tissue culture does not necessarily mean that it is incapable of self-differentiation or that it has lost the power of doing so.

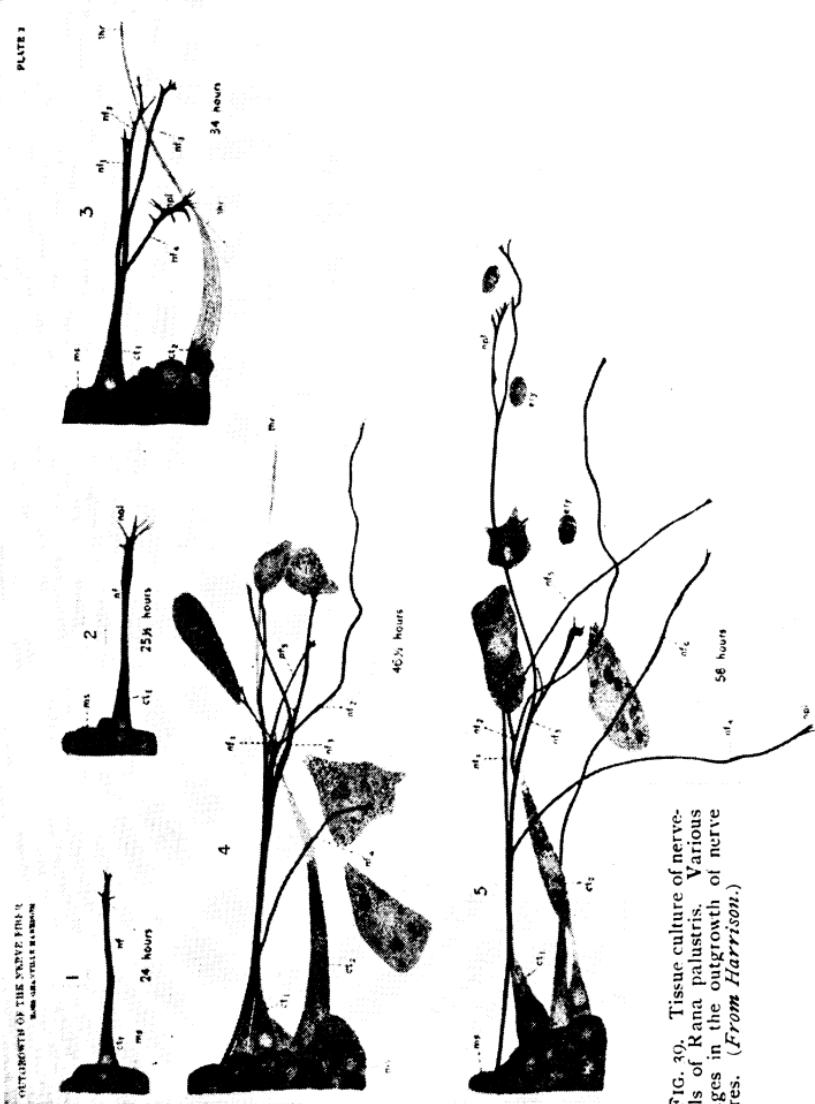


FIG. 39. Tissue culture of nerve cells of *Rana palustris*. Various stages in the outgrowth of nerve fibres. (From Harrison.)

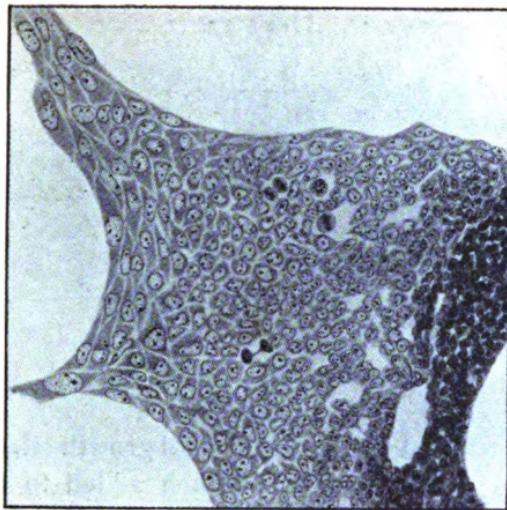
So many factors normally present in an animal may be absent in tissue culture that even if a tissue is self-differentiating *in vivo*, it need not be so *in vitro*.

Cartilage ordinarily dedifferentiates *in vitro*, but it has been shown that if the tissue is very carefully sub-cultured, the centre of the piece of tissue may be prevented from showing cell division, and in these cases differentiation into cartilage will actually take place *in vitro*. (112) This experiment also suggests that active cell division prevents differentiation.

Kidney tissue of the chick embryo carefully cultured *in vitro* will not only develop tubules, but it will also produce glomeruli and capillaries; this is a striking example of self-differentiation. (113)

Loss of differentiation does not mean loss of potency to differentiate. A portion of articular cartilage of the chick was sub-cultured twenty-three times to make sure that no portion of the original tissue remained, and was quite undifferentiated. When grafted back under the skin of the wing of a chick, this cultured tissue redifferentiated into typical cartilage. (112) A culture of mouse epithelial tissue dedifferentiated *in vitro* and grafted back into a mouse reassumes the characteristic structure of epithelium, even to the development of a horny layer. Similar results are obtained with cultures of intestine. Although not visibly differentiated, these tissues must have been chemodifferentiated, the effect of which was not shown owing to some factors absent *in vitro* but present *in vivo*.

With regard to the ability of certain tissues to show cell division *in vitro*, mere removal from the animal in some cases appears to suffice to enable cells to divide (e.g. nerve-cells), although in the animal they would normally not have divided after reaching the adult stage. In this connexion it is interesting to note that a portion of a non-malignant tumour grown *in vitro* rapidly assumed



1



2

FIG. 40. 1. Pure tissue culture or mouse carcinoma of the breast, growing as a sheet of undifferentiated cells. 2. Differentiation of mammary gland acini in a tissue such as that shown in 1, by addition of a different tissue to the culture. (From Drew.)

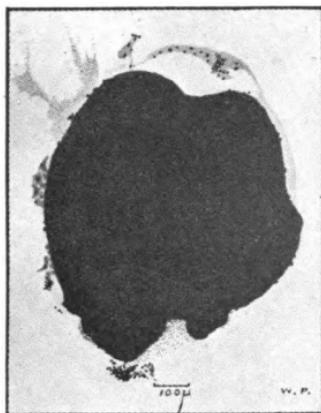
the appearance of malignant tissue, and grew much more rapidly. (114)

Certain substances have the property of stimulating cells to divide. Among these is an extract which can be obtained from embryos, and hence termed 'embryo extract'. (115) Addition of this substance to the medium in which tissues are cultured increases the cell divisions.

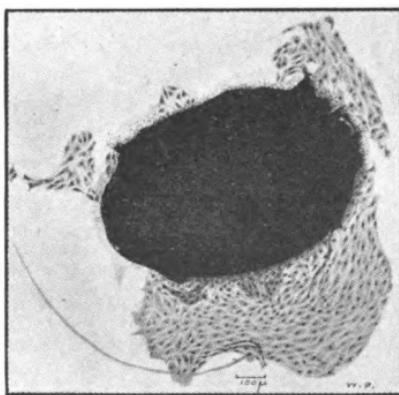
Extracts made from ordinary adult tissues contain no growth-promoting substance. On the other hand, adult tissue which is damaged and incubated at body temperature (autolysed) produces a very powerful growth stimulant. (111) The effects of this substance when added to cultures *in vitro* is remarkable, and it is very similar to that produced by extracts of malignant tumours. In the latter case the efficacy of the extract in accelerating cell division is proportional to the malignancy of the tumour. The autolysed extract is a substance altogether different from the embryo extract.

The growth obtained by addition of autolysed extract is very rapid, but after two days it ceases and the stimulating effect is worn off. Now in the ordinary process of repair of tissues in an organism (physiological regeneration) the damaged cells are replaced. It is possible that this is due to the liberation of the same substance as is found in autolysed extract, the substance being produced by incubation of the damaged cells at body temperature, and its effects being to stimulate neighbouring cells to divide sufficiently to replace the damaged ones. Tumours appear to be able to form this substance continuously, possibly owing to the lack of circulation and the too great rapidity of growth.

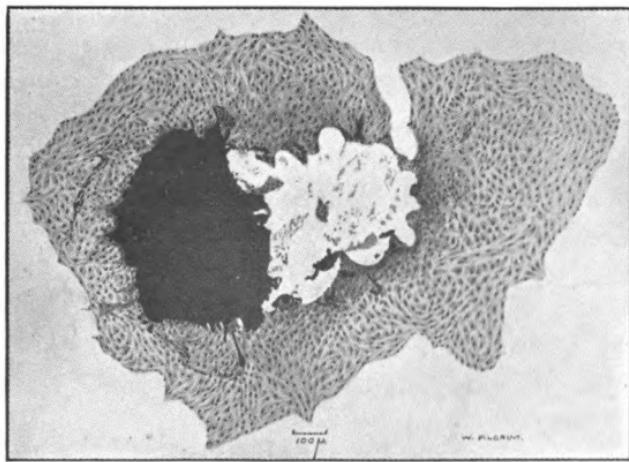
Growth resulting from damage to cells also occurs in plants. For example, the ovum may be made to develop parthenogenetically by pricking and damaging the external cells of the pistil. (116)



1



2



3

FIG. 41. 1. Tissue culture of adult rat kidney with embryo extract. Slight growth after 11 days. 2. The same tissue with autolysed extract 48 hours' growth. 3. The same tissue with malignant tumour extract 48 hours' growth. (*From Drew.*)

## XIX

### HORMONES AND DEVELOPMENT: AMPHIBIAN METAMORPHOSIS

DURING the period of functional differentiation the organism is a system of correlated parts. The co-ordination of the different parts implies action of one on another, often at a considerable distance, and involving a mechanism capable of carrying stimuli over such a distance. One mechanism of this kind is the transmission of excitability, either through ordinary protoplasm, or through specially differentiated conductors.

But there is also a mechanism involving the transportation of substances from one part to another. These substances, or hormones, produce certain definite effects in certain places, and since they have a definite chemical constitution this mechanism is known as chemical correlation. From the present point of view its importance lies in the fact that it not only assists to control and maintain the adult type of structure when once it is reached, but it is also essential in certain ways for the development of that structure. Its study therefore claims a place in experimental embryology.

The effect of hormones on development will be illustrated here by a consideration of the metamorphosis which amphibia undergo between their larval and adult conditions.

Frogs and newts alike begin life in the water in which their eggs hatch, and develop into larvae adapted to an aquatic mode of life. This means that they possess gills, tails, and tail-fins. The frog tadpole before it becomes a frog has to resorb its tail, develop four limbs, develop its lungs and tongue, modify its intestine, and close its gill

slits. There are also changes in the skeleton, liver, and pancreas. The newt undergoes a less sensational but still striking metamorphosis, in that it develops lungs and reduces its gills and tail-fin; its tail it keeps, and its limbs are present in the larval condition.

Now it is found that a diet of thyroid-gland substance fed to frog tadpoles has the property of making them undergo metamorphosis very quickly, and long before they would naturally have done so. (117) The consequence is that it is possible to obtain tiny frogs, perfect in every detail, but only about one-tenth the length of normal just metamorphosed frogs (in *Rana temporaria*).

The next step is to show that the tadpole's own thyroid is responsible in nature for bringing about its own metamorphosis. This can be done by extirpating the rudiment of the thyroid at an early stage. (120) It is then found that such tadpoles do not metamorphose: instead, they go on growing and reach a relatively huge size. If, however, to such thyroid-ectomized tadpoles thyroid is fed, they respond by metamorphosing. (121) These experiments definitely prove that the thyroid of a tadpole is an agent for its own metamorphosis. This did not necessarily follow from the first experiment alone (feeding thyroid substance to normal tadpoles) any more than the supposition that digitalin, which has a specific effect on the heart, must



FIG. 42. Frogs with and without thyroid glands. On the left a thyroidectomized tadpole, which grew but did not metamorphose; on the right a metamorphosed frog with normal thyroid, brother of the other and of the same age. (From Allen.)

have the same physiological significance in the fox-glove.

The tadpoles of the bull-frog do not metamorphose until the third season. They possess a thyroid gland which if grafted into tadpoles of smaller species of frogs induces precocious metamorphosis. (122) This shows, therefore, that the thyroid gland of the bull-frogs is active long before they metamorphose, which raises the question why this should be.

Metamorphosis in frog tadpoles is not due to the reaching of a certain size in itself, as the large thyroidectomized tadpoles show. Neither is it due to mere presence of a thyroid gland, as the bull-frog tadpole (and several newts) shows. It is due, however, to the relative sizes of the thyroid and the whole body.

For any given temperature there is a certain size at which metamorphosis normally takes place, and this size differs with a difference in temperature. (123) At low temperatures the size is greater. This is what one would expect if the temperature coefficient of the thyroid differed from that of the rest of the body. It has been found that different geographical races of the same species of frog have different rates of development of their thyroid glands; those from cold climates have larger thyroids than warm-climate races, when developed at the same temperature. (124) Experiments have also shown that frog tadpoles developed in the cold grow to a larger size and have larger thyroids than normal before undergoing metamorphosis.

Taken together these experiments show that varied proportions of thyroid and body size can be obtained at different temperatures, and that the larger the body is, the larger, relatively, must the thyroid be also to produce metamorphosis. There is therefore a balance between body size and thyroid, such that when a certain ratio is reached metamorphosis ensues.

There are other things to consider, however; it takes two to produce a reaction, viz. a reagent and material. Thyroid is the reagent, the tissues of the body are the

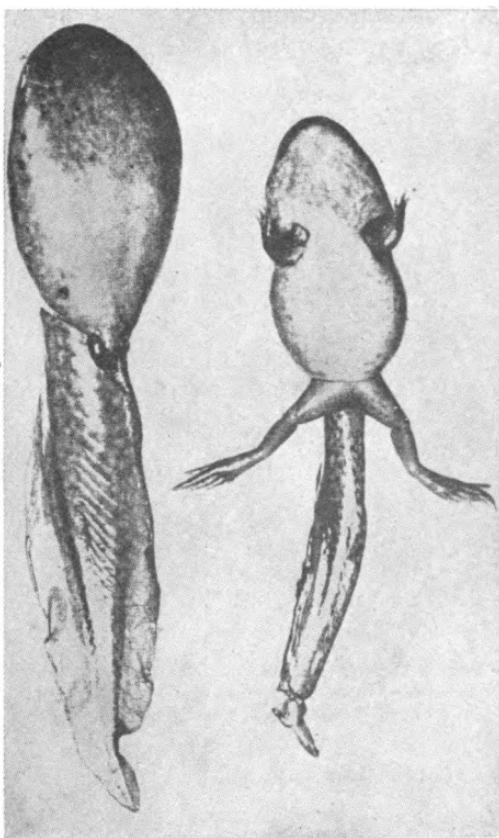


FIG. 43. On the left a normal frog tadpole; on the right a similar animal fed on thyroid gland substance and induced to metamorphose precociously. (*From Swingle.*)

material. Variations in the reagent naturally affect the reactions, but so do variations of the material. It is necessary therefore from this point of view to examine

the susceptibilities of the tissues to the action of the thyroid-gland substance.

A diet of thyroid will induce metamorphosis in Axolotl, Triton, and Salamandra, but no amount of thyroid feeding will produce a result in Necturus or Proteus, (131) which never metamorphose at all, but reach sexual maturity in the larval condition. At the same time the

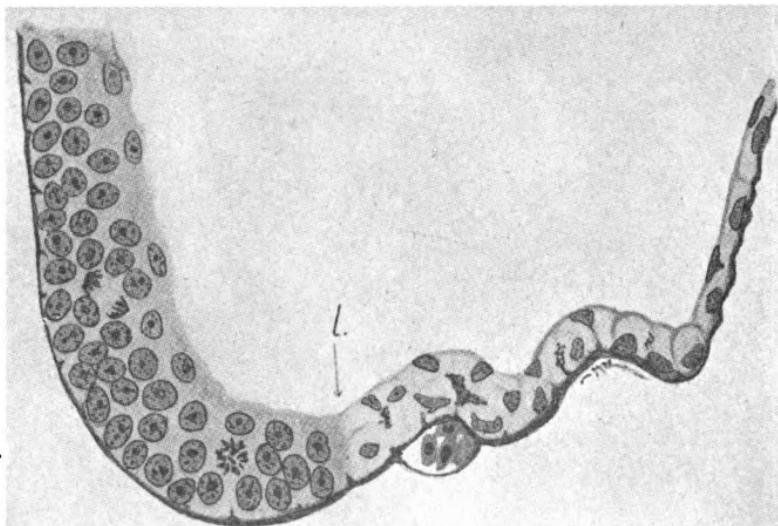


FIG. 44. Section through the skin of a frog tadpole treated with thyroid extract and undergoing precocious metamorphosis; I. transition between limb-rudiment region with active cell-division, and degenerating gill region. (From Champy.)

thyroid gland of Necturus is functional, for if grafted into a frog tadpole it will produce metamorphosis. (122) The difference between Necturus, Proteus, and the other amphibia therefore lies in that the tissues of the former are not sensitive to the thyroid hormone.

Between the tailed and tailless amphibia this difference in susceptibility can be observed, for thyroid in newts does not affect either the tails or the limbs, whereas it does so markedly in frogs. (126)

In frog tadpoles which have been fed on thyroid, histological examination shows that certain definite regions respond by an increase in the rate of cell division. These regions comprise the rudiments of the limbs, the tongue, and the lungs, and they are sharply marked off from the neighbouring regions. (127) The increased rate of cell division in these regions lasts until metamorphosis; in other regions such as the tail and the gills, cell division is retarded and degeneration sets in.

It follows that certain tissues (limb, lung) require a high concentration of thyroid hormone, others (tail, gills) will not survive except in a low concentration. A relative difference in the proportions of body size and thyroid affects this concentration, and is therefore able to bring about this structural change. In this case morphogenesis is definitely under experimental control by means of thyroid hormone.

In the light of these experiments which show that there can be not only variation in thyroid concentration but also in susceptibility of the tissues, it is possible to construct a scheme which roughly covers most of the cases of metamorphosis met with in amphibia. At first one may consider relative rates of development of the thyroid and times at which the necessary relative concentration for metamorphosis is reached, the susceptibility being regarded as constant. (128)

| <i>Rate.</i> | <i>Time.</i>   | <i>Example.</i>            |
|--------------|----------------|----------------------------|
| Very rapid.  | Early summer.  | <i>Bufo lentiginosus.</i>  |
| Moderate.    | Late summer.   | <i>Rana temporaria.</i>    |
| Slow.        | Second season. | <i>Rana clamitans.</i>     |
| Very slow.   | Third season.  | <i>Rana catesbeiana.</i>   |
| Nil.         | Never.         | Thyroidectomized tadpoles. |

In this series the final maximum size of the tadpoles becomes progressively larger.

Or the different susceptibility of the tissues may be considered, and the thyroid concentration regarded as constant.

| <i>Susceptibility.</i> | <i>Metamorphosis.</i> | <i>Example.</i>      |
|------------------------|-----------------------|----------------------|
| Very high.             | Occurs, far-reaching. | Anura.               |
| High.                  | Occurs.               | Axolotl, Salamandra. |
| Low.                   | Does not occur.       | Necturus, Proteus.   |

There are other directions in which experiments on amphibian metamorphosis throw light on problems of development. It has been found that addition of the inorganic element iodine to the water in which frog tadpoles are living causes them to metamorphose. (129) In other words, iodine produces an effect similar in final result, and iodine must be regarded as one of the necessary constituents of the hormone.

Now iodine will also produce metamorphosis in frog tadpoles from which the thyroid has been removed, only more slowly than in normal animals. (130) The capacity for elaborating the thyroid hormone out of iodine and other substances is therefore possessed not only by the thyroid gland, but also by the other tissues of the body. These, however, do it less quickly than the gland which is specialized for the purpose.

Iodine will not cause metamorphosis in the Axolotl, (131) but accelerates the metabolism of the thyroid.

So far only one gland and hormone has been considered in relation to metamorphosis, viz. the thyroid. It is now time to turn to the effects of another—the pituitary.

Injection of extracts from the anterior lobe of the pituitary body will bring about metamorphosis in frog tadpoles. (122) On the other hand in frog tadpoles from which the hypophysis has been removed so that no pituitary gland is formed, there is no metamorphosis. (78) In these cases it seems that the pituitary exerts its effect

by controlling the thyroid, for the latter gland is very small and undeveloped in hypophysectomized tadpoles, whereas its normal condition is reattained if a pituitary is grafted into it.

But in the Axolotl it has been found that injection of anterior lobe pituitary extract will produce metamorphosis even in thyroidectomized specimens. (125) From this it follows that the pituitary must also be capable of inducing metamorphosis directly, and independently of the thyroid.

With this short exposition of what is known concerning the morphogenetic functions of the thyroid and pituitary hormones this chapter will close. It may be pointed out that other hormones, such as those of the reproductive organs, have been experimentally proved to have marked effects on the development of certain organs. Sufficient will, however, by now have been said to show the importance of hormones in morphogenesis.

One remark is worth making. The various reactions which have been described in previous sections partake of a qualitative nature. Thus it is known that in some species the eye-cup exerts an effect in determining a differentiation of a lens. Nothing is known as to the quantitative nature of the necessary stimulus. In amphibian metamorphosis, however, it has been necessary to consider relative rates of development and concentrations of chemical substances. This means that the door is open to the employment of physical and chemical methods in experimental embryology, or, in other words, that having become susceptible of quantitative analysis, it is becoming an 'exact' science.

## XX

### NERVES AND THEIR RELATIONS TO MUSCLES DURING DEVELOPMENT

It is a remarkable fact that particular nerves are very faithful to the particular muscles, &c., which they innervate, although in development they arise from quite separate rudiments. This constancy has led to the advancing of a number of theories on primitive connexion between nerve and muscle. It has been imagined, for example, that there exist primitive protoplasmic paths at very early stages between the nerve-cord and the myotomes, and that the nerves in their development are guided by these paths. On the other hand, there is the view that the development of a nerve from the spinal cord is a perfectly free out-growth, not preformed in any way.

Fortunately, it has been possible to attack this problem by the experimental method.

If the nerve-cord of a newt embryo is removed, any possible connexion between the nerve-cord and the limbs is destroyed. Nevertheless if the limb buds ('aneurogenic') of this newt are transplanted into the proper place on a normal newt, the limbs become innervated perfectly normally. (132) Here then, at any rate, no previous connexion between nerve-cord and muscle is necessary for the nerve to develop and find its proper muscle.

It has also been found that if limb buds are grafted in not quite the right place, they will still become innervated by the proper nerves provided that they are not too far away from the normal position. (133) On the other hand, if the position of the implant is entirely different from the normal, as in the case of a limb planted in the head,

innervation takes place by an altogether strange nerve; in this case the trigeminus. (76)

When the rudiment of the eye of the newt *Amblystoma* is transplanted to the region of the ear, the developing optic nerve may become connected with the facial, glossopharyngeal, or vagus nerves: all abnormal connexions. Similarly the rudiment of the nose so transplanted will form



FIG. 45. A transplanted eye in *Amblystoma*. The optic nerve has established connexion with the glossopharyngeovagal ganglion. (From May and Detwiler.)

nervous connexions with the brain that could not possibly have existed in the form of predetermined paths. (172)

These experiments all contradict the theory that there is a previous connexion along which nerves develop, and suggest on the contrary that the process is one of free outgrowth.

Experiments on transplanting placodes, areas of ectoderm from which cells are contributed to form ganglia of certain nerves, lead to the same conclusion. In the newt

Amblystoma the placode which gives rise to the ophthalmic ganglion can be transplanted to a new position and grafted just above the normal ophthalmic placode of another animal. (136) The graft gives rise to a ganglion from which fibres develop and innervate regions of the skin normally supplied by the host's own nerve. Further than this, when an ophthalmic placode is grafted in place of the placode from which cells contribute to the gasserian ganglion, the transplant gives rise to a ganglion which partly replaces the gasserian in both form and function.

The behaviour of nerve-cells and the development of axons when cultured *in vitro* lend strong support to this view. In this case the nerve-fibre can actually be observed to grow out freely, armed at the tip with a cap of protoplasm. (107)

When a portion of the brain and of the trunk region of the embryo of a chick are grafted together on to the chorio-allantoic membrane, it can be observed that nerve-fibres develop and enter such structures as kidney and muscle, which appear to exert an attraction upon them. (134) On the other hand, they will not grow out far if only mesenchyme is present without any other structures.

It has already been mentioned that an electric field determines the direction of outgrowth of nerve-fibres in tissue cultures. (108)

There can be no doubt, therefore, that nerves grow out freely, and that they are directed in their development by certain stimuli. It will be remembered that fibres were found to grow out along the lines of force of the galvanic field and at right angles to a current passing through a conductor. Now one of the manifestations of the axial gradient of metabolic rate in organisms was found to be a difference of electric potential, observable in the undifferentiated spinal cord, and in the direction from head to tail. (58)

It is probable, therefore, that in development the nerve-fibres which form the white matter of the spinal cord are directed in their growth by this gradient. Further, the stimulation of a nerve-fibre and the conduction of an impulse through it results in a current passing through that fibre. Once established, therefore, the neurons in the spinal cord act as the conductor through the tissue culture described above. The tendency will then be for axons to grow out at right angles to the spinal cord. This they do in forming the segmental, cranial, and spinal nerves. The fundamental architecture of the central nervous system, therefore, has an experimentally supported basis in the reactions of nerve-fibres to axial gradients, a particular case of 'neurobiotaxis'. (135)

## XXI

### THE BLASTOPORE AND THE PRIMITIVE STREAK CONCRESCENCE

THE relations of the blastopore and the comparison between the conditions present in fish and amphibia on the one hand, and reptiles, birds, and mammals on the other, form one of the most important subjects in comparative embryology.

In the former group (*Anamnia*) the blastopore is the aperture of the archenteron formed during the process of gastrulation, either by invagination or epiboly; and it is open for a considerable time. In the other group (*Amniota*) there is an open blastopore in the reptiles, but it is a diminutive structure, and the others lack it. But characteristic of this group is a straight line of proliferating cells, the primitive streak, which gives rise to structures in exactly the same way as the rim of the blastopore.

The primitive streak has come to be regarded as a blastopore which is closed by the coming together and fusing of its lateral lips. This theory, known as concrecence, holds that the lateral portions of the would-be blastopore grow in towards the middle line, and fusing together there, add to the length of the primitive streak. Upon this theory others have been built, such as the 'prostoma', which holds that the blastopore is homologous with the aperture of the gastro-vascular cavity of coelenterates, and that this has been divided into two by nipping across and 'concrecence' of its lateral lips. In this way it is supposed that an explanation can be obtained for the fact that in some groups the blastopore occupies the position of the mouth, and in others of the anus. Applied to vertebrates

the prostoma theory indicates that the axial structures (nerve-cord, notochord, &c.) are developed in the region of the fusion of the lateral lips of the mouth of the coelenterate ancestor.

Now unfortunately for the theories of concrescence and

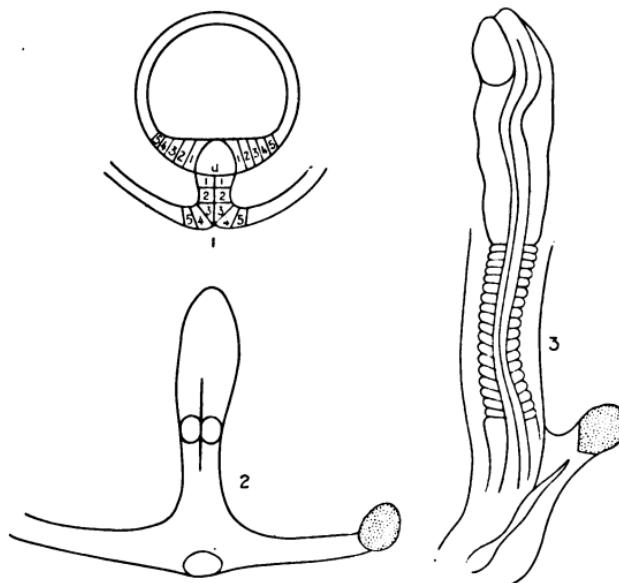


FIG. 46. 1. Diagram of the closure of the blastopore according to the theory of concrescence, whereby the regions marked 1 to 5 are supposed to move in towards the middle line. 2. A trout embryo with an injury to the edge of the blastoderm (dotted) to one side of the middle line. 3. Development of such an embryo. The injured area is still to one side, showing that the middle line of the embryo has not been formed by concrescence of the edge of the blastoderm. (From Kopisch.)

the prostoma, experiments which have been made on the mode of closure of the blastopore give results which are entirely opposed to concrescence. A short mention will be made of them here because of the importance which has been assumed by the theories which they disprove.

In shark embryos, before the blastopore closes, its edge grows back over the yolk so that the closure takes place

from before backwards. Now if concrescence occurs, injury to the edge of the blastopore to one side of the middle line should produce an injury to the structures in the middle line, at the spot where the lateral edges fused together. This is, however, not the case. (137) The middle line structures form quite normally, and the injury remains to one side. There is therefore no concrescence in the closure of the blastopore in Elasmobranchs. The same conclusion emerges from experiments performed on developing embryos of the trout. Furthermore, injury to the middle line should not, on the concrescence theory, prevent the formation of axial structures by concrescence of the lateral edge of the blastopore farther back. It does, however, prevent it completely. (138)

Lastly, in a developing newt it has been possible to mark definite spots by injecting stains into certain living cells. By this means the fate of these spots could be followed. It was found that the spots did not move into the middle line, on the contrary they moved along meridional lines, i. e. parallel with the mid dorsal line. (139)

The closure of the blastopore in those forms in which it can be observed has therefore been experimentally proved not to take place by concrescence. The evidence is therefore entirely against the assumption that the primitive streak arises by concrescence, and the somewhat extravagant theories which have been based on this view.

## XXII

### DEDIFFERENTIATION AND REDUCTION

THE work done by growth and differentiation may be negated by the opposite processes of reduction and dedifferentiation. This may happen to whole organisms, and it is a common feature as regards parts of organisms. So the tails of amphibian and of ascidian tadpoles, the gills of the former, the arms of the pluteus larva of sea-urchins undergo reduction and resorption, while the rudiment of the adult develops in another direction.

By starvation a planarian may be reduced in size from 30 to 3 mm. length, while keeping approximately the same proportions of parts. (140) The jelly-fish *Aurelia*, when starved, undergoes reduction from 50 mm. to 3 mm. diameter, at the same time undergoing marked changes of shape because the various regions are not reduced at the same rate. (141) The umbrella of the medusa decreases in size much faster than the oral arms, leading to a curious appearance. There is also histological dedifferentiation of the tentaculocysts and gonads.

Echinoderm larvae subjected to hunger or to dilute poisonous solutions undergo reductional changes. In the case of the pluteus larva of the sea-urchin, the arms, skeleton, and ciliated band round the body disappear, and the mouth and the anus close. (179) During the process a kind of competition takes place between the various organs for the available food and space. (142)

The sea-squirt *Clavellina* when exposed to unfavourable surroundings shrinks down completely to a more or less spherical mass. Of this animal (a near relative of the vertebrates) nervous, reproductory, and excretory systems,

heart, stomach, pharynx, and gills, all is dedifferentiated. If returned to a more suitable environment this formless mass redifferentiates into a normal individual, identical with its former self, only rather smaller. (143, 180)



FIG. 47. Dedifferentiation of pluteus larvae of the sea-urchin *Strongylocentrotus*. 1, normal larva; 2 to 6, stages of dedifferentiation in  $N/8$  million corrosive sublimate. 2 after one day, 3 and 4 after four days, 5 and 6 after six days. Note closure of mouth and anus, reduction of arms and skeleton, and assumption of spherical form.

It looks as if regions and organs which are highly differentiated have difficulty in maintaining themselves in the face of adverse circumstances, while less differentiated

tissue succeeds. This phenomenon has already been considered in connexion with the method of demonstrating axial gradients of metabolic rate by the differential sus-

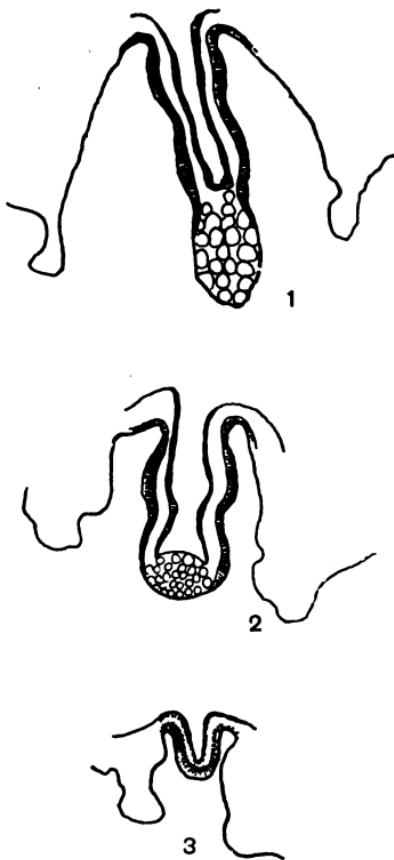


FIG. 48. Stages in the dedifferentiation of the tentaculocyst of *Aurelia*, by starvation. 1, normal; 2, after twelve days; 3, after twenty-three days. (From de Beer and Huxley.)

ceptibility of tissues to poisonous solutions. The maintenance of a differentiated form entails a higher expenditure of energy, and therefore a higher metabolic rate. *Clavellina*

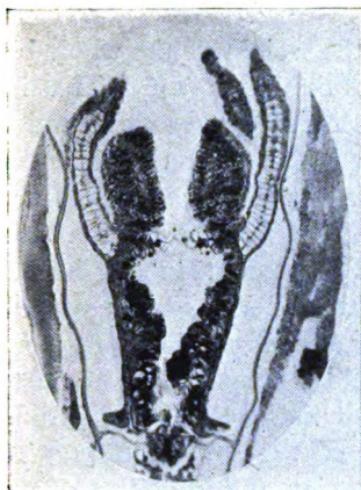
can therefore survive evil times by reducing itself to the dedifferentiated state.

This is the explanation of the state of affairs in *Obelia* and *Campanularia* when exposed to unfavourable circumstances. The hydranth, or polyp, is relatively highly differentiated, the stolon undifferentiated. The unfavourable environment causes dedifferentiation and complete resorption of the hydranth, while the stolon continues to grow normally. (144) A differential effect can therefore be produced between the hydranth systems on the one hand, and the stolon systems on the other.

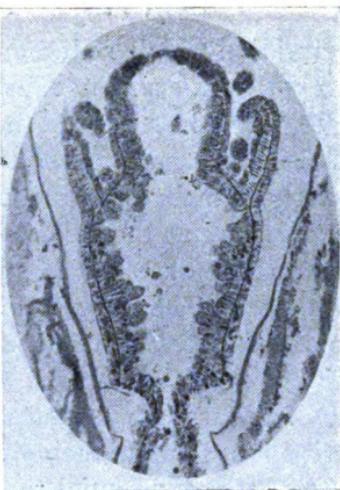
The interaction between two such systems can best be shown in the sea-squirt *Perophora*, which consists of individuals, or zooids, and a stolon. In clean sea-water the zooid will maintain itself and even grow at the expense of the stolon. On the other hand, if the water becomes dirty, the zooid is reduced, and the stolon grows: the former being resorbed into the latter. (145) The zooid and the stolon are therefore balanced in such a way that normally the zooid maintains its differentiation and dominates over the stolon. Variations in the environment may upset this balance.

The equilibrium between parts which is so easily demonstrated in *Perophora* is of wide occurrence in the later stages of development, during functional differentiation, when the organism is a system of co-ordinated parts. The competition which arises for space and available food in the organism has been termed the 'struggle of the parts'. (181)

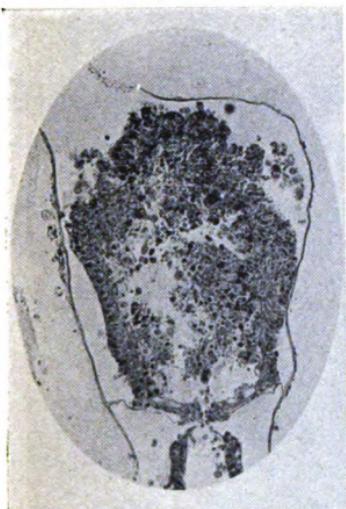
The various parts of an organism may be related and in equilibrium either by being in competition, or by one being dominant over the other. In the latter case, when there is insufficiency of food or space, the result is that some parts have 'preference'. This is well shown in the case of the testis of rats which are starved. A period



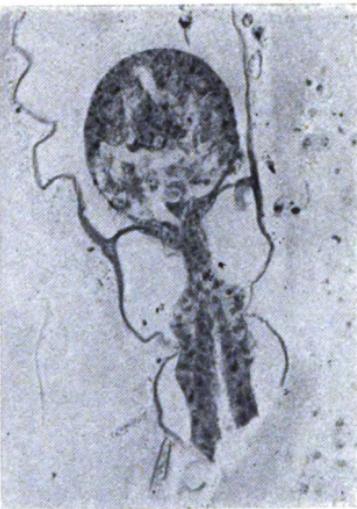
1



2



3



4

FIG. 49. Stages in the dedifferentiation and resorption of the polyp of *Obelia*. 1, normal; 2 to 4, progressive stages of resorption. (From Huxley and de Beer)

of starvation which reduces the weight of the whole body by 40 per cent. scarcely affects the testis. (146) That is to say, that the loss affects the other tissues first, and the testis is not drawn upon to make good the deficiency. Similarly, a malignant tumour in a human being may continue to grow at the expense of a body which is reduced by starvation or old age. The tumour in fact becomes dominant over the body. On the other hand, in a pregnant female mammal with a tumour, the developing embryo may dominate over the tumour, which does not grow, just as some of the ordinary tissues of the mother are laid under contribution for the benefit of the foetus. (183) This aspect of the differential reduction and inhibition of parts leads to the consideration of regulation, and the mutual relations between regions of different protoplasmic rate of activity.

## XXIII

### REGULATION

THERE have already been several opportunities for observing the fact that animals tend to approach a definite and characteristic form, which is the type of their species, and to return to it as far as possible if for some reason they are forced to depart from it.

Regeneration itself is a phenomenon partaking of this nature, for as a rule that which is replaced is just what was missing to complete the specific form. The fact that some animals do not regenerate in no way detracts from the positive value of the statement of the fact that regeneration as a process of regulation is of widespread occurrence.

A simple and striking case of regulative regeneration is that of the dissociated sponge. In a sponge which has been teased to pieces or passed through a sieve, all the differentiated tissues are separated and broken up, and the bottom of the jar in which the experiment is performed is strewn with isolated debris. These cells do not remain separated and die ; on the contrary they group themselves together and proceed to reorganize, the result being the redifferentiation of the sponge. (147)

If the flat-worm *Bipalium* be cut into short pieces, each fragment will undergo dedifferentiation and reorganization. Its shape alters, as can be seen by the change of shape of the coloured bands on its surface. No food can be taken in until the alimentary canal is re-formed. Meanwhile the pieces live on their own resources and use parts of their own selves as food. Eventually, as a result of this process of remodelling, termed morphallaxis, each fragment is

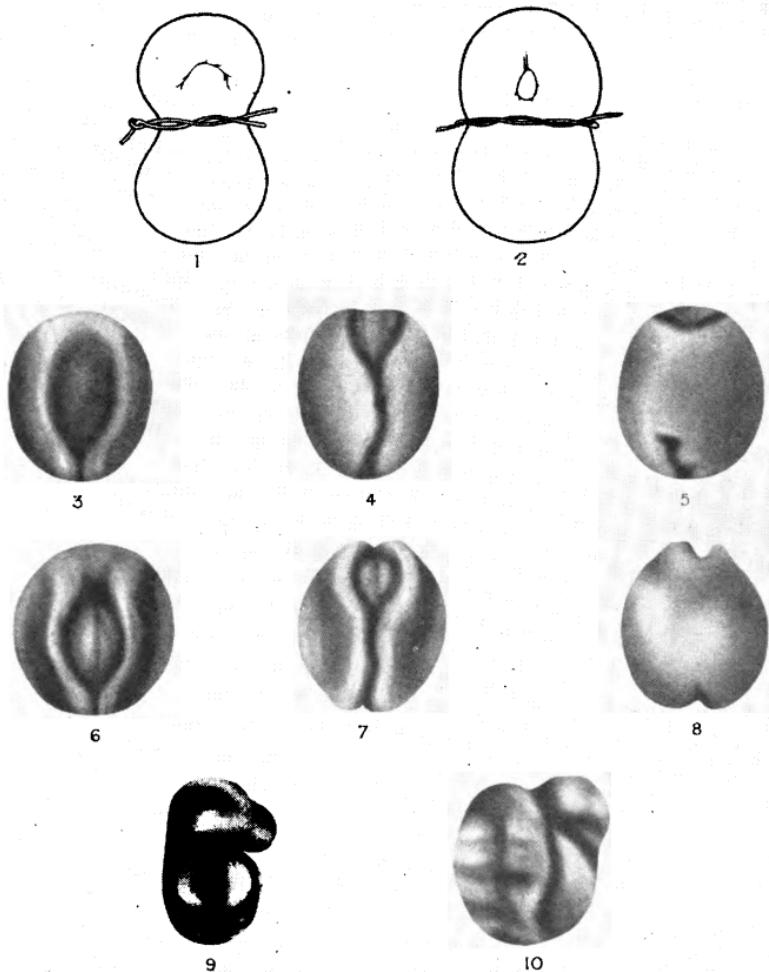
turned into a perfect little Bipalium. (148) This is of general occurrence in Planarians and many other forms.

The production of perfect embryos from isolated blastomeres, and the formation of single embryos from two fused eggs likewise show regulation at work in the production of specific form. (31)

If before the appearance of the gut-invagination in the blastula of the sea-urchin, a slice of cells is removed from the upper (animal) pole, the wound closes and the whole blastula is smaller than before the operation. When the gut-invagination does form, however, it is of the correct size relative to the diminished blastula. The same correctness of proportions is apparent in the three divisions into which the gut becomes separated. In this case the gut (endoderm) is diminished although only external (ectoderm) cells were removed. (149) This shows that the cells of the blastula are still totipotent, since they may or may not contribute to the gut, and it also shows that there is an harmonic relation between the relative proportions of the parts. For these reasons, this blastula is described as an harmonic equipotential system. This is already one step farther in the analysis of regulation.

If the removal of the slice of cells from the upper pole of the sea-urchin embryo takes place later, in the early gastrula stage, the rudiment of the gut is already determined and it develops relatively too large for the ectoderm: i. e. the ectoderm has been reduced by the removal of cells, but the endoderm has not been affected. However, within the gut itself harmonic equipotentiality remains, for if a portion of it is removed the remainder will divide into three regions of relatively correct proportions. The gut is therefore an harmonic equipotential *partial* system.

A similar state of affairs has been shown also to obtain in amphibia. By tying a fine hair round the gastrula of a newt and drawing it tight, the gastrula can be divided.



**FIG. 50.** Embryos of Triton divided by constriction with a hair; 1. in the early gastrula stage (blastopore crescentic); 2. in the late gastrula stage (blastopore closed and circular). Views of an embryo produced from the dorsal half of 1, from in front (3), above (4), and beneath (5). Regulation has taken place and the medullary folds are of the normal relative size, as can be seen by comparison with corresponding views of a normal embryo (6, 7, and 8). 9 and 10. Views of an embryo produced from the dorsal half of 2. Regulation has not taken place, and the medullary folds which are relatively much too large cannot close over to form a tube. The ventral halves of 1 and 2 do not develop. (*From Ruud and Spemann.*)

That half which contains the organizer or dorsal lip of the blastopore will continue developing. If this operation is performed at the early gastrula stage, the neural folds, when they form, are proportionately correct in size for the reduced organism. In other words, there ensues a regulation of the size of the neural folds relatively to the whole. On the other hand, if the division is carried out in the late gastrula stage, the neural folds are already determined. They then develop relatively too large for the reduced size of the organism. At this stage, therefore, the organism is no longer a complete harmonic equipotential system. (157)

The bud of the forelimb of the newt is equipotential. (75) A whole limb develops out of a part of a bud, and a single whole may arise from two fused rudiments. It is even possible to unite two rudiments belonging to different species and of different colours. In these cases 'chimaeras' are obtained comparable to the similarly named occurrence in variegated plants. (158, 159)

In the same way half the rudiment of the heart in an early amphibian embryo can regulate and give rise to a normal and whole heart. (173)

In the case of the limb bud of the chick (of four days' incubation), grafted on to the chorio-allantoic membrane, it is found that a fragment of the thigh rudiment will regulate to form a perfect femur, but no bones belonging to the other regions of the leg. (84) At this stage, therefore, the chick's limb bud consists of a number of harmonic equipotential partial systems, each system being able to regulate within itself.

The effect of time on plasticity and regulation is well seen in certain experiments on frogs' eggs. If a small region of an egg is injured with a hot needle up to as many as forty minutes after fertilization, normal development is not interfered with. If the injury is done an hour after fertilization, the embryo is complete but slightly

asymmetrical, being smaller on the injured side. (155) The deficiency of tissue caused by the injury has therefore not been made good. Injury an hour and a half after fertilization results in absence of some region of the embryo which would normally have been formed by the injured portion of the egg. Thus although the fate of any tissue is not irrevocably determined until chemo-differentiation sets in, the actual potency of the egg can be shown to diminish with time.

These experiments show that the power of regulation of the whole organism falls off during development. The complete harmonic equipotential system becomes broken up into a number of partial systems, and this takes place before and during the period of independent, visible, self-differentiation of the various regions, when no regeneration is possible.

Processes of reduction also show regulation, as in the case of the Planarian reduced to one-tenth of its former length. (140)

Grafting experiments are very illuminating. If a short piece of the tentacle-bearing extremity of a Hydra is grafted into the side of another normal Hydra, the two tentacle heads will, as it were, share the body between them, and when they separate again the two bodies are of the same size. (150) Similarly, if the number of tentacles of a normal Hydra is increased by grafting on others, the total number of tentacles will be reduced to normal by the resorption of some and the fusing together of others.

As to what this process of regulation may be, an explanation must be looked for in the axial gradients of 'metabolic' rate. Qualitative differences of tissue arise from quantitative differences of rate activity.

Now the gradient embraces the whole embryo, so it may be imagined that when the determination of the various regions take place this happens with regard to

the embryo as a whole. By the conception of quantitative variations along the axial gradients, it is possible to understand how the determined regions depend on a certain 'potential difference' of rate between different parts on the gradient.

Before chemo-differentiation, the gradients will there-

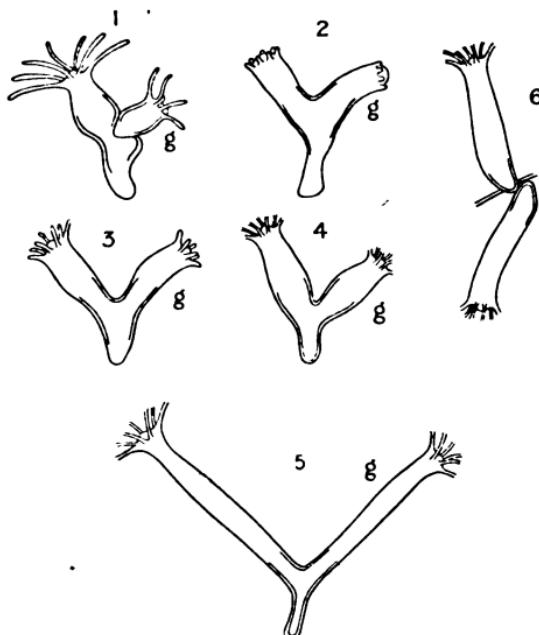


FIG. 51. Regulation in *Hydra* grafts. 1 to 6, stages in the process. Note dedifferentiation of the tentacles; the two zoids become almost equal in size and separate. (From Rand.)

fore produce harmonic equipotential systems. But after chemo-differentiation, the differences are qualitative between the various parts, the equipotentiality falls off to smaller and smaller partial systems, with consequent lack of power of regulation until the period of co-ordination and functional differentiation is reached.

It may be worth while to give some concrete examples

of the part played by axial gradients in regulation. The nerve-cord of the newt *Ambystoma* is a tapering cone, having a larger diameter in front than behind. There is, therefore, a gradient in size of the cord from head to tail. If at an early stage a piece of the cord representing trunk-segments 3, 4, and 5 be cut out, turned round, and re-planted in the order 5, 4, 3, the resulting cord will nevertheless show the normal taper. This means that 3 has been reduced to the normal size of 5, and 5 increased to the normal size of 3. This is understandable if the axial gradient of metabolic rate is regarded as controlling the gradient in size of the nerve-cord. (174)

*Stylaria* is a small worm; the head of which occupies the first five segments of the body; the crop and oesophagus are in segments six to eight. Now, amputation in the head region is followed by regeneration of the number of segments removed, up to and including five. But after self-division, or cutting *anywhere* behind the head region, five segments are regenerated forming a head, and in the three segments immediately behind this, the crop and oesophagus are formed by dedifferentiation and regulative transformation of the intestine. (151) An axial gradient runs from head to tail, and since this regulation can take place anywhere along the length of the body, the only explanation is that the crop and oesophagus are determined at a particular 'level' on that gradient.

Experiments on *Planaria* point to the same conclusion, especially those on the regeneration of pieces of the body behind the pharynx. In these no pharynx is regenerated unless first of all a head is regenerated, and then the pharynx forms at a definite level behind it. (56) Similarly in the hydroid polyp *Tubularia* the relative distances between various structures can be shown to be under the control of the axial gradients. *Tubularia* has two rows of tentacles round the hydranth, and if the latter is cut off,

the stem just beneath it undergoes reorganization into a hydranth without any growth of new tissue. In this region four zones may be distinguished : (a) between the apex and the distal tentacles ; (b) the belt of distal tentacles ; (c) between the distal and the proximal tentacles ; and (d) the belt of proximal tentacles. The widths of these zones normally bear a definite proportion to one another, but they may be increased or reduced by altering the conditions of experiment and the activity-rate of the apex. (152)

The relegating of a particular organ to a certain 'level' or distance from the apex of an organism is described as the effect of the range of dominance of a region of high activity-rate. At all levels, other than the correct one, the region of high rate inhibits other regions of lower rate. This is particularly well shown in plants in the phenomenon known as 'correlation'. (153) The dominant region in a plant is the growing point of the stem, and it inhibits the development of buds lower down on the same stem, for a certain distance. Below that level the buds are freed from the dominance of the apical region, and can and do develop. The range of dominance can be reduced by subjecting a belt of the stem to certain conditions, such as cold or narcotics, which decrease the vital activities of protoplasm. If the apical growing point is destroyed, the buds immediately below it develop at once. Conduction of excitation along the stem of *Mimosa* has been shown to be due to the diffusion of chemical substances, or hormones. (56, 182)

In newts the limb buds show a similar state of affairs. If a bud is removed and then planted back again on the same side of the same animal, but a little distant from the original position, some of the cells of the original bud may remain, and these being equipotential will regulate to produce a small limb rudiment. This small rudiment, however, will not develop if the large transplanted limb

bud is near to it, while its capacity for developing increases with the distance which separates it from the large bud. (133) The large bud therefore inhibits the other, within a certain range in which it is dominant.

In the lower animals the range of dominance appears to coincide with the limits of the individual. Planaria often divides by transverse fission. A secondary region of high metabolic rate develops (and can be experimentally demonstrated) half-way down the body. The posterior half of the body is thus physiologically isolated and has a certain degree of separate individuality, which it hastens to make complete by separating itself off from the anterior half, or stock. (154) This separation can be prevented by stimulating the head of the stock and thus raising its activity. Its range of dominance then spreads right through the secondary individual which it thus retains in its allegiance. On the other hand, by decreasing the range, which can be done quite simply by removing the head, the separation of the posterior portion takes place much sooner.

In higher forms the range of dominance is greater than the organism itself, and the delimitation of the individual is due to other causes.

Mistakes in regulation (heteromorphoses) occur when the metabolic rate is insufficiently high, so that the gradient is not steep enough. This accounts for the cases in which a tail is regenerated instead of a head in worms. Or the structure depended upon for differentiation may be absent, as, for example, the nerve in the case of the regenerating newt's limb, or the optic ganglion in the regeneration of the stalked eye of the rock-lobster.

All these cases have taken for granted that the external environment is normal. If it is not, then regulation will be affected. This is well shown by the effects of various chemical substances on the development of sea-urchin

larvae, and by the differential resorption of zooid and stolon in Perophora.

On the whole it may be concluded that the living matter of a species is only in equilibrium with its normal environment when it is in the specific form and shape of that species. When disturbed the equilibrium tends to be restored. The processes whereby this regulation is effected depend largely on axial gradients of metabolic rate from a dominant region, and are only possible when the tissues are capable of dedifferentiation or overriding a previous determination to differentiate in a particular manner. Regulation, therefore, can occur before chemo-differentiation and after the period of functional differentiation.

## XXIV

### REVIEW OF DEVELOPMENT

THESE brief descriptions of experiments and their results may with advantage be brought to a close with a short consideration of the main points which it has been possible to make in the study of animal development.

Some eggs are, to start with, totipotent and undifferentiated except for an axis, about which they are radially symmetrical. The sperm in some cases at least determines the plane of bilateral symmetry of the embryo. The other results of fertilization are biparental inheritance and activation of the egg. The nucleus is the bearer of factors which influence, among other things, the more minute characters of the organism; the broader features of the embryo are controlled by factors in the cytoplasm of the egg.

Cleavage is a process of fragmentation of the substance of the egg into small units, in which the ratio of nuclear material to cytoplasm is increased.

The earliest determination in the amphibian egg is that of the organizer in the dorsal lip of the blastopore. The various regions of the body are then invisibly determined by chemo-differentiation, at definite levels on axial gradients of metabolic rate.

There ensues a period of differentiation without function, in which the organism is an assemblage of regions most of which are self-differentiating; others undergo differentiation dependent on the former. During this period power of regulation is in abeyance.

This differentiation is partly histological, due to chemical predetermination leading to the production of certain

tissues, and partly morphological and based on considerations of available material and space. (80)

Lastly, there is a period of functional differentiation and co-ordination of the organism in which a struggle of the parts occurs. The correlation takes place by means of nerves and by hormones, by functional hypertrophy and atrophy, and by the struggle of the parts.

It is interesting in the light of present knowledge to review the old controversy as to whether preformation or epigenesis occurs during development. Preformation of course implies simply an unfolding of pre-existing complexity, whereas epigenesis means the progressive production of complexity of structure as development proceeds.

That a frog's egg should develop into no other animal than a frog is due in part to the presence of certain inherited factors which determine the first processes of the normal line of development characteristic of frogs. In so far as a kind of determination exists in the presence of these factors, one might say that there was preformation of a sort, but it is not the spatial preformation which would regard the egg as a miniature adult only waiting to be unfolded and expanded.

On the other hand, it has been conclusively shown that all the factors of development are not present as such in the egg, but that they are constantly arising as a result of mutual interaction of parts, of the effects of previous factors, and of the relation of the organism to its environment. This part of development is more nearly an epigenesis. The frog's egg will not even develop into a frog unless these factors arise and co-operate with those innate in the egg, always in relation to the external factors of the environment.

Given the initial inherited predetermination, therefore, development is a series of processes of differentiation, of producing form where none was, and each stage follows

from the previous stage as irrevocably as it is followed by the subsequent one.

The fact that nuclear division is not qualitatively unequal during cleavage prevents the possibility of regarding differentiation as due to an unequal distribution of hereditary factors in the nucleus. The Roux-Weismann theory of mosaic development is therefore untenable. On the other hand, since cases do occur in which precocious chemo-differentiation of organ-forming substances takes place, the cytoplasmic divisions during cleavage can in some animals be regarded as qualitatively unequal. The mosaic development of the so-called mosaic-eggs is therefore due to cytoplasmic localization.

The inability of organisms to regulate during the period of self-differentiation of separate organs can be illustrated by drawing the analogy from an army, whose staff at the outset of a campaign has determined and assigned the duties of the various corps. It loses control of these while they are independently performing their allotted tasks, and regains it again later when intercommunication is established. Now this lack of regulative power is of great interest from another point of view. It is the amazing results of regulation in *Clavellina*, sea-urchin blastomeres, &c., which have led to the belief that living processes cannot be explained by physics and chemistry. This form of vitalism has been developed at the hands of Driesch and others. It is therefore interesting to find that organisms do not always regulate.

As to whether physics and chemistry contain categories of phenomena adequate to explain the behaviour of living organisms is at present beside the point. Just as the properties and behaviours of chemical substances had to be determined before any great advance could be made in that science, so in biology the experimental method is revealing phenomena which one must call 'biological

properties' of living matter; such as the organization of the axial structures by the dorsal lip of the blastopore or the formation of a lens by the activity of the eye-cup. There is yet time to know whether these processes require non-physical categories to contain them. Meanwhile the processes of development are being analysed into 'complex components'. These are organic processes, based on fundamental properties of living matter. By means of them the sequence of events in development can be sorted out into chains of happenings causally connected with one another.

There is no hiding the fact that most of the complex components of development are as yet unintelligible, but by the experimental method the analysis is gradually splitting them up. The development of the frog's egg into the frog has already been split up into dozens of complex components, a few of which it has been the purpose of this book to describe.

The analysis of development by the experimental method, or *Entwicklungsmechanik* as it was called by its founder and organizer Wilhelm Roux, derives its importance from the fact that it is able to carry the analysis a step farther than the comparative method. It is based on the anatomical knowledge of developing organisms, and is indeed helpless without it. For this reason there must exist a partnership between the two. They both study the same problems and differ only in that one uses razor, microscope, and microtome, while the other, in addition, makes use of varied and controlled organic, chemical, and physical conditions.

With regard to the term 'explanation' it must be remembered that ultimately nothing can be really explained. It is idle and absurd to imagine that experimental embryology will explain the development of organisms. What it can do is to show that the processes involved therein, however

complicated and dissimilar they may be, obey certain definite laws ; it can define these activities of living matter, and show with their help that the kaleidoscopic tangle of events following one another in development can be unravelled to form simpler skeins. These activities of living matter are part of its function, and by combining knowledge of function with that of form of organisms and parts of organisms a much truer picture is obtained than is possible by the help of either alone.



## LIST OF THE EXPERIMENTS DESCRIBED

| Page Nc.   | Exp. No. | Experiment.   | Experimenter.                  | Reference.  |
|------------|----------|---|--------------------------------|---|
| 10         | 1        | Separation of two agents in tumour formation.                   | Gye, W. E.                     | <i>Lancet</i> , 209, '25, p. 109.   |
| 14         | 2        | Engulfing of sperm by filament from egg.                        | Chambers, R.                   | <i>J. Gen. Phys.</i> 5, '23, p. 821.  |
| 15, 21     | 3        | Fertilizing ability of sperm, &c.                               | Lillie, F. R., and Just, E. E. | In <i>General Cytology</i> , Section 8. Chicago, '25.   |
| 16         | 4        | Necessity of egg cortex for activation.                         | Chambers, R.                   | <i>Biol. Bull.</i> 41, '21, p. 318.   |
| 19         | 5        | Asters and monospermy.  | Brachet, A.                    | <i>Arch. Zool. Exp. et Gén.</i> 6, '10, p. 1, and <i>Arch. Ent. Mech.</i> 30, I, '10, p. 261. |
| 21         | 6        | Parthenogenesis by butyric acid and hypertonic sea-water.       | Loeb, J.                       | In <i>Artificial Parthenogenesis and Fertilization</i> . Chicago, '13.                        |
| 22         | 7        | Parthenogenesis by tannin and ammonia.                          | Delage, Y.                     | <i>Arch. Zool. Exp. et Gén.</i> 7, '08, p. 445.   |
| 22, 30, 39 | 8        | Parthenogenesis by pricking.                                    | Bataillon, E.                  | <i>Arch. Zool. Exp. et Gén.</i> 6, '10, p. 101.   |
| 24, 30, 39 | 9        | Spindle length and nuclear size, and position of grey crescent. | Herlant, M.                    | <i>Arch. de Biol.</i> 26, '11, p. 103.  |
| 24, 30     | 10       | Spindle length and cell size.                                   | Conklin, E. G.                 | <i>J. Exp. Zool.</i> 12, '12, p. 1.   |
| 24, 30     | 11       | Speed of division and spindle length.                           | Teichmann, E.                  | <i>Arch. Ent. Mech.</i> 16, '03, p. 243.  |
| 26         | 12       | Electrical conductivity of eggs.                                | Gray, J.                       | <i>Phil. Trans. Roy. Soc. B.</i> 207, '16, p. 481.  |

|            |    |   |                  |
|------------|----|---|------------------|
| 26         | 13 | Oxygen consumption and fertilization.       | Shearer, T. C.   |
| 26         | 14 | Oxygen consumption and division.            | Warburg, O.      |
| 27         | 15 | Larval hybrids.                             | Baltzer, F.      |
| 28         | 16 | Larval hybrids, nuclear influence.          | Boveri, T.       |
| 28         | 17 | Larval hybrids, cytoplasmic influence.      | Godlewski, E.    |
| 30         | 18 | Nuclear size and cell size.                 | Boveri, T.       |
| 32         | 19 | Nuclear size and cell size.                 | Herbst, C.       |
| 32         | 20 | Nuclear surface-area and chromosome number. | Hertwig, G.      |
| 33         | 21 | Nucleo-cytoplasmic ratio.                   | Dolley, D. H.    |
| 34         | 22 | Value of different chromosomes.             | Boveri, T.       |
| 36, 42,    | 23 | Suppression of cleavage.                    | Lillie, F. R.    |
| 36, 42,    | 24 | Pressure and direction of spindle.          | Hertwig, O.      |
| 37         | 25 | Frog.                                       | Hertwig, O.      |
| 38         | 26 | Cleavage and yolk.                          | Jenkinson, J. W. |
| 38         | 27 | Axis of the egg.                            | Roux, W.         |
| 39, 41, 53 | 28 | Symmetry of the egg.                        | Jenkinson, J. W. |
| 42         | 29 | First furrow and symmetry plane.            | Driesch, H.      |
| 43         | 30 | Pressure and cleavage. Sea-urchin.          | Wilson, E. B.    |
| 44, 122    | 31 | Pressure and cleavage. Nereis.              | Driesch, H.      |
| 46         | 32 | Isolation sea-urchin blastomeres.           | Zofja, R.        |
| 46         | 33 | Isolation Coelenterate blastomeres.         | Zeleny, C.       |
| 46         | 34 | Isolation Nemertine blastomeres.            | Wilson, E. B.    |

LIST OF EXPERIMENTS DESCRIBED (cont.)

| Page No. | Exp. No. | Experiment.                              | Experimenter.    | Reference.  |
|----------|----------|--|------------------|---|
| 46       | 35       | Isolation frog blastomeres.              | Brachet, A.      | <i>Arch. de Biol.</i> 21, '05, p. 103.                        |
| 48       | 36       | Isolation blastomeres Ctenophores.       | Fischer, A.      | <i>Arch. Ent. Mech.</i> 7, '98, p. 557.                       |
| 48       | 37       | Separation of lobe of Ilyanassa.         | Crampton, H. E.  | <i>Arch. Ent. Mech.</i> 3, 96, p. 1.                          |
| 48       | 38       | Separation of lobe of Dentalium.         | Wilson, E. B.    | <i>J. Exp. Zool.</i> 1, '04, p. 197.                          |
| 48       | 39       | Separation of lobe of Myzostoma.         | Driesch, H.      | <i>Arch. Ent. Mech.</i> 4, '97, p. 75.                        |
| 48       | 40       | Separation blastomeres Ascaris.          | Stevens, N. M.   | <i>Arch. Ent. Mech.</i> 27, '09, p. 622.                      |
| 48, 50   | 41       | Separation blastomeres Ascidians.        | Conklin, E. G.   | <i>J. Exp. Zool.</i> 2, '05, p. 145.                          |
| 50       | 42       | Centrifuging visible substances in eggs. | Morgan, T. H.    | <i>J. Exp. Zool.</i> 9, '10, p. 593.                          |
| 51       | 43       | Frog, half embryo.                       | Roux, W.         | <i>Gesammelte Abhandlungen</i> , ii. 22, '95.                 |
| 8, 52    | 44       | Frog's egg and gravity.                  | Roux, W.         | <i>Gesammelte Abhandlungen</i> , ii. 19, '95.                 |
| 52       | 45       | Inverted eggs and cleavage planes.       | Pflüger, E.      | <i>Flügner's Archiv</i> , 84, '83, p. 311.                    |
| 53       | 46       | Inverted eggs and streaming.             | Born, G.         | <i>Arch. Mikr. Anat.</i> 24, '85, p. 475.                     |
| 53       | 47       | Differential growth and differentiation. | Edwards, C. L.   | <i>Amer. J. Phys.</i> 6, '02, p. 351.                         |
| 54       | 48       | Heat and cell division.                  | Driesch, H.      | <i>Zeit. Wiss. Zool.</i> 55, '93, p. 1.                       |
| 54       | 49       | Temperature and development.             | Bodine, J. H.    | <i>J. Exp. Zool.</i> 42, '25, p. 91.                          |
| 56       | 50       | Electric polarity in Obelia.             | Lund, E. J.      | <i>J. Exp. Zool.</i> 89, '24, p. 357.                         |
| 56       | 51       | Osmosis, collar-cell spheres.            | de Beer, G. R.   | <i>Arch. Zool. Exp. et Gén. Notes et Rev.</i> 61, '22, p. 47. |
| 58, 79   | 52       | Effect of chemicals.                     | Jenkinson, J. W. | <i>Arch. Ent. Mech.</i> 21, '06, p. 367.                      |
| 58       | 53       | Cyclopic fish.                           | Stockard, C. R.  | <i>J. Exp. Zool.</i> 6, '09, p. 285.                          |
| 58       | 54       | Chemicals.                               | Herbst, C.       | <i>Arch. Ent. Mech.</i> 5, '97, p. 649, and 9, '00, p. 424.   |
| 62       | 55       | Frog's egg made parasitic.               | Beloglowsky, G.  |   |

|                      |    |                  |  |
|----------------------|----|------------------|--|
|                      |    | Axial gradients. | Child, C. M.   |
| 56                   |    |                  |  |
| 63                   |    |                  |  |
| 65, 127,<br>128, 132 |    |                  |  |
| 64                   | 57 | 59               | Tashiro, S.<br>Hyman, L. H., and<br>Bellamy, A. N.   |
| 55, 65, 110          | 58 | 60               | Harrison, R. G.<br>Spemann, H.<br>Huxley, J. S.<br>Mangold, O.   |
| 69                   |    | 61               | Harrison, R. G.<br>Mangold, H.<br>Spemann, H.  |
| 71, 75               |    | 62               | Harrison, R. G.<br>Mangold, H.<br>Spemann, H.  |
| 72                   |    |                  | Harrison, R. G.<br>Brachet, A.<br>Spemann, H.<br>Spemann, H.   |
| 74                   | 63 | 63               | Lewis, W. H.<br>Spemann, H.<br>Lewis, W. H.<br>Spemann, H.<br>Streeter, G. L.<br>Luther, A.  |
| 74                   |    | 64               | Lewis, W. H.<br>Spemann, H.<br>Lewis, W. H.<br>Ear vesicle.<br>Ear vesicle.<br>Ear vesicle.  |
| 75                   |    | 65               | Lens.<br>Lens.<br>Cornea.<br>Ear vesicle.  |
| 76                   |    | 66               | Lens.  |
| 78                   |    | 67               | Cornea.  |
| 78, 79               |    | 68               | Ear vesicle.   |
| 78                   |    | 69               | Ear vesicle.   |
| 79                   |    | 70               | Ear vesicle.   |
| 80                   |    | 71               | Ear vesicle.   |
| 80                   |    | 72               | Ear vesicle.   |
| 80                   |    | 73               | Ear vesicle.   |
| 80                   |    | 74               | Ear vesicle.   |
| 80, 124              |    | 75               | Limb-buds, amphibian.  |
| 82, 109              |    | 76               | Limb in eye.   |
| 82                   |    | 77               | Hole in operculum.   |
|                      |    |                  |  |
|                      |    |                  | <i>In individuality in Organisms</i> . Chicago,<br>'15. <i>The Origin and Development of the Nervous System</i> , '21.<br><i>Physiological Foundations of Behavior</i> , '24.<br><i>A Chemical Sign of Life</i> . Chicago, '17.<br><i>Biol. Bull.</i> 43, '22, p. 313.   |
|                      |    |                  | <i>Arch. Mikr. Anat.</i> 63, '04, p. 35.<br><i>Arch. Ent. Mech.</i> 43, '18, p. 448.<br><i>Nature</i> , 113. Febr. 23, '24, p. 273.<br><i>Arch. Mikr. Anat. und Ent. Mech.</i><br>100, '23, p. 198.<br><i>J. Exp. Zool.</i> 32, '21, p. 1.<br><i>Arch. Mikr. Anat. und Ent. Mech.</i><br>100, '23, p. 599.<br><i>Brit. J. Exp. Biol.</i> 2, '25, p. 493.<br><i>Arch. de Biol.</i> 33, '23, p. 343.<br><i>Verh. Deut. Zool. Ges.</i> 16, '06, p. 195.<br><i>Zool. Jahrb. (Abt. Zool. und Phys.)</i><br>32, '12, p. 1.<br><i>Amer. J. Anat.</i> 3, '04, p. 505.<br><i>Zool. Anz.</i> 31, '07, p. 379.<br><i>J. Exp. Zool.</i> 2, '05, p. 431.<br><i>Arch. Ent. Mech.</i> 30, II, '10, p. 437.<br><i>J. Exp. Zool.</i> 16, '14, p. 149.<br><i>Soc. Scient. Fennica. Comm. Biol.</i><br>2, '24, p. 1.<br><i>J. Exp. Zool.</i> 25, '18, p. 413.<br><i>Nachr. K. Ges. Wiss. Göttingen,</i><br><i>Math.-Phys. Kl.</i> 15, '13, p. 210.<br><i>Morph. Jahrb.</i> 35, '06, p. 509. |

LIST OF EXPERIMENTS DESCRIBED (cont.)

| <i>Page No.</i>           | <i>Exp. No.</i>      | <i>Experiment.</i>   | <i>Experimenter.</i>   | <i>Reference.</i>  |
|---------------------------|----------------------|--|--|--|
| 82, 106<br>83<br>83, 132  | 78<br>79<br>80       | Hypophysis and infundibulum.<br>Blastoderm grafts.<br>Head self-differentiated.  | Smith, P. E.<br>Danckhoff, V.<br>Murray, P. D. F., and<br>Huxley, J. S.  | <i>Amer. Anat. Mem.</i> 11, '20.<br><i>Anat. Rec.</i> 28, '22, p. 14.<br><i>Brit. J. Exp. Biol.</i> 3, '25, p. 9.  |
| 83<br>83<br>83<br>83, 124 | 81<br>82<br>83<br>84 | Eye, ear, nose self-differentiated.<br>Other organs self-differentiated.<br>Metanephros self-differentiated.<br>Femur self-differentiated. | Hoadley, L.<br>Hoadley, L.<br>Atterbury, R. R.<br>Murray, P. D. F., and<br>Huxley, J. S.   | <i>Biol. Bull.</i> 46, '24, p. 281.<br><i>J. Exp. Zool.</i> 42, '25, p. 143.<br><i>Amer. J. Anat.</i> 31, '23, p. 409.<br><i>J. Anat.</i> 59, '25, p. 379. |
| 85                        | 85                   | Blood-vessels.   | Oppel, A., and Roux,<br>W.   | <i>Vorträge und Aufsätze über Ent.</i><br><i>Mech.</i> 10, '10.<br><i>Amer. J. Anat.</i> 29, '21, p. 341,<br>and 32, '24, p. 475.                          |
| 85                        | 86                   | Dog's bladder.   | Carey, E. J.   | <i>Growth and Form.</i> Cambridge,<br>, 17.  |
| 87                        | 87                   | Bone spicules.   | Thompson, D'A. W.  | Stockard, C. R.<br>Dirken, B.<br>Steinitz, E.<br>Babak, E.<br>Spurling, R. G.<br>Towie, E. W.<br>Naville, A.   |
| 88                        | 88                   | Cyclopic fish.   | <i>Amer. J. Anat.</i> 10, '10, p. 369.<br><i>Zeit. Wiss. Zool.</i> 99, '12, p. 189.<br><i>Arch. Ent. Mech.</i> 20, '06, p. 537.<br><i>Biol. Zentralbl.</i> 23, '03, p. 477.<br><i>Arch. Rec.</i> 26, '23, p. 41.<br><i>Biol. Bull.</i> 2, '01, p. 289. |  |
| 87                        | 89                   | Hind brain and limbs.  |  |  |
| 87                        | 90                   | Eye and optic lobes.   |  |  |
| 87                        | 91                   | Intestine and diet.  |  |  |
| 88                        | 92                   | Chick limb regeneration.   |  |  |
| 88                        | 93                   | Newt limb regeneration.  |  |  |
| 88                        | 94                   | Frog tail regeneration.  |  |  |
| 88                        | 95                   | Lizard tail regeneration.  |  |  |
| 89                        | 96                   | Newt lens regeneration.  |  |  |
| 89                        | 97                   | Lineus gut regeneration.   |  |  |
|                           |                      |  |  | Dawyodoff, C.<br>Also <i>Zool. Ans.</i> 36, '10, p. 1.<br>Nusbaum, J., and <i>Arch. Ent. Mech.</i> 30, 1, '10, p. 74.<br>Oxner, M.                         |

|         |     |  |  |
|---------|-----|--|--|
|         |     | Allolophora ganglia regeneration.                      | Nuzum, M. F., and Biol. Bull. 47, '24, p. 213. |
| 89      | 98  | Nerve and limb regeneration.                           | Rand, H. W.                                    |
| 90      | 99  | Nerve and limb regeneration.                           | Wolf, G.                                       |
| 91      | 100 | Nerve and taste-bud regeneration.                      | Olmsted, J. M. D.                              |
| 91      | 101 | Worm head regeneration.                                | Morgan, T. H.                                  |
| 91      | 102 | Frog tail regeneration and notochord.                  | Morgan, T. H., and Davis, S. E.                |
| 91      | 103 | Lobster eye and feeler regeneration.                   | Herbst, C.                                     |
| 93      | 104 | Ten-year strain of cells <i>in vitro</i> .             | Ebeling, A. H.                                 |
| 93      | 105 | Tissue culture.  | Drew, A. H.                                    |
| 93      | 106 | Moth testis <i>in vitro</i> .                          | Goldschmidt, R.                                |
| 93, 110 | 107 | Axon formation <i>in vitro</i> .                       | Harrison, R. G.                                |
| 94, 110 | 108 | Electric current and axons.                            | Ingvar, S.                                     |
| 94      | 109 | Dedifferentiation <i>in vitro</i> .                    | Champy, C.                                     |
| 94      | 110 | Dedifferentiation <i>in vitro</i> . Effect of tissues. | Champy, C.                                     |
| 94      | 111 | Redifferentiation <i>in vitro</i> .                    | Drew, A. H.                                    |
| 96      | 112 | Redifferentiation.                                     | Strangeways, T. S. P.                          |
| 96      | 113 | Differentiation <i>in vitro</i> .                      | Rienhoff, W. F.                                |
| 98      | 114 | Tumour <i>in vitro</i> .                               | Champy, C., and Coca, F.                       |
| 98      | 115 | Embryo extract.  | Cärrel, A.                                     |
| 98      | 116 | Wound-hormones in plants.                              | Haberlandt, G.                                 |
| - 101   | 117 | Frog and thyroid.                                      | Gudernatsch, J. F.                             |
| 75      | 118 | Organizer in different species.                        | Geinitz, B.                                    |

LIST OF EXPERIMENTS DESCRIBED (cont.)

| Page No.  | Exp. No. | Experiment.                             | Experimenter.                     | Reference.  |
|-----------|----------|---|-----------------------------------|---|
| 83        | 119      | Blastoderm differentiation.             | Hoadley, L.                       | <i>J. Exp. Zool.</i> 43, '26, p. 151.   |
| 101       | 120      | Frog thyroidectomized.                  | Allen, B.                         | <i>J. Exp. Zool.</i> 24, '18, p. 499.   |
| 101       | 121      | Frog thyroidectomized and thyroid.      | Swingle, W. W.                    | <i>J. Exp. Zool.</i> 24, '18, p. 521.   |
| 102, 104, | 122      | Bull-frog and <i>Necturus</i> thyroids. | Swingle, W. W.                    | <i>Anat. Rec.</i> 23, '22, pp. 41, 100,<br>and 106.                                 |
| 106       |          |   |                                   | <i>J. Gen. Phys.</i> 1, '19, p. 473.  |
| 102       | 123      | Size at metamorphosis.                  | Uhlenhuth, E.                     | <i>Pflüger's Arch.</i> 164, '16, p. 1.  |
| 102       | 124      | Temperature and metamorphosis.          | Adler, L.                         | <i>Proc. Roy. Soc. B.</i> 94, '22, p. 204.  |
| 107       | 125      | Thyroid, pituitary and metamorphosis.   | Hogben, L. T.                     |   |
| 104       | 126      | Different susceptibility to thyroid.    | Uhlenhuth, E.                     | <i>Biol. Bull.</i> 42, '22, p. 143.   |
| 105       | 127      | Thyroid and cell division.              | Champy, C.                        | <i>Arch. de Morph. Exp. et Gén.</i> 4, '22.   |
| 105       | 128      | Metamorphosis.                          | Huxley, J. S.                     | <i>Science Progress</i> , 68, '23, p. 606.  |
| 106       | 129      | Frogs and iodine.                       | Swingle, W. W.                    | <i>J. Gen. Phys.</i> 2, '20, p. 161.  |
| 106       | 130      | Thyroidectomized tadpoles and iodine.   | Swingle, W. W.                    | <i>J. Exp. Zool.</i> 27, '19, p. 397.   |
| 104, 106  | 131      | Iodine and axolotls.                    | Huxley, J. S., and Hogben, L. T.  | <i>Proc. Roy. Soc. B.</i> 93, '22, p. 36.   |
| 108       | 132      | Aneurogenic limbs.                      | Harrison, R. G.                   | <i>J. Exp. Zool.</i> 4, '07, p. 239.  |
| 108, 129  | 133      | Nerve and transplanted limbs.           | Detwiler, S. R.                   | <i>J. Exp. Zool.</i> 31, '20, p. 117.   |
| 110       | 134      | Nerves in grafts.                       | Hoadley, L.                       | <i>J. Exp. Zool.</i> 42, '25, p. 163.   |
| 111       | 135      | Neurobiotaxis.                          | Kappers, C. A.                    | <i>Brain</i> , 44, '21, p. 125.   |
| 110       | 136      | Transplanted placodes.                  | Stone, L. S.                      | <i>J. Comp. Neur.</i> 38, '24, p. 73.   |
| 114       | 137      | Concrecence, sharks.                    | Kastchenko, N.                    | <i>Anat. Anz.</i> 8, '88, p. 445.   |
| 114       | 138      | Concrecence, trout.                     | Kopsch, F.                        | <i>Verh. Anat. Ges. Berlin</i> , 10, '90,<br>p. 113.                                |
| 114       | 139      | Concrecence, newt.                      | Goodale, H. D. Also Goerttler, K. | <i>Amer. J. Anat.</i> 12, '11, p. 173.<br><i>Arch. Ent. Mech.</i> 106, '26, p. 503. |

|         |     |                                   |  |
|---------|-----|-----------------------------------|--|
|         |     |                                   | In <i>Senescence and Rejuvenescence</i> .                |
| 115, 25 | 140 | Child, C. M.                      | Chicago, '15, p. 35.                                     |
|         | 141 | de Beer, G. R., and Huxley, J. S. | <i>Q. J. Micr. Sci.</i> 68, '24, p. 471.                 |
| 115     | 142 | Runnström, J.                     | <i>Arch. Ent. Mech.</i> 43, '18, p. 223.                 |
| 116     | 143 | Driesch, H.                       | <i>Arch. Ent. Mech.</i> 14, '02, p. 247.                 |
| 118     | 144 | Huxley, J. S., and de Beer, G. R. | <i>Q. J. Micr. Sci.</i> 67, '23, p. 473.                 |
| 118     | 145 | Huxley, J. S.                     | <i>Q. J. Micr. Sci.</i> 65, '21, p. 643.                 |
| 120     | 146 | Siperstein, D. M.                 | <i>Anat. Rec.</i> 20, '21, p. 355.                       |
| 121     | 147 | Huxley, J. S.                     | <i>Phil. Trans. Roy. Soc. B.</i> 202, '11, p. 165.       |
|         | 148 | Morgan, T. H.                     | <i>Arch. Ent. Mech.</i> 9, '09, p. 563.                  |
| 122     | 149 | Driesch, H.                       | <i>Arch. Ent. Mech.</i> 2, '96, p. 169.                  |
| 122     | 150 | Rand, H. W.                       | <i>Arch. Ent. Mech.</i> 9, '09, p. 161.                  |
| 125     | 151 | Harper, E. H.                     | <i>Biol. Bull.</i> 6, '04, p. 173.                       |
| 127     | 152 | Child, C. M.                      | <i>Arch. Ent. Mech.</i> 23, '07, p. 415.                 |
| 128     | 153 | Mogk, W.                          | <i>Arch. Ent. Mech.</i> 38, '14, p. 584.                 |
| 128     | 154 | Child, C. M.                      | <i>Arch. Ent. Mech.</i> 30 (ii), '10, p. 159.            |
| 129     | 155 | Brachet, A.                       | <i>Arch. Ent. Mech.</i> 22, '06, p. 325.                 |
| 125     | 156 | Spemann, H.                       | <i>Sitzber. Ges. Nat. Freunde, Berlin</i> , '16, p. 306. |
| 76      |     |                                   | <i>Arch. Ent. Mech.</i> 52, '23, p. 95.                  |
| 77, 124 | 157 | Ruud, G., and Spemann, H.         | <i>Arch. Ent. Mech.</i> 48, '21, p. 533.                 |
| 124     | 158 | Spemann, H.                       | <i>Genetica</i> , 4, '22, p. 339.                        |
| 124     | 159 | Schäxel, J.                       | <i>Zool. Jahrb., Festj.-Spengel</i> , 3, '12,            |
| 72      | 160 | Spemann, H.                       | p. 1.  |
|         |     |                                   | <i>Arch. Ent. Mech.</i> 39, '14, p. 328.                 |
| 79      | 161 | Ekmann, G.                        | <i>Arch. Mikr. Anat. und Ent. Mech.</i>                  |
| 79      | 162 | Filatow, D.                       | 104, '24, p. 50.   |
| 79      | 163 | le Cron, W. L.                    | <i>Amer. J. Anat.</i> 6, '06, p. 245.                    |

LIST OF EXPERIMENTS DESCRIBED (*cont.*)

| Page No. | Expt. No. | Experiment.  | Experimenter.                  | Reference.   |
|----------|-----------|--|--------------------------------|--|
| 82       | 164       | Operculum hole.<br>Regeneration and sympathetic.                                   | Weber, A.<br>Schotte, O.       | C. R. Assoc. Anat. 19, '24, p. 287.<br>C. R. Soc. Phys. et Hist. Nat. Genève, 39, '22, p. 137. |
| 90       | 165       | Lens, Rana and Bufo.<br>Liver, pancreas, gut-wall, and heart self-differentiation. | Filatow, D.<br>Holtfreter, J.  | Arch. Ent. Mech. 105, '25, p. 475.<br>Arch. Ent. Mech. 105, '25, p. 330.                       |
| 79       | 166       | Limb-bud polarity.   | Harrison, R. G.                | Arch. Ent. Mech. 106, '26, p. 469.   |
| 80       | 167       | Axial gradients and ultra-violet light.  | Hinrichs, M. A.                | J. Expt. Zool. 41, '25, p. 21.   |
| 74       | 168       | Parthenogenesis and fertilization.   | Gray, J.                       | Q. J. Micr. Sci. 66, '22, p. 419.  |
| 65       | 169       | Snail eye regeneration.  | Nonne, F.                      | Arch. Ent. Mech. 105, '25, p. 430.   |
| 22       | 170       | Optic nerve and transplantation.   | May, R.                        | J. Expt. Zool. 43, '26, p. 83.   |
| 91       | 171       | Frog heart regulation.   | M., and<br>Detwiler, S. R.     | Arch. Ent. Mech. 106, '26, p. 409.   |
| 109      | 172       | Axial gradients and nerve-cord.  | Stöhr, P., jun.                | Arch. Ent. Mech. 106, '26, p. 320.   |
| 124      | 173       | Axial gradients and nerve-cord.  | Ekman, G.                      | J. Expt. Zool. 42, '25, p. 333.  |
| 127      | 174       | Axial gradients.   | Detwiler, S. R.                | Anat. Rec. 31, '25, p. 369.  |
| 65       | 175       | Cell-volume.   | Child, C. M.                   | Biol. Zentralbl. 44, '24, p. 145.  |
| 32       | 176       | Vein and artery.   | Wettstein, F. von.             | Frankfurt. Zeit. f. Path. 3, '09, p. 1.  |
| 85       | 177       | Axial gradients and regeneration.  | Fischer, B., and Schmidien, V. | J. Expt. Zool. 20, '16, p. 99.   |
| 67       | 178       | Pluteus reduction.   | Hyman, L. H.                   | Biol. Bull. 43, '22, p. 210.   |
| 115      | 179       | Clavellina reduction.  | Huxley, J. S.                  | Mitt. Zool. Stat. Neapel.  |
| 116      | 180       | Struggle of parts.   | Huxley, J. S.                  | Der züchtende Kampf der Teile.   |
| 118      | 181       | Correlation and hormones.  | Roux, W.                       | Leipzig ('81).   |
| 128      | 182       | Tumours and Pregnancy.   | Snow, R.                       | Proc. Roy. Soc. B. 96, '24, p. 349,<br>and Proc. Roy. Soc. B. 98, '25,<br>p. 188.              |
| 120      | 183       |  |                                | Slye, M.   |

# INDEX

Figures in dark type following an author's name refer to the List of Experiments described on pages 136 to 144, from which the page references can be obtained. Where a subject is treated on two or more consecutive pages, reference is here made to the first of such pages.

- |  |  |
|--|--|
| <p>Acclimatization, 65.<br/>     Adler, L., 124.<br/>     Agglutination, 16.<br/>     Allen, B., 120.<br/> <b>Allolobophora</b>, ganglion regeneration, 89.<br/>     Ammonia, and parthogenesis, 22.<br/> <b>Amphibia</b>, blastomeres, 46, 51.<br/>     —, regulation, 124.<br/> <b>Amphioxus</b>, blastomeres, 46.<br/>     'Aneurogenic' limbs, 108.<br/> <b>Antedon</b> hybrids, 28.<br/> <b>Antenna</b> and eye regeneration, 91.<br/> <b>Artery</b> and vein transplantation, 85.<br/> <b>Ascaris</b>, fertilization, 14.<br/> <b>Ascidians</b>, blastomeres, 48.<br/> <b>Asters</b>, 14, 22, 39.<br/> <b>Atterbury</b>, R. R., 83.<br/> <b>Aurelia</b>, reduction, 115.<br/>     Autolysed extract, 98.<br/>     Autotomy, 88.<br/> <b>Axis</b>, of frog's egg, 8, 38, 52.<br/>     —, of organism, 65.<br/>     Axon differentiation, 56, 93, 110.</p> | <p>Brachet, A., 5, 35, 66, 155.<br/>     Brain, and limb bud, 87.<br/>     — and eye, 87.<br/>     Braus, H., 77.<br/>     Butyric acid, and parthogenesis, 21.</p> <p>Calcium, effects of, 60.<br/>     Campanularia reduction, 118.<br/>     Cane sugar, effects of, 58.<br/>     Carey, E. J., 86.<br/>     Carnivorous diet, effects of, 87.<br/>     Carrel, A., 115.<br/>     Cartilage, <i>in vitro</i>, 94.<br/>     Cell division, and differentiation, 96.<br/>     — and thyroid, 105.<br/>     Cell volume, 24, 30.<br/> <b>Chaetopterus</b>, fertilization, 13.<br/>     —, cleavage, 36.<br/> <b>Chambers</b>, R., 2, 4.<br/> <b>Champy</b>, C., 109, 110, 127.<br/>     and Coca, F., 114.<br/>     Chemical composition of medium, 58.<br/> <b>Child</b>, C. M., 56, 140, 152, 154, 175.<br/>     'Chimaeras', 124.<br/>     Chlorine, effects of, 60.<br/>     Chorio-allantoic grafts, 82.<br/>     Chromosome number, 32.<br/>     Clavellina reduction, 115, 133.<br/>     Cleavage, 26, 30.<br/> <math>\text{CO}_2</math> and sperm, 15.<br/>     Coca, F., Champy, C., and —, 114.<br/>     Coelenterate blastomeres, 46.<br/>     Collar-cell spheres, 56.<br/>     Complex components, 134.<br/>     Conditions, 9.<br/>     Connective tissue, <i>in vitro</i>, 94.<br/>     Conklin, E. G., 10, 41.<br/>     Controls, 10.<br/>     Copulation path, 41.<br/>     Cornea, differentiation, 80.</p> <p>C<br/>     C<br/>     C<br/>     L<br/>     C<br/>     '</p> |
|--|--|

- Danchakoff, V., 79.  
 Davis, S. E., Morgan, T. H., and —, 102.  
 Dawydoff, C., 97.  
 de Beer, G. R., 51.  
     and Huxley, J. S., 141.  
     Huxley, J. S., and —, 144.  
 Dedifferentiation, 94.  
 Delage, Y., 7.  
 Dentalium, polar lobe, 48.  
 Dependent differentiation, 78, 92.  
 Detwiler, S. R., 133, 174.  
     May, R. M., and —, 172.  
 Dinophilus, fertilization, 13.  
 Dolley, D. H., 21.  
 Dorsal lip of blastopore, 38, 46, 68, 75.  
 Drew, A. H., 105, 111.  
 Driesch, H., 29, 31, 39, 48, 143, 149.  
 Dürken, B., 76, 89.  
 Ear differentiation, 80, 83.  
 Ebeling, A. H., 104.  
 Echinoderm larvae reduction, 115.  
 Echinoidea, fertilization, 13, 14.  
 Echinus, hybrids, 28.  
 Ectoderm, transplantation, 73.  
 Edwards, C. L., 47.  
 Ekman, G., 161, 173.  
 Electricity, effects of, 55.  
 Electric conductivity of egg, 26.  
 Electric potential, and gradients, 65.  
 Embryo extract, 98.  
*Entwicklungsmechanik*, 134.  
 Epigenesis, 132.  
 Epithelium, *in vitro*, and transplanted, 71, 78, 94.  
 Equipotential systems, 46, 122.  
 Exogastrula, 58.  
 Eye, determination, 72.  
     —, differentiation, 77, 83.  
     —, extirpation, 87.  
     —, regeneration, 90.  
 Fertilization membrane, 14, 21.  
 Fertilizin, 17, 20.  
 Filament, and sperm, 14.  
 Filatow, D., 162, 166.  
 First furrow of cleavage, 39, 52.  
 Fischel, A., 36, 96.  
 Fischer, B., and Schmieden, V., 177.  
 Frog's egg cleavage, 42.  
 Fundulus, cyclopic eye, 58.  
 Ganglion regeneration, 89.  
 Geinitz, B., 118.  
 Germ layers, 7, 74.  
 Godlewski, E., 17.  
 Goerttler, K., 139.  
 Goldschmidt, R., 106.  
 Goodale, H. D., 139.  
 Gravity, effects of, 8, 39, 52.  
 Gray, J., 12, 170.  
 Grey crescent, 38, 76.  
 Gudernatsch, J. F., 117.  
 Gye, W. E., 1.  
 Haberlandt, G., 116.  
 Harmonic equipotential systems, 122.  
 Harper, E. H., 151.  
 Harrison, R. G., 59, 63, 75, 107, 132, 168.  
 Heart differentiation, 80, 124.  
 Herbst, C., 19, 54, 103.  
 Herlant, M., 9.  
 Hertwig, G., 20.  
 Hertwig, O., 24, 25.  
 Heteromorphosis, 91, 129.  
 Hinrichs, M. A., 169.  
 Histological differentiation, 84, 131.  
 Hoadley, L., 81, 82, 119, 134.  
 Hogben, L. T., 125.  
     Huxley, J. S., and —, 131.  
 Holtfreter, J., 167.  
 Huxley, J. S., 61, 128, 145, 147, 179, 180.  
     Murray, P. D. F., and —, 80-4.  
     de Beer, G. R., and —, 141-4.  
     and Hogben, L. T., 131.  
 Hydra, graft regulation, 125.  
 Hyman, L. H., 178.  
     — and Bellamy, A. N., 58.  
 Hypertonic sea-water, 21, 56.  
 Hypophysis extirpation, 82.  
 Hypotonic sea-water, 56.  
 Ilyanassa, blastomeres, 48.  
 Infundibulum, 82.  
 Ingvar, S., 108.  
 Insects, fertilization, 15.  
 Intestine, area and diet, 87.

- Intestine, differentiation, 83, 96.  
 Iodine effects of, 106.  
 Iso-agglutinin, 16.
- Jenkinson, J. W., 26, 28, 52.  
 Just, E. E., Lillie, F. R., and —, 3.
- Kappers, C. A., 135.  
 Kastchenko, N., 137.  
 Kidney, *in vitro*, 94.  
 Kopsch, F., 138.
- Lateral line organs, 68.  
 Le Cron, W. L., 163.  
 Lens, differentiation, 78.  
 —, regeneration, 89.  
 Lewis, W. H., 69, 71.  
 Light, effects of, 39.  
 Lillie, F. R., 23.  
 — and Just, E. E., 3.  
 Limb bud, determination, 74.  
 —, differentiation, 80, 83, 124.  
 —, extirpation, 87.—  
 —, regeneration, 88.  
 Limb regeneration, 88.  
 Lineus, gut regeneration, 89.  
 Lithium, effects of, 58.  
 Liver differentiation, 80, 83.  
 Lizard, tail regeneration, 88.  
 Loeb, J., 6.  
 Lumbricus, gradients, 67.  
 Lund, E. J., 50.  
 Luther, A., 74.
- Magnesium, effects of, 58.  
 Mangold, H., and Spemann, H., 64.  
 Mangold, O., 62.  
 Maturation, 13.  
 May, R. M., and Detwiler, S. R., 172.  
 Mesonephros, and metanephros differentiation, 83.  
 Mogk, W., 153.  
 Morgan, T. H., 42, 101, 148.  
 —, and Davis, S. E., 102.  
 Morphallaxis, 121.  
 Morphological differentiation, 84, 132.  
 'Mosaic egg', 49.  
 Mosses, cell volume, 32.  
 Murray, P. D. F., and Huxley, J. S., 80, 84.  
 Myzostoma, blastomeres, 48.
- Naville, A., 94.  
 Nematode, blastomeres, 48.  
 Nemertine, blastomeres, 46.  
 Nereis, fertilization, 13, 21.  
 —, cleavage, 42.  
 Nerve and regeneration, 90.  
 Nerve-cord, 58, 71, 127.  
 Neural crest, differentiation, 83.  
 Neurobiotaxis, 111.  
 Nonne, F., 171.  
 Nose, differentiation, 83.  
 Notochord, 58, 91.  
 Nuclear, membrane, 16.  
 —, volume, 24, 30.  
 Nucleo-cytoplasmic ratio, 33.  
 Nusbaum, J., and Oxner, M., 97.  
 Nuzum, M. F., and Rand, H. W., 98.
- Obelia, polarity, 55.  
 —, reduction, 118.  
 Olmsted, J. M. D., 100.  
 Operculum hole, 82.  
 Oppel, A., and Roux, W., 85.  
 Organ-forming substances, 48, 74.  
 'Organizer', 75.  
 Osmosis, 56.  
 Oxner, M., Nusbaum, J., and —, 97.  
 Oxygen consumption, 26, 63.
- Pancreas, differentiation, 80.  
 Penetration path, 41.  
 Perophora, reduction, 118.  
 Pflüger, E., 45.  
 Pituitary, differentiation, 82.  
 —, and metamorphosis, 106.  
 Placode, transplantation, 109.  
 Planaria, gradients, 65.  
 —, reduction, 115.  
 —, regulation, 127.  
 Polyspermy, 15.  
 Polyzoa, fertilization, 15.  
 Potassium, effects of, 58.  
 Preformation, 132.  
 Pressure, effect on cleavage, 36, 42.  
 —, — bladder, 85.  
 —, — bone, 86.  
 Pronephros, differentiation, 83.
- Rand, H. W., 150.  
 —, Nuzum, M. F., and —, 98.

- 'Regulation' egg, 49.  
 Rhabditis, 27.  
 Rienhoff, W. F., 113.  
 Rous tumour, 11.  
 Roux, W., 27, 43, 44, 181.  
 —, Oppel, A., and —, 85.  
 Runnström, J., 142.  
 Ruud, G., and Spemann, H., 157.
- Schaxel, J., 159.  
 Schmieden, V., Fischer, B., and —, 177.  
 Schotté, O., 165.  
 Sea-urchin, cleavage, 42.  
 —, blastomeres, 44.  
 —, regulation, 122.  
 Self-differentiation, 77, 96.  
 Semi-permeable membrane, 56.  
 Shearer, T. C., 13.  
 Siperstein, D. M., 146.  
*Situs inversus*, 78.  
 Size and metamorphosis, 102.  
 Slye, M., 183.  
 Smith, P. E., 78.  
 Snow, R., 182.  
 Specificity, 17.  
 Spemann, H., 60, 65, 67, 68, 70,  
 72, 156, 158, 160.  
 —, Mangold, H., and —, 64.  
 —, Ruud, G., and —, 157.  
 Sphaerechinus, hybrids, 27.  
 Spindle, 23, 30, 36, 39.  
 Sponge, regulation, 121.  
 Spurling, R. G., 92.  
 Steinitz, E., 90.  
 Stevens, N. M., 40.  
 Stockard, C. R., 53, 88.  
 Stöhr, P., jun., 173.  
 Stone, L. S., 136.  
 Strangeways, T. S. P., 112.  
 'Streaming meridian', 53.  
 Streeter, G. L., 73.  
 Striated muscle, 85.  
 Strongylocentrotus hybrids, 27, 31.  
 Struggle of the parts, 118.
- Styela, 50.  
 Styilaria, regulation, 127.  
 Sub-culturing, 12, 93.  
 Surface-area of nucleus, 32.  
 Susceptibility, 65, 104.  
 Swingle, W. W., 121, 122, 129, 130.  
 Sympathetic system and regenerations, 90.
- Tail regeneration, 88.  
 Tannin and parthenogenesis, 22.  
 Tashiro, S., 57.  
 Taste-bud, regeneration, 91.  
 Teichmann, E., 11.  
 Temperature coefficient, 53.  
 Thomson, D'A. W., 87.  
 Thyroid, and metamorphosis, 9,  
 101.  
 Towle, E. W., 93.  
 Trochosphere and gradients, 65.  
 Tubularia, regulation, 127.  
 Tumour, cells *in vitro*, 94, 96.  
 —, extract, 98.  
 —, formation, 11.  
 —, and pregnancy, 120.
- Uhlenhuth, E., 123, 126.  
 Ultra-violet light and gradients, 65.  
 Urea, effects of, 58.
- Vegetarian diet, effects of, 87.  
 Vein transplantation, 85.  
 Vertebrates, fertilization, 13.  
 Vitalism, 133.
- Warburg, O., 14.  
 Weber, A., 164.  
 Wettstein, F. von, 176.  
 Wilson, E. B., 30, 34, 38.  
 Wolff, G., 99.  
 Woodland, W. N. F., 95.
- Yolk, and axis, 8, 52, 67.  
 —, and cle
- Zeleny, C.  
 Zoja, R.,







[REDACTED]

3 9015 05844 3188

ction to  
embryo-

JUL 31  
1956  
26 1956





