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Jacques BENVENISTE
Homeopathy & Digital Biology

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http://en.wikipedia.org/wiki/Jacques_Benveniste

Jacques Benveniste

Jacques Benveniste (March 12, 1935–October 3, 2004) was a French immunologist. In 1979 he published a well-known paper on the structure of platelet-activating factor and its relationship with histamine. He was head of INSERM's Unit 200, directed at immunology, allergy and inflammation.

Benveniste was at the center of a major international controversy in 1988, when he published a paper in the prestigious scientific journal Nature describing the action of very high dilutions of anti-IgE antibody on the degranulation of human basophils, findings which seemed to support the concept of homeopathy. Biologists were puzzled by Benveniste's results, as only molecules of water, and no molecules of the original antibody, remained in these high dilutions. Benveniste concluded that the configuration of molecules in water was biologically active; a journalist coined the term water memory for this hypothesis. Much later, in the nineties, Benveniste also asserted that this "memory" could be digitized, transmitted, and reinserted into another sample of water, which would then contain the same active qualities as the first sample.

As a condition for publication, Nature asked for the results to be replicated by independent laboratories. The controversial paper published in Nature was eventually co-authored by four laboratories worldwide, in Canada, Italy, Israel, and in France [1]. After the article was published, a follow-up investigation was set up by a team including physicist and Nature editor John Maddox, illusionist and well-known skeptic James Randi, as well as fraud expert Walter Stewart who had recently raised suspicion on the work of Nobel Laureate David Baltimore [2]. With the cooperation of Benveniste's own team, the group failed to replicate the original results, and subsequent investigations did not support Benveniste's findings either. Benveniste refused to retract his controversial article, and he explained (notably in letters to Nature) that the protocol used in these investigations was not identical to his own. However, his reputation was damaged, so he began to fund his research himself as his external sources of funding were withdrawn. In 1997, he founded the company DigiBio to "develop and

commercialise applications of Digital Biology."

Benveniste died in Paris at the age of 69 after heart surgery. He was married twice and had five children.

Nature publication and investigation --Unusual conditions

Nature agreed to publish Benveniste's article in June 1988 with two unusual conditions: first, that Benveniste obtain prior confirmation of his results from other laboratories;[citation needed] second, that a team selected by Nature be allowed to investigate his laboratory following publication. Benveniste accepted these conditions; the results were replicated by four laboratories, in Milan, Italy; in Toronto, Canada; in Tel-Aviv, Israel and in Marseille, France.[citation needed]

Unusual disclaimer

Following replication, the article was then published in *Nature*, which printed an editorial titled "When to believe the unbelievable" in the same issue of the journal and attached the following disclaimer to the article: "Editorial reservation: Readers of this article may share the incredulity of the many referees. . . There is no physical basis for such an activity. . . *Nature* has therefore arranged for independent investigators to observe repetitions of the experiments." The last time such a disclaimer had been added was in 1974 to an article on Uri Geller.

Critical investigation

A week after publication of the article, *Nature* sent a team of three investigators to Benveniste's lab to attempt to replicate his results under controlled conditions. The team consisted of Nature editor and physicist Sir John Maddox, American scientific fraud investigator and chemist Walter Stewart, and skeptic and former magician James Randi.

The team pored over the laboratory's records and oversaw seven attempts to replicate Benveniste's study. Three of the first four attempts turned out somewhat favorable to Benveniste; however the *Nature* team was not satisfied with the rigor of the methodology. Benveniste invited them to design a double blind procedure, which they did, and conducted three more attempts. Before fully revealing the results, the team asked if there were any complaints about the procedure, but none were brought up. These stricter attempts turned out negative for Benveniste. In response to Benveniste's refusal to withdraw his claims, the team published in the July 1988 edition of *Nature* [3] the following critiques of Benveniste's original study:

1. Benveniste's experiments were "statistically ill-controlled", and the lab displayed unfamiliarity with the concept of sampling error. The method of taking control values was not reliable, and "no substantial effort has been made to exclude systematic error, including observer bias"

2. "interpretation has been clouded by the exclusion of measurements in conflict with the claim". In particular, blood that failed to degranulate was "recorded but not included in analyses prepared for publication". In addition, the experiment sometimes completely failed to work for "periods of several months".

3. There was insufficient "avoidance of contamination", and, to a large extent, "the source of blood for the experiments is not controlled".

4. The study had not disclosed that "the salaries of two of Dr Benveniste's coauthors of the published article are paid for under a contract between INSERM 200 and the French company Boiron et Cie."

5. "The phenomenon described is not reproducible". "We believe that experimental data have been uncritically assessed and their imperfections inadequately reported."

Response

In the same issue of the journal *Nature*, and in subsequent commentary, Benveniste derided the *Nature* team's "mockery of scientific inquiry" and warned other scientists not to permit such investigations into their own labs.[citation needed] He claimed that such "Salem witchhunts or McCarthy-like prosecutions will kill science." Some of his criticisms included:

1. "Lip service is paid to our honesty; yet accusation of cheating was rampant". For example, the *Nature* team implied that the lab's partial funding from the homeopathy industry was cause for concern, even though industry funding - both homeopathic and non-homeopathic - of research is commonplace.

2. The team of non-biologists displayed "amateurism", failed to "get to grips with our biological system", created an atmosphere of "constant suspicion", and their member James Randi played tricks and pulled stunts such as taping information to the ceiling to prevent tampering.

3. The team arrived without a prior plan, and based on one week of work "would blot out five years of our work

and that of five other laboratories".

4. The blinded attempts likely failed due to "erratic controls", the excessive work-load, and the team's experimental design.

5. Benveniste totally rejected the team's allegations of unfamiliarity with sampling error, and of the unreliability of his control values.[3]

Attempts to replicate Benveniste's results

Academy of Sciences

In 1991, Benveniste found the French Academy of Sciences willing to publish his latest results, obtained under the supervision of a statistician, in its weekly Proceedings. Eric Fottorino writing in Le Monde relates how the remorseful Academy of Science noticed that an earlier edition contained a study critical of the memory of water. Seizing on this opportunity, the Academy ordered the printing to stop and the already printed copies destroyed, so that it could print a revised edition, in which Benveniste's article was labeled a mere "right of reply" - downgraded from the status of an article.

Although the new findings fell substantially short of confirming the patterns previously claimed by Benveniste, writer Yves Lignon quotes study co-author and statistician Alfred Spira, who said that "the transmission of information persisted at high dilution", and acknowledged that a "weakness in the experimental procedure was possible".

Ovelgonne et al.

A group of Dutch researchers reported their failure to duplicate the results in Experientia in 1992:

"In fact, in our hands no effect of extreme dilutions was shown at all. We conclude that the effect of extreme dilutions of anti-IgE, reported by Davenas et al., needs further clarification and that in this process the reproducibility of results between experimenters should be carefully determined."

Hirst et al.

A group of English researchers reported another failure to duplicate the results in Nature in 1993:

"Following as closely as possible the methods of the original study, we can find no evidence for any periodic or polynomial change of degranulation as a function of anti-IgE dilution."

However, Benveniste in a 1994 letter to *Nature* argued that the study neglected to faithfully follow his methods. The study has also been criticized on the grounds that its results were more favourable to Benveniste's claims than the study authors acknowledged in their conclusion.[4][5]

Josephson and the APS

Benveniste gained the public support[6] of Brian Josephson, a Nobel physicist with a reputation for openness to paranormal claims. Time magazine reported in 1999 that, in response to skepticism from physicist Robert Park, Josephson had challenged the American Physical Society (APS) to oversee a replication by Benveniste, using "a randomized double-blind test", of his claimed ability to transfer the characteristics of homeopathically diluted water over the Internet. The APS accepted and offered to cover the costs of the test, and Benveniste wrote "fine by us" in his DigiBio NewsLetter in response to Randi's offer to throw in the $1 million challenge prize-money if the test succeeded. However, Randi in his Commentary notes that Benveniste and Josephson did not follow up on their challenge.

Ennis et al.

An article published in *Inflammation Research* in 2004 brought new media attention to the issue with this claim:

"In 3 different types of experiment, it has been shown that high dilutions of histamine may indeed exert an effect on basophil activity. This activity observed by staining basophils with alcian blue was confirmed by flow cytometry. Inhibition by histamine was reversed by anti-H2 and was not observed with histidine these results being in favour of the specificity of this effect We are however unable to explain our findings and are reporting them to encourage others to investigate this phenomenon."[7]

Following up on a study they had published in 1999 in the same journal, the researchers concluded that an effect did exist. Some of the researchers had not been involved in homeopathic research before, while others had, such as former Benveniste collaborator Philippe Belon, Research Director at the homeopathic company Boiron. It was Madeleine Ennis who received the most attention in the media. Ennis led the activities at the British lab, with other labs in Europe, running a variation of Benveniste's water memory experiments. Ennis states that she

began the research as a skeptic, but concluded that the "results compel me to suspend my disbelief and start searching for rational explanations for our findings."[8]

BBC Horizon

In 2002 *BBC Horizon* broadcast its failed attempt to win James Randi's $1 million prize to prove that a highly diluted substance could still have an effect. Prominent spokespersons on both sides of the debate were interviewed, including Benveniste. See water memory.

Digital Biology

With the support of Brian Josephson, increasingly odd experiments continued, culminating in a 1997 paper claiming a water memory effect could be transmitted over phone lines.[9] This culminated in two additional papers in 1999[10] and another on remote-transmission in 2000.[11]

Intrigued by Benveniste's claims that biological interactions could be digitized, the US Defence Advanced Research Projects Agency (DARPA) asked Dr. Wayne Jonas, homeopath and then director of the US National Center for Complementary and Alternative Medicine, to organize an attempt at independently replicating the claimed results. An independent test of the 2000 remote-transmission experiment was carried out in the USA by a team funded by the US Department of Defense. Using the same experimental devices and setup as the Benveniste team, they failed to find any effect when running the experiment. Several positive results were noted, but only when a particular one of Benveniste's researchers was running the equipment. Benveniste admitted to having noticed this himself, and offered a variety of reasons to explain away what appeared to be another example of experimenter effect. The experiment is also notable for the way it attempted to avoid the confrontational nature of the earlier Maddox test.[12] The study implemented "A social and communication management process that was capable of dealing with conflicting interpersonal dynamics among vested parties in the research effort." One of Benveniste's machines was used, and, in the design and pilot project phase in 2001, Benveniste and other members of his DigiBio lab participated as consultants. Interviews at the time indicated study participants were satisfied with the way the study was being conducted. In the end, the authors reported in the FASEB Journal in 2006 that "Our team found no replicable effects from digital signals".

INSERM

The July 1989 edition of *Nature* reported that INSERM placed Benveniste on probation following a routine evaluation of his lab. Although INSERM found that his laboratory activities overall were exemplary, it expressed severe discomfort with his high dilution studies, and criticized him for "an insufficiently critical analysis of the results he reported, the cavalier character of the interpretations he made of them, and the abusive use of his scientific authority vis-à-vis his informing of the public".[13]

Benveniste and homeopathy

Nearly all conventional scientists believe that there is no credible evidence to support claims that homeopathic remedies actually work, nor is there a plausible mechanism to explain how homeopathy could work.[14] Indeed, skeptics often dismiss homeopathy out of hand, citing internal inconsistencies in the hypothesis, and the fact that biological reactions require the presence of chemicals, whereas homeopathic remedies are so diluted that they are equivalent to pure water. Homeopathists respond to the latter that this is a straw man argument, since they have long acknowledged the absence of chemicals in their products. Homeopathists have instead based their claims on some other yet-to-be-discovered mechanism.

Benveniste's 1988 article attracted attention in large part because it hinted at a potential mechanism that could be used by proponents of homeopathy to explain how homeopathy might work. This is the idea that water may somehow retain a memory of a substance that it no longer contains.

Conventionally, pure water is pure water, regardless of whether it once contained a substance in the past. Benveniste challenged this convention by claiming that water that had once contained antibodies but had had them removed could affect a basophil just as if the water still contained antibodies.

Miscellaneous

Benveniste has been awarded two Ig Nobel Prizes in Chemistry. They are a parody of the Nobel Prizes. The first in 1991 describes Jacques Benveniste as a "prolific proselytizer and dedicated correspondent of Nature, for his persistent belief that water, H2O, is an intelligent liquid, and for demonstrating to his satisfaction that water is able to remember events long after all trace of those events has vanished." The second in 1998 cites "his homeopathic discovery that not only does water have memory, but that the information can be transmitted over telephone lines and the Internet."[15]

Bibliography

* Benveniste, Jacques (2005) Ma vérité sur la 'mémoire de l'eau', Albin Michel. ISBN 2-226-15877-4

* Benveniste, Jacques. “Where is the Heresy?” Dec 1998
* Benveniste, Jacques. From "Water Memory" effects To "Digital Biology"
* Benveniste, Jacques, and Peter Jurgens. On the Role of Stage Magicians in Biological Research The Anomalist 1998
* Benveniste, Jacques. Electromagnetically Activated Water and the Puzzle of the Biological Signal INSERM Digital Biology Laboratory (March 10., 1999)
* Benveniste, Jacques, J. Aïssa, and D. Guillonnet. The molecular signal is not functional in the absence of "informed" water FASEB Journal 13 (1999) A163
* Benveniste, Jacques. "Put a match to pyre review" Nature 396 Dec 10 1998
* Benveniste, Jacques. "Further Biological Effects Induced by Ultra High Dilutions: Inhibition by a Magnetic Field", In P.C. Endler, ed.,Ultra High Dilution: Physiology and Physics. Dordrecht: Kluwe academic, 1994
* Benveniste, Jacques, et al.,"Activation of human neutrophils by electronically transmitted phorbol-myristate acetate.” Medical Hypotheses 54 2000
* Benveniste, Jacques, J. Aïssa and D. Guillonnet. “A simple and fast method for in vivo demonstration of electromagnetic molecular signaling (EMS) via high dilution or computer recording.” FASEB Journal 13:A163 (1999).
* Benveniste, Jacques, J. Aïssa, P. Jurgens and W. Hsueh. “Digital biology : Specificity of the digitized molecular signal.” FASEB Journal 12:A412 (1998).
* Benveniste, Jacques, L. Kahhak, and D. Guillonnet. “Specific remote detection of bacteria using an electromagnetic / digital procedure.” FASEB 13:A852 (1999).
* Benveniste, Jacques, P. Jurgens and J. Aissa. "Digital recording/transmission of the cholinergic signal." FASEB Journal 10:A1479 (1996) abstract
* Benveniste, Jacques, J. Aïssa, P. Jurgens and W. Hsueh. “Transatlantic transfer of digitized Antigen signaling at high dilution.” FASEB Journal A602 (1993)
* Benveniste, Jacques, "Transfer of Biological Activity by Electromagnetic Fields." Frontier Perspectives 3(2) 1993:113-15.
* Benveniste, Jacques, "Molecular signaling at high dilution or by means of electronic circuitry." Journal of Immunology. (1993 150:146A)
* Benveniste, Jacques, "Transfer of the molecular signal by electronic amplification." FASEB Journal (1994 8:A398).
* Benveniste, Jacques, "Electronic transmission of the cholinergic signal." FASEB Journal 1995 9:A683
* Benveniste, Jacques, "Direct transmission to cells of a molecular signal via an electronic device." FASEB Journal 1995 9: A227
* Benveniste, J. & Didier Guillonnet (1999) "III - Demonstration challenge, etc.", DigiBio NewsLetter 1999.2. Full text
* Benveniste, J., B. Ducot & A. Spira (1994) "Memory of water revisited", Nature, Letter to the Editor, 370(6488):322. Reference:[1]
* Benveniste, J., Davenas, E. & A. Spira (1991) Comptes Rendus de l'Académie des Sciences, January.
* Benveniste, J. (1988) "Dr Jacques Benveniste replies", News and views, Nature, 334:291. Full text

Notes

1. Davenas E, Beauvais F, Amara J, et al. (June 1988). "Human basophil degranulation triggered by very dilute antiserum against IgE". Nature 333 (6176): 816–8. doi:10.1038/333816a0. PMID 2455231.
2. Maddox J (June 1988). "Can a Greek tragedy be avoided?". Nature 333 (6176): 795–7. doi:10.1038/333795a0. PMID 3133566.
3. a b Maddox, John; James Randi and Walter W. Stewart (28 July 1988). "‘High-dilution’ experiments a delusion". Nature 334: 287–290. doi:10.1038/334287a0.
4. Experiments past and future Some remarks on the Memory of Water Controversy
5. ÉTUDE CRITIQUE ET PROJETS D'AVENIR Dr B. POITEVIN
6. molecule memories: Letter to New Scientist
7. Cumps, J.; Belon P, Cumps J, Ennis M, Mannaioni PF, Roberfroid M, Sainte-Laudy J, Wiegant FA (Received: 11 December 2002 Accepted: 12 November 2003 Published online: 21 April 2004). "Histamine dilutions modulate basophil activation". Inflammation Research (Birkhäuser Basel) 53 (5): 181–188. doi:10.1007/s00011-003-1242-0. PMID 15105967.
8. Milgrom, Lionel (March 15, 2001). "Thanks for the memory". Guardian Unlimited. http://www.guardian.co.uk/Archive/Article/0%2C4273%2C4152521%2C00.html.
9. J. Benveniste; P. Jurgens, W. Hsueh and J. Aissa (February 21–26, 1997). "Transatlantic Transfer of Digitized Antigen Signal by Telephone Link". Journal of Allergy and Clinical Immunology.
10. J. Benveniste; Aissa, J., Guillonnet. "The molecular signal is not functional in the absence of "informed water"". Medical Hypotheses 54 (A163 (abstr.)).
11. J. Benveniste; Thomas Y, Schiff M, Belkadi L, Jurgens P, Kahhak L (2000). "Activation of human neutrophils by electronically transmitted phorbol-myristate acetate". FASEB Journal 13 (1): 33–39. doi:10.1054/mehy.1999.0891. PMID 10790721.
12. Jonas, Wayne B.; John A. Ives, Florence Rollwagen, Daniel W. Denman, Kenneth Hintz, Mitchell Hammer, Cindy Crawford, and Kurt Henry (January 2006). "Can specific biological signals be digitized?". FASEB Journal 20 (1): 23–28. doi:10.1096/fj.05-3815hyp. PMID 16394263. http://www.fasebj.org/cgi/content/full/20/1/23. Retrieved 2007-06-05. — this paper includes an excellent references list.

13. Coles, Peter (1989). "Benveniste under review". Nature 340 (6229): 89. doi:10.1038/340089b0. PMID 2739750.
14. Report 12 of the Council on Scientific Affairs (A-97), American Medical Association. Accessed 10 April 2006
15. Benveniste, J.; P. Jurgens, W. Hsueh & J. Aissa (1997). "Transatlantic Transfer of Digitized Antigen Signal by Telephone Link" ([dead link]). Journal of Allergy and Clinical Immunology - Program and abstracts of papers to be presented during scientific sessions AAAAI/AAI.CIS Joint Meeting February 21–26, 1997. Poster. http://www.csicop.org/si/9801/sheaffer.html.

References

* BBC Horizon (2002) Homeopathy: The Test, first broadcast November 26, 2002. Summary and transcript. Rebroadcast on ABC Catalyst in 2003.[2]
* Beauvais, Francis (2007) L'Âme des Molécules - Une histoire de la mémoire de l'eau, Coll. Mille-Mondes [3], Ed. Lulu.com, Text in French, ISBN 978-1-4116-6875-1.
* Belon, P., J. Cumps, M. Ennis, P.F. Mannaioni, M. Roberfroid, J. Sainte-Laudy, & F.A. Wiegant (1999) "Inhibition of human basophil degranulation by successive histamine dilutions: results of a European multi-centre trial", Inflammation Research, 48(13):17-8. Reference:[4]
* Burridge, Jim (1992) "A Repeat of the 'Benveniste' Experiment: Statistical Analysis", Research Report 100, Department of Statistical Science, University College London, England. (early version of Hirst et al.)
* Chaplin, Martin (2000–2006) "Water Structure and Behavior London South Bank University
* Davenas, E., F. Beauvais, J. Arnara, M. Oberbaum, B. Robinzon, A. Miadonna, A. Tedeschi, B. Pomeranz, P. Fortner, P. Belon, J. Sainte-Laudy, B. Poitevin & J. Benveniste (1988) "Human basophil degranulation triggered by very dilute antiserum against IgE", Nature, 333(6176):816-18. Full text (source 1)(2)(3)(4)
* Fisher, Peter (1999) "The End of the Benveniste Affair?", British Homeopathic Journal, 88(4). Full text
* Fottorino, Eric (1997) Le Monde, January 21, 22 & 23, 1997.
* Hammer, M. & W. Jonas (2004) "Managing Social Conflict in CAM Research: The Case of Antineoplastons, ''Integr. Cancer Therapy'', 3(1)59-65.Full text
* Hirst, S.J., N.A. Hayes, J. Burridge, F.L. Pearce & J.C. Foreman (1993) "Human basophil degranulation is not triggered by very dilute antiserum against human IgE", Nature, 366(6455):527. Abstract
* Ives, John (2002) "Evaluating Unusual Claims and Devices Using a Team Approach: A Case Study", Subtle Energies & Energy Medicine, 13(1):39-59, based on Dr. Ives Keynote Address made at the Twelfth Annual ISSSEEM Conference The Co-Creation Process in Energy Medicine: A Synergy of the Sciences and the Healing Arts, June 14–19, 2002. Abstract, Full text
* Jaroff, Leon (1999) "Homeopathic E-Mail: Can the 'memory' of molecules be transmitted via the Internet?", Time, May 17. Full text
* Jonas, W. B., J. A. Ives, F. Rollwagen, D. W. Denman, K. Hintz, M. Hammer, C, Crawford & K. Henry (2006) "Can Specific Biological Signals be Digitized?", The Federation of American Societies for Experimental Biology (FASEB) Journal, 20(1):23-28.Full text
* Jonas, W. B. & J. Jacobs (1996) Healing with Homeopathy, Warner.
* Lignon, Yves (1999) "L'Homéopathie et la mémoire de l'eau", Les dossiers scientifiques de l'étrange, Chapter 21, Michel Lafon Publishing. ISBN 2-84098-482-2. Full text in French
* Maddox, John (1988) "Waves caused by extreme dilution", News and views, Nature, 335(6193):760-3.
* Maddox, John (1988) "When to believe the unbelievable", Nature, 333:787.
* Milgrom, Lionel (1999) "The memory of molecules", The Independent, March 19. Full text
* Ovelgonne, J.H., A.W. Bol, W.C. Hop & R. van Wijk (1992) "Mechanical agitation of very dilute antiserum against IgE has no effect on basophil straining properties", Experientia, 48(5):504-8. Abstract
* Park, Bob (1999) "The Challenge: Homeopathy Via the Internet", What's New, May 14. Full text (source 1)(2)
* Park, Bob (1997) "Alternative Medicine and the Laws of Physics", Skeptical Inquirer, 9/1/1997.Full text
* Randi, James. Commentary. January 26, 2001 "a Nobel Laureate reneges"[5]. September 5, 2003 "Benveniste and Josephson on Abandoning Science"[6].
* Targ, Russel & Harold Puthoff (1974) "Information transfer under conditions of sensory shielding", Nature, 251:602-7. Abstract
* Thomas, Y., M. Schiff, L. Belkadi, P. Jurgens, L. Kahhak & J. Benveniste (2000) "Activation of Human Eurtrophils by Electronically Transmitted Phorbol-Myristate Acetate", Medical Hypotheses, 54(1),33-39.Abstract
* Schiff, Michel. The Memory of Water: Homoeopathy and the Battle of Ideas in the New Science (Thorsons, 1995)
* Vithoulkas, George (2003) The controversy with the BBC program Horizon. Full text
* Walker, Martin (1993) "Dr Jacques Benveniste: The Case of the Missing Energy", Chapter in Dirty Medicine, Slingshot Publications, London. Chapter full text (source 1) (2)

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Patents

WO9417406
METHOD AND DEVICE FOR TRANSMITTING AS A SIGNAL THE BIOLOGICAL ACTIVITY OF A CARRIER MATERIAL TO ANOTHER CARRIER MATERIAL, AND FOR PROCESSING SAID SIGNAL, AND PRODUCT THEREBY OBTAINED

Abstract -- A method and a device for transmitting and processing a signal representative of the biological activity or behaviour specific to a predetermined substance, from a first carrier material having said biological activity to a second material physically separate from the first and free at first of any physical presence of said predetermined substance, as well as the material resulting from such a method, are disclosed. The method comprises amplifying the electrical or electromagnetic signal transmitted by the first substance and sensed by a sensor, and transmitting, to a transmitter, a signal representative of the biological activity or behaviour of the first material, then sensing in the second material a signal representative of the biological activity specific to said predetermined substance, and transmitted to said second material via high gain amplification means.

Present invention relates to a method and an apparatus of transmission in the form of a signal of the biological activity or specific biological behavior with a determined substance, starting from a carrying first material presenting the aforementioned biological activity, with a second initially free carrier material of any physical presence of the aforesaid determined substance. It also relates to an obtained product with such a method.

By “biological activity” one more conveniently understands any activity capable to be exerted by a biological substance with regard to an other substance refer target.

The target can be single or complex, such as for example a molecule, a body, a living being, in particular when the biological activity concerned does not imply the performing of a stable chemical bond between substance and the target.

The specific biological activity can be that of a natural substance or that of an artificial substance created by the man.

The expression “substance” such as it is used here for reasons of convenience of language, should not be regarded as applying only to one pure or individualized chemical molecule. It also must and particularly to be heard as including any complex reagent capable to express a biological activity which would be specific to the whole of the elements of which the reagent could be made up.

Like indicated right now above, and in short, it thus results, by what precedes that the expression “target” must as for it also be taken in its widest direction, to be operable as well and, according to case's, < as examples) for an individualized molecule, for example a specific substrate of an enzyme, when this one constitutes aforesaid “substance”, and for a body a living being when it is in its connection that “the biological activity” of “substance” to the study is tested.

The invention particularly finds an applying important although nonexclusive in the field of the manufacture of homeopathic drugs presenting a biological activity corresponding one at one or more active principles.

The invention puts at profit an extraordinary property of the material which was clarified by a certain number of experiments whose results are further described, namely that it is possible to transmit by electronic means or electromagnetic the expression of a specific biological activity of a material presenting it at another material not presenting initially the aforementioned activity.

The physical base of the method in accordance with the invention is still unknown. Perhaps it is explained by the following assumption: the manifestation of any biological activity of molecular origin would implement at the very least partially, activity of an electrical or electromagnetic type.

Observations as the inhibition of the biological activity by a magnetic field consolidate this assumption, [L.Hadji, B. Arnoux, J.Benveniste (1991)

Effect off dilute histamine one coronary flow off guinea-pig isolated heart. Inhibition by has magnetic field, Faseb J. 5: A1583. See also: J.C. Weaver, R.D. Astumian (1990) Tea response off living room concealments to very weak electric fields: thermal tea noise limit.

247:459462 science; R. Pool (1990) Electromagnetic fields: tea biological evidence. 249:1096 science - 1098; Smith C.W., Best S. (1989) Tea

Electromagnetic Man. J.M. Tooth and Sounds Ltd., Avon,
The U.K.].

With this aim the invention proposes particularly a transmission method in the form of a characteristic signal of the manifestation of the biological activity or specific biological behavior with a determined substance, starting from a carrying first material presenting the aforementioned biological activity at a second material physically separated from the first material and initially free of any physical presence of the aforesaid determined substance, this method comprising at the same time the exposure respectively carrying first material, presenting the biological activity, with a signal sensor electrical or electromagnetic, and the exposure of the second electrical or electromagnetic material to a signal transmitter, connected to the sensor via transmitting means and of amplifying with high profit, pendent a sufficient time to allow - amplifying of the electromagnetic electrical signal collected by the sensor and the transmission with the transmitter of a characteristic signal of the manifestation of the biological activity or biological behavior presented by the first material, - detection in the second material of a characteristic signal of the manifestation of the specific biological activity with the aforementioned substance determined and transmitted to this second material via the amplifying means with high profit.

The detection of the manifestation characteristic of the activity or the specific biological behavior of determined substance is révélable by the action which the transmitted signal can exert on a substrate (organism or reagents) at the time the implementation of a tentative protocol adapted to make identical or similar with that normally allowing the setting in evidence of the presence in the first material carrying of the aforesaid determined substance, thanks to the action exerted by this last on the same substrate.

The transmitting means and of amplifying with high profit comprise a medium or carrier medium suitable to convey a coherent flow of information to electromagnetic or electrical characters. This medium includes/understands for example a conducting cable of the electricity or means allowing the exploitation of a light beam carrier of coherent light.

By “amplifying means at high one sheathed hears characterized means by a coefficient of amplifying of an electrical signal or electromagnetic important, particularly great at 1000 and preferably great to 10.000.

For example the tension is amplified of 100 microvolts to 6 Volts and, simultaneously the intensity of 100nA with 150 my.

As an indication, one will mention that with such apparatuses of transmission and amplifying with high profit, the higher durations are at least about 10 mn, with preferably about 15 minutes.

Also advantageously the first and/or the second carrier material are, or contain, of said solvents “protonic”, i.e. capable to release and/or collect protons, such as for example water, the ethanol or any product presenting a linked labile proton at an electronegative atom, of formula of the type R - X - H.

Advantageously, the carrying first material and/or the second carrier material are specifically of water, or the aqueous products.

It can (or they can) be consisted all impregnatable materials by water, same if this one is present only in low proportions.

In the case of water, this one is advantageously consisted distilled water, which previously was heated at a great temperature with about 70 " C pendent a great time with about 20 minutes.

Advantageously single of the electromagnetic signals are collected, amplified and transmitted between the sensor and the transmitter.

In an advantageous embodiment the method in accordance with the invention is applied with the water treatment, for example with the depollution of used or biologically contaminated water.

Still advantageously the second material is a living material, for example a nonhuman body.

Advantageously the transmitted signal is stored in an intermediate way (before being transmitted to the second material) on an electromagnetic storage medium of known type in him same, like a magnetic tape, or after processing by an analogue/digital converter on a digital storage medium such as an optical disc, a computerized memory etc

Also advantageously the transmitted signal is treated by known methods of treatment digital or analogue into they-same, in order to be modified and to thus correspond to the biological activity of a substance presenting an active principle modified, optimized, amplified, purified or without secondary effects.

One thus can and particularly to influence (to increase, to be opposed, see removing) a determined biological

activity.

To optimize or modify such signals in order to obtain a different result of that obtained by the corresponding signal with the initial active principle, one will proceed for example by testing the active principle modified thanks to the implementation of different tentative protocols known or easy to work out for the person skilled in the art and whom it has at his disposal to account for the activity of a determined or improved active principle.

Advantageously the transmitted signal corresponds to that transmitted by several present substances in the first material and it is treated by known methods of treatment digital or analogue into they-same, to analyze and measure of the aforesaid substances among the others, such as for example the blood rate of glucose or alcohol.

In an advantageous embodiment, the determined substance is present in homeopathic amount in the carrying first material.

The homeopathic amount used is then advantageously optimized in order to allow a manifestation maximum of the desired biological effect, and this in a known way in it same, starting from numerous treaties written and published in the species, such as for example “the practical homeopathy” of Doctor C.BINET published with the editings of ANGLES (1979).

In an advantageous embodiment the determined substance is present in homeopathic amount in the carrying first material, with great dilutions in extreme cases indicated by the number of Avogadro.

In an advantageous mode of performing, dilution is a dilution about - log41M (theoretical).

Advantageously the second material is consisted homeopathic granules.

The homeopathic granules are often containing impregnated lactose of water molecules.

The invention also relates to a material in a volume finite and carrying information characteristic of the specific biological behavior to a determined substance, but in the total absence of this determined substance in the aforementioned material, this information being with electrical or electromagnetic character because of its capacitance to being transmissible by electric means or electromagnetic, this manifestation being normally révélable by the action exerted by this material in finite volume with regard to a specific substrate with determined substance in a tentative protocol identical or similar with that which one would implement to account for the presence of the aforesaid determined substance in a medium which would contain it.

A method particularly advantageous for the producing of such a carrier material is a method comprising the setting in electrical or electromagnetic relation of the same material, however not initially carrying the aforesaid information and free of any physical presence of the aforesaid determined substance, with a medium containing this last, via electromagnetic or electrical signal transmission means comprising an apparatus provided with receiver means, average amplifiers with high profit.

The invention also proposes an apparatus implementing the method in accordance with the invention, comprising average electrical or electromagnetic signal sensors transmitted by a first material and characteristics of a specific manifestation of a biological activity of a determined substance contained in this first material, of the average amplifiers with high profit of the aforesaid signals and of the emitter means suitable also to transmit signals characteristics of the biological activity to a second material otherwise deprived of any contact with the first.

In modes particular of performing, one has moreover recourse to the one and/or the other of the following provisions - the average amplifiers comprise an electronic circuitry of amplifying to high profit, in the form of discrete elements or of the semiconductor type; - the electronic circuitry of amplifying includes/understands a mounted output transistor out of common-emitter; - the sensor and the transmitter comprise electromagnetic coils - the supply of the apparatus is done by battery, which makes it possible to avoid the possible perturbations of the sector.

But a supply starting from the alternating array 220 Volts converted in low continuous tension, for example 9 Volts, is also completely operable; - the apparatus includes/understands moreover of average the electromagnetic storage medium of the transmitted signals, of same known type in them, such as for example a magnetic tape; ; - the apparatus comprises moreover of the average converters analogue/digital of the transmitted signals and storage means on a datum storage medium digital of the aforesaid signals, such as for example an optical disc, a computerized memory etc T - the apparatus includes/understands processing means digital or analogue transmitted signal, to modify the said signal to make it correspond to that of a substance presenting an active principle modified, optimized, amplified, purified or without secondary effects - the apparatus includes/understands processing means digital or analogue arranged to analyze and measure the transmitted signals corresponding one with a substance among others, such as for example the rate of glucose in the alcohol blood or rate - the second coil is arranged to emit towards a living material, such as a nonhuman body.

The present invention will be included/understood better with the reading of the description which follows of an embodiment given as nonrestrictive example, and within sight of the examples and results supplied hereafter in a nonrestrictive way.

Description also refers to the drawings which accompany it, in which -

Figure 1 is a scheme of the apparatus according to an embodiment of the invention.

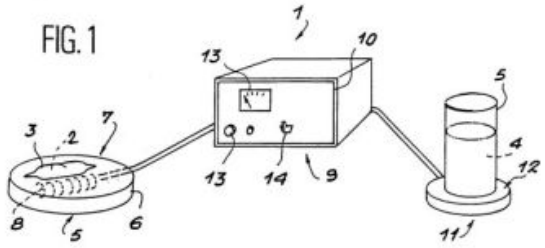


Figure 2 is an electrical scheme of the apparatus of transmission of figure 1.

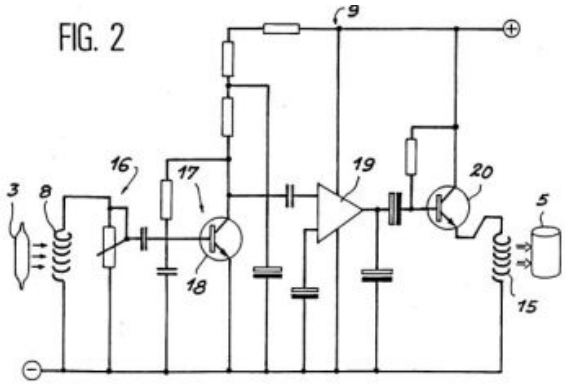


Figure 1 watch an apparatus 1 of transmission of the specific biological activity to a determined substance, for example of histamine or ovalbumin, a carrying first material 2, for example consisted distilled water placed in an ampoule preferably out of glass 3 from 1 to 10 ml, with a second material 4 also consisted water distilled and placed in an ampoule from 1 to 10 ml or a container 5 for example of 500 ml, even more, also and preferably out of glass.

Material 2 can comprise in its breast the physical presence of determined substance, in or not homeopathic quantity, or can simply comprise information characteristics of the activity or the specific biological behavior with determined substance.

The physical presence can for example be revealed by a method of the type spectrometry or spectrofluorometry.

Information characteristics of a manifestation itself characteristic of the specific biological behavior to a determined substance can, when with it, being normally révélable at the time of the action which it can exert on a medium containing this determined substance with regard to a target (organism or reagents) implemented in a tentative protocol adapted to account for the presence in this medium of the aforesaid determined substance.

Material 4 is initially free in its breast of any physical presence of the aforesaid determined substance, and is not initially carrying information characteristics of the specific biological behavior with determined substance.

Apparatus 1 includes/understands an electromagnetic sensor 5 comprising an housing 6, provided with a tray 7 on which ampoule 3 is deposited.

In the embodiment particularly described here, the tray is for example made up out of transparent plastic with the electromagnetic waves, of low thickness (for example 2 millimeters).

Inside housing 6 the electromagnetic sensor itself, for example consisted a receiver coil 8 is as one will see it in reference on figure 2.

Sensor 5 is connected, by electrical conducting cable, with a circuit 9 amplifier with high profit placed in an housing 10.

Circuit output 9 is connected, also by electrical conducting cable, with a sensor transmitter 11, of configuration similar to sensor 5 but arranged for the transmission, and on the tray 12 whose is placed the ampoule or

container 5 of retention of second material 4.

Circuit 9 includes/understands means 13 of setting of the power (potentiometer, dial, etc) and of powering 14 (switch) apparatus known in themselves.

One represented accurately on figure 2 electronic circuitry 9 of apparatus 1 according to the embodiment of the invention particularly described here.

Circuit 9 is connected on one side to electromagnetic coil 8 to high impedance (receiving) (for example a coil made up of approximately 600 yarn coils enamelled of 5/100) belonging with sensor 5, and other side with electromagnetic coil 15 with high impedance (transmitting) (for example a coil made up of 100 yarn coils enamelled of 20/100) belonging with sensor 11.

Circuit 9 includes/understands a filter cuts high 16 (for example of 10khi) connected to coil 8 and one pre-amplifier 17 comprising an amplifier transistor 18.

Pre-amplifier 17 is connected of outputted to the operational amplifier 19 which can be connected directly to transmitter coil 15, or as represented on figure 2, via a mounted output transistor 20 out of common-emitter to generate an output current of stronger intensity.

Such a change makes it possible to treat the more important liquid volumes in the same time, the alternating tension of outputted being in addition and for example from 4 to 5 Volts peak with peak.

If not the provision above guarantees to outputted equivalent signal with a tension from 3 or 4V and a current from at least 20 my.

In an advantageous variant the amplifier is with variable profit for example of < lmV with > 3/4 V and of < 10 microamperes with > 20 my.

The supply of the circuit is done advantageously exclusively by batteries (not represented), which makes it possible to avoid the uncontrolled changes of the structure of the signal due to unpredictable perturbations of supply network 50 Hz (sector).

One now will make reference, with illustrative titre, two specific examples of transfer.

In the first case (example N " L), it was about a transfer starting from water distilled, of the active principles of ovalbumin (Ova) or the endotoxine of E.Coli (Endo) towards one second distilled water material also made up, and in the second case (example N " 2) of a transfer always between two materials made up of distilled water, of the principles of the endotoxine of E.Coli (Endo) or of histamine (Hista).

A summary table several experiments carried out by the inventors to date, who thus could validate the method and the apparatus of the invention, is also presented.

Detection method of the biological activity used in the experiments carried out, of which those corresponding with the two ciaprès examples, is the following one.

Male hearts of guinea-pigs of Hartley of approximately 400 G are mounted on an apparatus known under denomination ANDERSON for infusion of heart and perfusés at 37 " C with a buffer solution Krebs-Henseleit (KHB into initial Anglo-Saxon for Krebs-Henseleit Buffer) (lmN Ca2+) with a pH of about 7,4. The solution is ventilated permanently with a mixing O2/CO2 to 95,5%.

The coronary flow is controlled permanently for example using an apparatus of known automatic weighing in itself, connected to computer means of processing and restitution of the measured flows, in graphic form.

The maximum and minimum systolic contractions, the heart rate and the values of dp/dt (speed of the muscular contraction) measured and are recorded permanently via a transducer, for example a known transducer under reference ELI-SO45-35 of company ENRA Technologies: 53 bld of the General Martial Vallin - 75015 Stakes (France).

The active principles (histamine, ovalbumin or endotoxine of E.Coli) which made the object of a transfer, were diluted starting from concentration of lmM with distilled water.

Between each dilution, the solutions were violently agitated in a pendent vortex 15 S.

The solutions are injected at the base of the aorta with an electrical syringe (6 ml 1 ml/mn).

EXAMPLE NR " 1

The transfer of ovalbumin (Ova), endotoxine of E.Coli (Endo) and, as control, of distilled water, out of sealed

ampoules of 2 ml, was carried out towards water sealed ampoule-girls distilled of 2 ml.

The concentration in theory active was in the “transmitting” ampoules of 1 X 10-8 Moles per liter.

The ampoule-girls were then diluted to the 1/1000 and 20 ml of dilutions were divided into tubes of 50 ml.

The tubes were tested at blind (in the order, from 1 to 12) on July 11, 1992 out of two isolated hearts of pre guinea-pigs immunized with ovalbumin.

The results are the following ones

Tubes Active principle % variation of the Active principle NR " transferred coronary flow detected in (ampoule-mother) tube receptor
(ampoule-girl)
Heart A Heart B
Heart A Heart B
1 Endo 50 17 +
2 Endos 55 21 +
3 Ova 75 93 +
4 H20Tr* O 0
5 Ova -50.-53 +
6 H20 ** 0 0
7 H20Tr O 0
8 H20 0 0
9 H20 0 0 10 H20Tr O 0 11 H20 11 10 12 Ova -37.-42 + * water having received information water ** water of origin slightly variable result but nevertheless
acceptable. I1 is probably explained by one
bacterial contamination of the tube, giving one
reaction of type endotoxine.

The effects of the 5 active tubes (water having received Ova information or Endo) and the absence of effect of 7 controls (water of origin or having received information water) are net and reproducible.

One finds these differences on the mechanical effects (not shown here).

This experiment in accordance with the invention illustrates the transmission of biological activities to water by an electronic circuitry or electromagnetic.

EXAMPLE NR " 2:

The tubes were tested on September 23, 1992.

The operating conditions are identical with those of example 1.

The results are the following ones
Tubes Active principle % variation of the Active principle NR " transferred coronary flow detected in (ampoule-mother) tube receptor
(ampoule-girl) 1 H2OTR 0 1 H20TR heated O2 H2OTR 0 3 HistaTR -10 + 4 OvaTR -94 +
SUMMARY TABLE
The following table gives the effects on coronary flow in % of variation of the flow (in + or in -) on hearts of guinea-pigs previously immunized to ovalbumin (in the presence of Alum like adjuvant) at the end of October 1992.

with
C1: not transmitted water
C2: transmitted water, i.e. which has received one
neutral information (background noise of the apparatus)
Hist: water with transmitted histamine, i.e. which has
received information “Histamine”
Ova: water with transmitted ovalbumin, i.e. which has
received information “Ovalbumin”
Endo: water with endotoxine transmitted, i.e. which has
received information “Endotoxine”.

As one can note it coronary flows vary in a way significant and systematic at the time of the corresponding information transfer to histamine, ovalbumin or the endotoxine, whereas in the presence of water (transmitted or not), of low variations or substantially any variation are observed, which illustrates the present invention.

The use of second material in which appears the transmitted activity can be done for example by oral route,

injection, even same impregnation by contact between the skin of the individual to be treated and a container containing the aforementioned second material.

As it goes without saying, and as it results besides from what precedes, the present invention is not limited to the embodiments of the invention particularly not described. It relates to on the contrary all the variants of them and particularly those where: - the carrier materials are not 1 'pure water, but of the aqueous mixtures, or materials pasty or solid, - the sensors are not of electromagnetic type but of the electrical type, i.e. they are arranged to detect a difference in potential. They can then be metallic plates connected between them via an amplifier to high profit, the first and second materials or their containers being particularly placed at contact with the sensors, - the active principles are different those particularly tested. All types of biological molecules capable to be contained in all natural substance types or artificial acting on the living beings, species animal or vegetal, are in fact concerned. They can be for example present substances in the blood or any other liquid body or biological tissue of the animals or in vitro men, ex vivo, in vivo.

The revelation of the presence of information corresponding one to an active principle could then be done differently, in a known way in itself by a person skilled in the art for the active principle concerned.

WO2005119271
METHOD AND SYSTEM FOR PROVIDING A SUBSTANCE WITH RECEPTIVE AND/OR TRANSMISSIVE PROPERTIES FOR A SIGNAL

Also published as: JP2008500894 // FR2870993 // EP1756589

Abstract -- The invention relates to a method and system for providing a substance (101) with receptive and/or transmissive properties, which permit the substance (101) to receive or transmit a signal, acquired by receiving an electromagnetic field coming from a source substance. The substance (101) is subjected to an electromagnetic field and/or a sound (102), emitted at one or more given frequencies for a given period. The invention permits the substance (101), initially non-receptive and/or non-transmissive to be given receptive and/or transmissive properties.

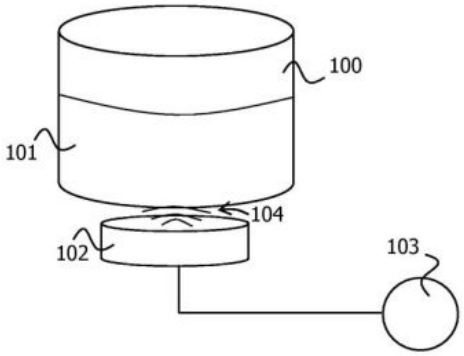


FIG. 1

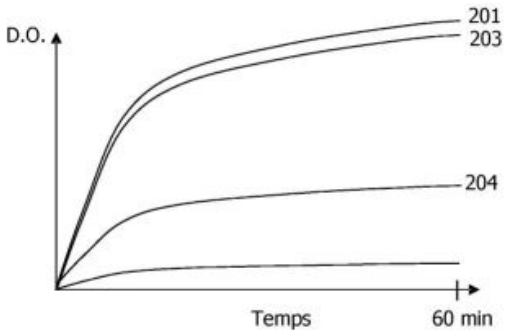


FIG. 2

US2004038937
Method and device for avoiding alteration of a substance having biological activities

Present invention relates to a method and a system allowing to ensure, that after an application of a generated signal starting from a field electromagnetic generated by a substance source, a substance, especially of water, present an active characteristic of the substance source.

It is known, especially patent FR 2783606, which it is possible to produce a substance having an active characteristic of a substance source, while applying to substance, initially inactive, a generated signal to be left, and in function, electromagnetic field generated by the substance source.

Such an active characteristic can be a chemical biological activity and/or or a biological and/or chemical behavior.

It was observed that the application with substance of such a signal does not guarantee that the substance, subsequently to this application, will acquire the active characteristic of the substance source. Large changes as for the capacity of a substance to acquire an active characteristic of a substance source by application of a signal are observed.

Especially, it happens that water subjected to a generated signal to leave, and in function, electromagnetic field generated by a substance active source present step of specific activity. The signal then seems not to have acted on water so as to make it active. Thus, the capacity of water to record, keep a trace of the signal to which it is subjected thus varies experiment in experiment from 0% to 100% same when it is a specific robot which carries them out handlings. That poses especially an obvious problem of reproducibility.

The invention solves this problem of reproducibility.

It consists in previously treating substance with the application of the signal. For that, the invention relates to a method to confer on a substance, especially of water, properties receptive and/or diffusing making it possible substance to be informed and/or to diffuse a signal, especially an acquired signal by collecting an electromagnetic field coming from a substance source, the aforementioned method comprising the step to subject, using a transmitter, substance with an electromagnetic field and/or an emitted sound at one or more included frequencies in a pendent frequency spectrum predetermined one predetermined duration.

Indeed, in accordance with the invention, the substance, initially nonreceptive and/or nondiffusing, is made adapted to receive and/or diffuse a signal.

According to other performings', the method comprises moreover a step of agitation of substance, the aforementioned step of successive or simultaneous agitation being with the step to subject substance to an electromagnetic field and/or an emitted sound.

The agitation especially makes it possible to decrease the predetermined duration pendent which the substance is subjected to an electromagnetic field and/or an emitted sound. This agitation consists, for example, with the creation of a vortex in the solution.

The invention also relates to a system to implement the method like presented above, a substance made receptive and/or diffusing according to a method of the invention and the use of such a substance for the producing of a substance presenting an active characteristic of a substance source.

Other characteristics and advantages of the invention will appear with description made below, this last being carried out with descriptive and nonrestrictive titre by making reference with the drawings hereafter on which:

Figure 1 represents a system in accordance with the invention.

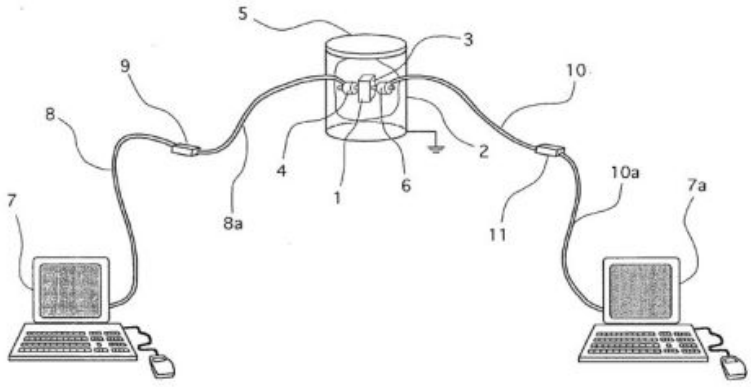


Fig. 1

Figure 2 is a diagram illustrating the presence or not active characteristic in substances obtained or not in accordance with the invention.

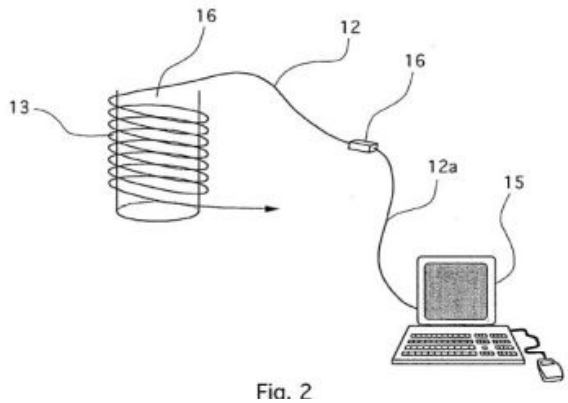


Fig. 2

According to figure 1, a system in accordance with the invention comprises a transmitter 102 to subject substance 101 to an electromagnetic field and/or an emitted sound 104 at one or more included frequencies in a pendent frequency spectrum predetermined one predetermined duration.

The transmitter is for example an high speaker making it possible to subject substance to an applied frequency in sound form.

It is also possible that the transmitter is a transmitter of electromagnetic waves, for example an electromagnetic coil, subjecting, for example, the substance with a field of frequency 50 Hz, 500 Hz or others: white noise…

The frequencies are selected in a frequency spectrum predetermined by an electromagnetic generator 103 of field and/or sound 104.

It is possible to use one or of the given frequencies for the electromagnetic field and/or sound 104.

The subjecting of substance, of water in the experiment, at random frequencies covering at least the spectrum of the audible frequencies (20 Hz - 20000 Hz) made it possible to obtain a formatted said water, namely a water having a good receptivity and/or good diffusing qualities at a signal coming from a substance active source.

The random frequencies can especially be obtained by the diffusion of a music piece.

Examples of predetermined duration are given below. Generator 103 allows a control of the predetermined duration. For example, the water subjected to a music 104 pendent one present night a receptivity and/or good diffusing qualities.

According to another performing of the invention, the system comprises moreover means to agitate substance, for example of swirling manner.

The creation of an agitation in substance especially makes it possible to reduce the predetermined duration to which water must be subjected to be formatted. For example, a correct formatting of water and thus a good receptivity and/or good diffusing qualities are observed when water is subjected to a music 104 pendent two hours and successively agitated of swirling manner, for example pendent twenty seconds. It is also possible to simultaneously agitate water with the diffusion of the music piece.

A container 100 contains substance 101. This container is for example a cylinder in transparent plastic.

Any other form (tube…) allowing to accomodate substance is appropriate thus that any other material (glass, metal…) permeable with the sound and/or the electromagnetic waves can be used.

Advantageously, the container is placed on transmitter 102 but any other position making it possible substance to receive the electromagnetic waves and/or sound 104 is possible.

In another performing, the transmitter can be placed within substance, for example, immersed in water.

Figure 2 in accordance with the invention illustrates the action of a method on the capacity of water to being informed by a generated signal starting from an electromagnetic field generated by a substance source possessing an active characteristic.

The hirudine is an acting anticoagulant by direct inhibition of thrombin. At the site of action, the effect of the hirudine is immediate. In the given illustration, the substance source is the hirudine and the generated signal from electromagnetic field of the substance source is called signal hirudine.

A said method of information making it possible to obtain an informed substance, i.e. presenting the properties of an anticoagulant such as the east the hirudine, is described in the patent FR 2783606.

According to this method, the electromagnetic field coming from the hirudine, substance source, is transformed into an electrical signal using a transducer-receptor collecting the electromagnetic field. The electrical signal is then applied with a substance by means of a transducer-transmitter.

The substance to which the electrical signal is applied can or not be subjected to the method in accordance with the invention.

The experiment consists in in accordance with the invention comparing substances having undergone a method and others which did not undergo it, previously with the application of the method of information.

Like biological system allowing to reveal an anticoagulant effect of the hirudine or signal hirudine, one uses a solution of water, substance capable to undergo a method in accordance with the invention, including thrombin.

One mixture the solution water-thrombin with a fibrinogen solution which, under the effect of thrombin, ends in the formation of a fibrin clot, final step of coagulation.

In order to measure coagulation, one measuring change according to the time of the optical density of the solution resulting of the mixture. Present figure 2 thus of the curves of optical density (in ordinate).

Curve 201 represents the evolution of the optical density observed after the adding of a solution of water including of molecular thrombin in the absence of hirudine in a solution including of fibrinogen. The solution of water including of thrombin is carried out with a water which did not undergo any prior preparation (and thus not formatted according to the vocabulary defined above). It is checked that the optical density increases rapidly: there is coagulation.

On figure 2, is represented curve 202 corresponding one with the mixture of a solution of water including of the thrombin into which molecular hirudine was introduced with a solution including of fibrinogen. One observes only one low increase of the optical density in time: it is checked that there is no coagulation.

Then, for the requirements of the comparing, several solutions including of thrombin are prepared with water not having undergone a method of formatting in accordance with the invention and having undergone a method of information using the signal hirudine like described above.

It is observed that the curves of optical density obtained after mixture with a solution including of fibrinogen are sparingly reproducible. Thus it is possible to observe curves near of curve 201, intermediate curve 202 or curves. For example curve 203 is representative of a not formatted solution of water, not including a molecular hirudine but to which a signal hirudine was applied.

The anticoagulant effect of the signal hirudine is not observed.

Also, for the requirements of the comparing, several solutions including of thrombin are prepared with water having undergone a method of formatting in accordance with the invention and having undergone a method of information using the signal hirudine like described above.

Curve 204 represents the results obtained with such solutions of water including of molecular thrombin without hirudine but to which a signal hirudine was applied.

Such a curve is obtained in a reproducible way.

Lastly, a similar curve with curve 201 is obtained with solutions of water including of thrombin prepared with water having undergone a method of formatting in accordance with the invention and not having undergone a method of information using the signal hirudine.

Measuring repeated on water samples having undergone a method of formatting in accordance with the invention and on water samples not having undergone a formatting before the application of a signal of type hirudine shows an average anticoagulant activity much higher for the sample formatted according to the method of the invention. The table below watch measurement results of the activity anti coagulating after application of a signal of type hirudine with formatted water samples and not formatted water samples. The reported results correspond to the percentage of inhibition of thrombin at the end of thirty minutes. For comparing, measurement results of the coagulating anti activity of a solution of hirudine titrated with a M/L are presented. One thus observes an average value raised for the hirudine (70. 6%), as well as differential substantial enter the average observed for the water samples formatted in accordance with the invention (21. 4%) and that observed for the not formatted water samples (9.6%).

WO0204958
US6541978
METHOD FOR DETERMINING POTENTIAL ALTERATIONS OF A SUBSTANCE HAVING BIOLOGICAL ACTIVITIES

Also published as: FR2783605 // WO0017638 // EP1116025 // AU5867399

Abstract -- The invention concerns a method, a system and a device for producing, from a substance, electric signals characteristic of the biological activity of an active element contained in the substance. The method consists in: placing the substance in a zone subjected to a specific electric, magnetic and/or electromagnetic excitation field. The method further includes a step which consists in transforming the fields resulting form the interaction between the specific excitation filed and the substance, into signals, in particular electric signals, using a first transducer receiving the resulting fields.

Description

[0002] The present invention relates to a method, a system and a device for producing signals from a substance, in particular electric signals, characteristic of the biological and/or chemical activity or the biological and/or chemical behaviour of said substance or an active element contained in said substance. The invention also relates to a method and a system for controlling said signals. The invention also relates to the applications of said method, system and device in particular to the production of active substances and to the detection of defined substances. Finally, the invention relates to signals linked to a biological and/or chemical activity thus produced by said method, system and device.

[0003] It is known from the research works of Jacques Benveniste, in particular those described in the patent application WO 94/17406 published on Aug. 4, 1994, that one can pick up, from a biological and/or chemical active element such as a chemical compound, a cell or a micro-organism, or from a substance containing this active element such as a purified preparation, a biological sample, or a living being, an "electromagnetic signal characteristic of the biological and/or chemical activity or of the biological and/or chemical behaviour" of said substance and/or said active element contained in said substance.

[0004] It is also known that it is possible to transform, in particular by means of a transducer, such an electromagnetic signal into electric signals. In the following text one also means by "electric signals characteristic of the biological and/or chemical activity or of the biological and/or chemical behaviour of said substance or of an active element contained in said substance" the electric signals derived by signal digitising and/or processing. In this expression the word "characteristic" is used in the meaning where the physical parameters of the electric signals are specific to the substance or to the active element contained in said substance and that the application of these electric signals, via a transducer, to a biological control system makes it possible:

[0005] (i) to induce a biological and/or chemical activity on said biological control system relative to that of the substance of origin or the active element it contains;

[0006] (ii) to reveal a characteristic of the substance or the active element it contains, at the origin of said electric signals.

[0007] The patent application WO 94/17406 published on Aug. 4, 1994, describes a method and a device for picking up "an electromagnetic signal characteristic of a biological and/or chemical activity or of a biological and/or chemical behaviour" from a biological and/or chemical active element such as a chemical compound, a cell or micro-organism, o r from a substance containing this active element such as a purified preparation, a biological sample, or a living being.

[0008] Since then the inventors have discovered that it is possible to improve the quality of the electromagnetic signal picked up as well as the reliability of the method for producing these signals and that consequently it is possible to produce characteristic electric signals appropriate for industrial applications. The production of such characteristic electric signals implies an exceptional industrial importance.

[0009] It thus becomes possible to detect and characterise active elements present in low concentration or in very low concentration in a substance. As examples, it is thus possible to monitor the presence or absence of chemical compounds such as caffeine, ionophoretic-calcium, ovalbumin, propranolol or micro-organisms such as bacterium coli, streptococci, staphilocci whose presence is looked for.

[0010] It thus becomes possible to carry out remote tests at several thousands of kilometers since the characteristic signals are electric signals which can immediately be transmitted to the investigation centre of the control laboratory.

[0011] It is possible to modify the biological and/or chemical activity or the biological and/or chemical behaviour of a biological receptor system by submitting it to the effects of characteristic electric signals. It also becomes possible to produce new drugs such as solutions depending on signals from arnica, bradykinin, caffeine, nicotine. New production techniques for drugs can be implemented. For example, in the case of certain drugs such as antibiotics, anti-viruses, anti-parasites, anti-mitotics which, to act within bacteria, viruses or cells (tumour cells in particular), must breach the defensive barriers of the above, the signals of these drugs are applied directly into the heart of the bacteria, viruses or cells. In fact, the application of characteristic electric signals, via a n appropriate transducer, generates magnetic fields which penetrate into the bacteria, viruses or cells and modify their chemical and/or biological behaviour.

[0012] It is possible to store the characteristic electric signals in data banks, using computer techniques. Then, the spread of therapeutic resources, from one point to the other on the planet, is instantaneous according to needs.

[0013] The examples described above concern the medical domain. The chemical industry also, such as electronic components, will also be concerned by the new possibilities offered by the present invention. The use of electromagnetic fields, emitted by characteristic electric signals, to modify the behaviour of molecules and promote chemical reactions will open up new prospects concerning both the conception of new materials and their methods of production. Thus, for example, it will be possible to use them as catalysts able to influence the

stereochemistry of molecules.

[0014] The method according to the invention making it possible to improve the performances of characteristic electric signals comprises the stages:

[0015] of placing said substance in a zone submitted to a specific excitation field of electric, magnetic and/or electromagnetic nature,

[0016] of transforming the fields resulting from the interaction of the specific excitation field and the substance, into signals, in particular electric signals, by means of a first transducer receiving said resulting fields.

[0017] In fact, the inventors have noted that, in a surprising manner, the use of an excitation field such as for example an electromagnetic field of uniform power spectral density over a frequency spread (for example white noise of 1 Hz to 20 kHz) makes it possible to improve the performance of characteristic electric signals. As an example of such a first transducer, one can mention very sensitive small copper wire bobbins with an impedance of 300 Ohms; internal diameter of 6 mm, external diameter of 16 mm, length 6 mm, normally used as telephone receivers.

[0018] Preferably, the process according to the invention further comprises the stage for processing said signals derived from said first transducer, relative to second signals derived from a second transducer receiving the specific excitation field, in the absence of said substance. As an example, the processing can consist of subtracting these two signals by using two receiver bobbins connected in series and with opposite phases, one facing said substance and receiving the electromagnetic field through said substance and the other receiving the electromagnetic field directly. Thus, the part of the signals really characteristic of the biological and/or chemical activity or of the biological and/or chemical behaviour of said substance or said active element contained in said substance, is enhanced relative to that derived from the first transducer alone.

[0019] As an example, according to another embodiment of the invention, the processing can consist of recording consecutively the signals coming from said substance and then the signals coming from a neutral substance (water or physiological serum), then subtracting the first signals from the second (which serve as reference), this subtraction being carried out before or after processing the signals as described below (subtraction of amplitudes or power spectral densities).

[0020] Preferably, according to another embodiment of the invention, the process according to the invention comprises the stage of processing the signals derived from said first transducer, in function of the characteristics of the specific excitation field. For example, the signal processing consists of calculating the power spectral density using a Fourier transform, to narrow the useful frequency band (bandpass filter), to normalise the specific excitation field relative to the power spectral density, and to reconstitute a signal using an inverse Fourier transform. As in the case of the preceding embodiment, the part of the signals which are really characteristic of the biological and/or chemical activity or of the biological and/or chemical behaviour of said substance or said active element contained in said substance, are thus enhanced relative to that produced without processing.

[0021] Preferably, the specific excitation field has the characteristic of having a uniform power spectral density over a frequency band. As an example, the power spectral density is uniform over a frequency band from 1 Hz to 20 kHz. Thus, said substance is submitted to a neutral excitation field of the white noise type.

[0022] Preferably, furthermore, the zone submitted to the specific excitation field is insulated from parasitic fields from the environment.

[0023] The invention also relates to the applications of the signals produced. To this effect, the method further comprises the stage of applying said signals from said first transducer to a biological receiver, by means of a third transducer. In the case where said signals are processed, it is the signals processed in this way which are applied to the biological system receptor.

[0024] As an example, said third transducer will generate and emit an electromagnetic field in the direction of biological system receptors such as a carrier substance or a reactive medium producing stereochemical molecules. This electromagnetic field will modify the biological and/or chemical activity or the biological and/or chemical behaviour of the biological system receiver as a function of the nature of biological and/or chemical activity or the biological and/or chemical behaviour of said substance. Thus, for example, it is possible to send the message for caffeine into a water-based beverage to produce a dietetic drink or an alimentary supplement.

[0025] The invention also concerns the control of characteristic electric signals. For this, the process further comprises the stage for controlling the correlation between on the one hand, the signal derived from said first transducer or the processed signal and, on the other hand, the biological and/or chemical activity or the biological and/or chemical behaviour of said substance or said active element contained in said substance. This control is carried out by applying, by means of said third transducer, the signals derived from said first transducer to a biological control system and by verifying that said biological control system reacts in a specific

manner to the signals from said first transducer. In the case where said signals are processed, it is the signals thus processed which are applied to said biological control system. The reaction of said biological control system must be related to the nature of the biological and/or chemical activity or the biological and/or chemical behaviour of said substance or said active element contained in said substance whose signals are emitted from said first transducer. As an example, in particular one can cite as a biological control system: an isolated guinea-pig heart, a ligand/receptor couple in particular an antigen/antibody couple, the skin of a guinea-pig or a live rabbit which is submitted to a cutaneous injection test, isolated or cultured cells.

[0026] Surprisingly, it was noted that the method according to the invention for producing characteristic signals delivers exploitable signals from an active substance whose active element can even be contained in low or very low concentrations (less the 10<6 >moles per liter). The method according to the invention can thus be applied to characterise the presence of an active element at the trace level in a substance.

[0027] The invention also relates to a system for producing signals, in particular electric signals, characteristic of the biological and/or chemical activity or of the biological and/or chemical behaviour of a substance or an active element contained in said substance. The invention also concerns a system for implementing the properties of said signals. Said system comprises an emitter generating a specific excitation field of electric, magnetic and/or electromagnetic nature in a zone where said substance is located. As an example, one can cite an emitter with the following characteristics: bobbin with internal diameter 50 mm, length 80 mm, R=3.6 ohms, 3 layers of 112 turns of copper wire, field on the axis to the centre 44 Oe/A, and on the edge 25 Oe/A. Said system also comprises a first transducer receiving the fields resulting from the interaction of said specific excitation field and said substance, said first transducer transforming said resulting fields into signals, in particular electric signals. As an example, one can cite a transducer such as a very sensitive little bobbin of copper wire with an impedance of 300 Ohms, of internal diameter 6 mm external diameter 16 mm, length 6 mm, usually used for telephone receivers. In the case of this example the characteristics of the electric signals derived from the transducer are as follows: amplitude of about 200 mV crest to crest.

[0028] Said system also comprises means of emission for applying said signals derived from said first transducer to a biological system receptor. As an example of such means of emission, one can cite a transducer with the following characteristics: bobbin with internal diameter 50 mm, length 80 mm, R=3.6 ohms, 3 layers of 112 spirals of copper wire, field on the axis to the centre 44 Oe/A, and on the edge 25 Oe/A. Examples of biological receptor systems have been mentioned above.

[0029] Preferably, the system according to the invention further comprises means for processing said signals derived from said first transducer, in function of the signals derived from a second transducer receiving the specific excitation field, in the absence of said substance. Thus said processed signals are more characteristic of the biological and/or chemical activity or the biological and/or chemical behaviour of said substance or said active element contained in said substance.

[0030] Preferably, according to another variant of the invention, the system further comprises means for processing the signals derived from said first transducer, in function of the characteristics of the specific excitation field. In the case of this variant embodiment also, said processed signals are more characteristic of the biological and/or chemical activity or of the biological and/or chemical behaviour of said substance or said active element contained in said substance.

[0031] Preferably, said specific excitation field has the characteristic of having a uniform power spectral density over a frequency band.

[0032] Preferably, the system according to the invention further comprises means for isolating said zone from parasitic fields from the environment.

[0033] Preferably, the system according to the invention further comprises control means for controlling the correlation between, on the one hand, the signal derived from said first transducer or the processed signal and, on the other hand, the biological and/or chemical activity or of the biological and/or chemical behaviour of said substance or said active element contained in said substance. Said control means comprise a third transducer applying the signals derived from said first transducer to a biological control system. In the case where the signals are processed, it is the processed signals which are applied to the biological control system. Said control means further comprise means for verifying that the biological control system reacts in a specific manner to the signals derived from said first transducer, according to the nature of the biological and/or chemical activity or of the biological and/or chemical behaviour of said substance or said active element contained in said substance from which the signals derived from said first transducer are issued. As an example, one can cite as biological control system: an isolated guinea-pig heart, a ligand/receptor couple in particular an antigen/antibody couple, an injectable substance provoking cutaneous reactions, isolated or cultured cells.

[0034] Preferably, the system according to the invention is such that said substance contains a low concentration or very weak concentration of an active element.

[0035] The invention also relates to a device for producing signals, in particular electric signals, characteristic of the biological and/or chemical activity or of the biological and/or chemical behaviour of a substance or an active

element contained in said substance. Said device comprises an emitter generating a specific excitation field of electric, magnetic and/or electromagnetic nature in a zone where said substance is located. It also comprises a first transducer receiving the fields resulting from the interaction of said specific excitation field and said substance. Said first transducer transforms said resulting fields into signals, in particular electric signals. Said signals are characteristic of the biological and/or chemical activity or of the biological and/or chemical behaviour of said substance or said active element contained in said substance.

[0036] The device according to the invention further comprises means for processing said signals derived from said first transducer, relative to the signals derived from a second transducer receiving the specific excitation field, in the absence of said substance.

[0037] According to another embodiment variant of the invention the device further comprises means for processing the signals derived from said first transducer, in function of the characteristics of the specific excitation field.

[0038] Preferably, said specific excitation field has the characteristic of a uniform power spectral density over a frequency band.

[0039] Preferably, the device according to the invention further comprises means for isolating said zone from parasitic fields from the environment.

[0040] The invention also relates to the applications of the method, system or the device described above. More particularly, the invention concerns the production of active substances in particular the production of drugs. Said active substances are produced by applying said signals derived from said first transducer to a carrier substance. In the case where said signals are processed, it is the signals thus processed which are applied to the carrier substance.

[0041] The invention also relates to the application of the process, system or device which has the aim of establishing a table of correlation between the characteristics of a determined substance or an active element contained in said determined substance and the modifications they can induce on test biological systems. Such correlation tables also enter into the framework of the invention, as well as the use of such correlation tables for detecting said determined substance or said active element contained in said determined substance. This detection can in particular be carried out remotely, after transmitting said characteristic signal to a testing laboratory possessing test biological systems. The correlation tables can also be used for controlling the production of homeopathic products, by making it possible to verify the activity of the latter during successive phases of dilution.

[0042] The invention also relates to electric signals linked to a biological and/or chemical activity, obtained through implementing the method, the system or the device according to the invention. It is possible to characterise these signals from the effects they produce on a biological control system like that described above.

[0043] Other characteristics and advantages of the invention will become clear by reading the description of the variants of embodiments of the invention, given as indicative but non-limiting examples, and also by reading the examples of experiments making it possible to validate the method of production of characteristic electric signals, the aim of the present invention, and which refer to the attached drawings in which:

[0044] **FIG. 1** shows a diagram of an example of an embodiment of a system and a device for producing characteristic electric signals, said system comprising an applicator making it possible to apply the characteristic signals to a biological system receptor,

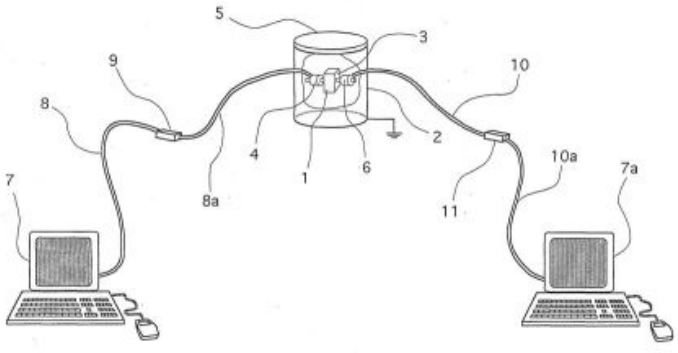


Fig. 1

[0045] **FIG. 1a** shows a detailed view in perspective of a part of the device for producing electric signals, showing the emitter of the excitation field and the transducer receiving the resulting fields,

[0046] **FIG. 1b** shows in diagrammatic form the type of micro-computer used either for generating the excitation fields, or for recording and transmitting under digitised form the characteristic electric signals, or for applying the characteristic electric signals to biological system receivers via transducers.

[0047] **FIG. 1c** shows a detailed view in perspective of a part of the applicator intended to apply the characteristic electric signals to biological system receptors,

[0048] **FIG. 2** shows a drawing of an example of an embodiment of an applicator making it possible to control the presence of the characteristic electric signals issued from a solution of acetylcholine by applying them to a biological control system constituted by an isolated perfused guinea-pig heart,

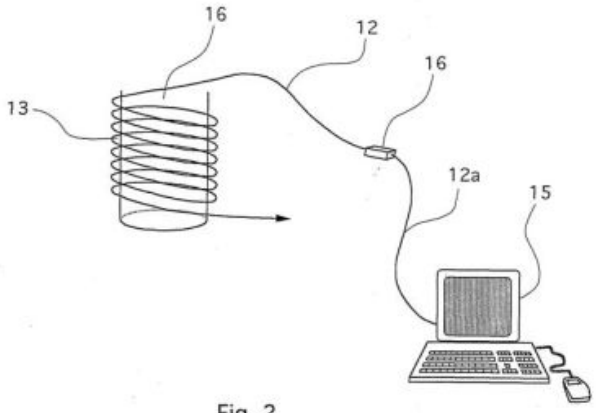


Fig. 2

[0049] **FIG. 3** shows a drawing of an example of an embodiment of an applicator making it possible to apply the characteristic electric signals issued from a solution containing as active biological element, Escherichia coli K1, Streptococcus or an antibody directed against the polysaccharidic antigen of Escherichia coli K1.

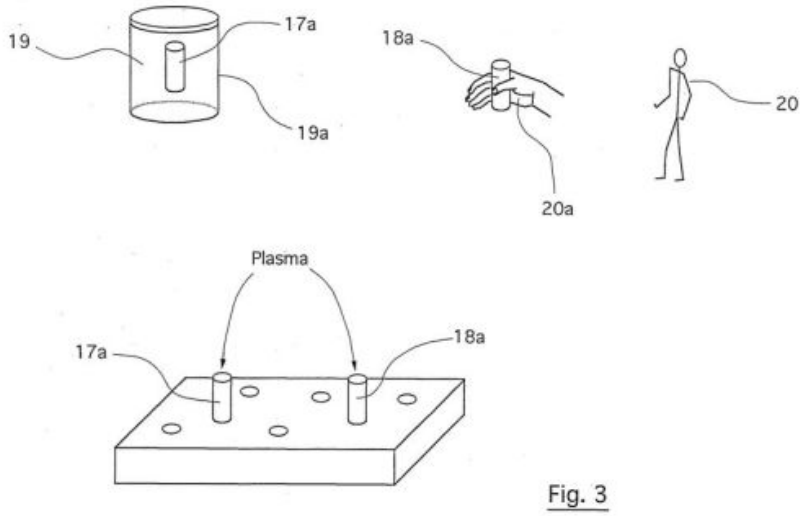
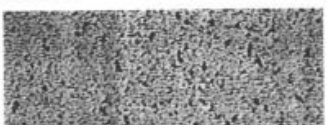


Fig. 3

[0050] **FIG. 3a** shows a black and white image of 320 pixels*240 pixels of precipitates formed during the precipitation reaction between the polysaccharidic antigen of Escherichia coli K1 and an antibody directed against this antigen, after application of characteristic electric signals coming from a biological system containing Streptococcus,

[0051] **FIG. 3b** shows a black and white image of 320 pixels*240 pixels of precipitates formed during the precipitation reaction between the polysaccharidic antigen of Escherichia coli KP1 and an antibody directed against this antigen, after application of characteristic electric signals coming from a biological system containing Escherichia coli K1,

FIG. 3a



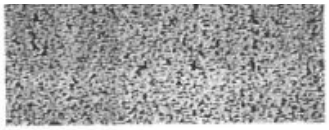
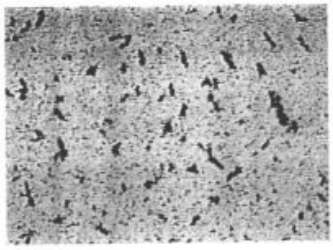


FIG. 3b



[0052] **FIG. 3c** shows a black and white image of 320 pixels*240 pixels of precipitates formed during the precipitation reaction between the polysaccharidic antigen of Escherichia coli K1 and an antibody directed against this antigen, after simultaneous application of characteristic electric signals coming from a biological system containing Streptococcus and coming from a biological system containing Escherichia coli K1,

[0053] **FIG. 3d** shows a black and white image of 320 pixels*240 pixels of precipitates formed during the precipitation reaction between the polysaccharidic antigen of Escherichia coli K1 and an antibody directed against this antigen, after simultaneous application of characteristic electric signals coming from a biological system containing Escherichia coli K1, and coming from a biological system containing a n antibody directed against Escherichia coli K1.

FIG. 3c

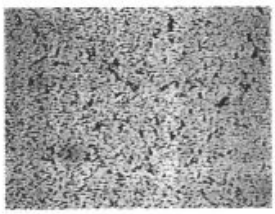
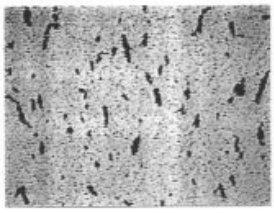


FIG. 3d



[0054] **FIG. 4** shows an image of the sub-cutaneous allergic reaction of a skin of a guinea-pig after injection of 0.1 ml distilled water, the distilled water having previously been submitted to an applicator of characteristic electric signals coming from a neuromediator such as acetylcholine (ACh).

[0055] Below is described an example of an embodiment of a system and of a device for producing characteristic electric signals, with reference to FIGS. 1, 1a, 1b and 1c. In these figures, a schematic drawing is given of a variant of an embodiment of a system making it possible to produce characteristic electric signals and to implement them for industrial purposes. The signals are characteristic, in the meaning of the present invention, of the biological and/or chemical activity or of the biological and/or chemical behaviour of a substance.

[0056] The system comprises a device 10 for producing electric signals characteristic of the biological and/or chemical activity or of the biological and/or chemical behaviour of a substance 1 or of an active element contained in said substance. In the case of the variant described with reference to FIGS. 1, 1a, 1b, 1c, said substance 1 is a solution of caffeine 10<-6 >M.

[0057] The device 10, located in Paris, for example, produces characteristic electric signals which are digitised after digital-analog conversion. The signals thus digitised are, in a known manner, transmitted remotely, for example by a computer communication network of the Internet type using radio links 11. The digitised signals thus transmitted are received by an applicator 12, located in New York for example, comprising 10 emission means 13. The emission means 13 make it possible to apply the characteristic signals (after digital-analog

conversion) to a biological system receptor. In the case of the embodiment described with reference toFIG. 1, 1a, 1b and 1c, the biological system receptor is a dietetic beverage. The digitised signals can be processed 27, recorded and stored 33, before their remote transmission and/or before having been applied to a biological system receiver.

[0058] The device for producing the signals 10 comprises a chamber 2 provided with electric and magnetic shielding isolating it from parasitic fields from the environment. The shielded cylindrical chamber is composed of three superposed layers: copper, soft iron, permalloy, made from sheets 1 mm thick. The chamber has an internal diameter of 65 mm, and a height of 100 mm. The chamber is closed by a shielded lid 5. An emitter 4 is situated inside the chamber. It generates a specific excitation field of electromagnetic nature. The emitter is supplied by a generator, 14. In the chamber 2 is placed a glass container 3 with the dimensions 10 mm*10 mm*4.5 mm. This container 3 holds 1 ml of the substance 1. The emitter 4 comprises a bobbin advantageously completed by a magnetic core in soft iron. The emitter bobbin 4 has an impedance of 300 ohms, an internal diameter of 6 mm, an external diameter of 16 mm, and a length of 6 mm. The magnetic core in soft iron is placed in contact with the external walls of the container 3. Said substance is thus submitted to an excitation field emitted by the emitter 4. The generator 14 is designed to generate a low frequency signal especially square or sinusoidal low frequency signals, of pink noise or, advantageously, white noise. The spectrum of the excitation signal supplying the emitter bobbin 4 corresponds closely to the spectrum of audible frequencies (20 Hz-20,000 Hz). The generator 14 can be a generator of an analog signal of known type, using for example a read-only memory (ROM, PROM, EPROM, EEPROM) containing the digital signal of the desired noise. This memory is linked in a known way to a digital-analog converter. A microcomputer 14 can also b e used, provided with a sound card 25 comprising a digital-analog converter 41. For example, one can use a computer 14 of the PC type, operating under the WINDOWS(R) 95 operating system from MICROSOFT and comprising, apart from the sound card 25 a microprocessor 27, an input/output interface 29, a controller 31 for mass storage 33 and a video interface 35 linked by one or several bus 37. The digital-analog converter 41 of the sound card 25 comprises an output terminal 8. The output terminal 8 of the sound card of the microcomputer 14 is linked to the input terminal 8' of the emitter 4, via an amplifier 15 whose specifications are the following: passband from 10 Hz to 20 kHz, gain 1 to 10, input sensitivity +/-1 V. Among the sound cards 25 which can be used, one can cite, for example the Soundblaster 16 card sold by the CREATIVELABS Company.

[0059] The transducer 6, situated inside the chamber 2, receives the fields resulting from the interaction between said specific excitation field and said substance 1. The transducer 6 transforms said resulting fields into electric signals. These electric signals arrive at the output terminals 9' of the transducer 6 under the form of a variable difference of potential or of an electric current of variable intensity. The transducer 6 comprises a bobbin with a soft iron core. This bobbin has an impedance of 300 ohms, an internal diameter of 6 mm, an external diameter of 16 mm, and a length of 6 mm. The magnetic core in soft iron is placed in contact with the external walls of the container 3.

[0060] Advantageously, the characteristic electric signals available at the output from the transducer 6 are amplified by a preamplifier 16. The amplifier-preamplifier 16 has the following specifications: passband from 10 Hz to 20 kHz, gain 50 to 100 for an input sensitivity of +/-100 mV or gain 500 to 2000 for an input sensitivity of +/-5 mV (to be used in the case of an "opposition series" connection of a second transducer). The characteristic electric signals can b e recorded 31, stored 33, transferred 11, 29, remotely by implementing technologies of electronics, computers and telecommunications known to those skilled in the art.

[0061] The recording of characteristic electric signals, or that of electric signals derived after amplification or processing, can be carried out in analog by a signal recorder, in particular o n magnetic tape, adapted to the frequencies of the characteristic electric signals at the output from the transducer 6. Since the passband used corresponds to the audio band, one can i n particular use a tape recorder. The output terminal 9' of the device for producing signals 10 is linked to the microphone input or to the line input of such a tape recorder. During play, the characteristic electric signals recorded are collected at an output terminal, in particular at the line output or at the loudspeaker output of the tape recorder. Preferably, digital recording of the characteristic electric signals is carried out after analog-digital conversion of said signals. In order to d o this, a micro-computer 17 is used, provided with a signal acquisition card 25. For example, one can use a PC 17 type computer, operating on the WINDOWS(R) 95 operating system from MICROSOFT. This microcomputer can be of the same type as that used to generate the excitation field. It can be the same microcomputer. In this case it comprises, apart from the sound card, an acquisition card 25, a microprocessor 27, an input/output interface 29, a controller 31, a mass storage 33 and a video interface 35 linked by one or several bus 37. The acquisition card 25 comprises a analog-digital converter 39 possessing, preferably, a resolution higher than 12 bits, and advantageously equal to 16 bits, as well as a sampling frequency double the maximum frequency one wishes to be able to digitise, for example 44 kHz. The output 9' of the transducer 6 is linked to the input 9 of the digital-analog converter 39 via the preamplifier 16.

[0062] All links consist of shielded cable. All the apparatus is earthed.

[0063] Advantageously, in order to process the characteristic electric signals or the signal derivatives, one uses the Matlab software from the company "The MathWorks". The output of the device 10 for producing characteristic electric signals is connected to the input 9 of the analog-digital converter 39 of the card 25 of the computer 17. One proceeds with a n acquisition of characteristic electric signals for a length of time for example

of between 1 and 60 sec (for example 6 sec) and the digital file is saved in a mass storage 33, for example under the form of a sound file with the WAV format. This file can later undergo digital processing, as for example digital amplification for calibrating the signal level, filtering for eliminating unwanted frequencies, or be transformed into its spectrum by a discrete FOURIER transform, preferable by the algorithm of FFT "Fast Fourier Transform".

[0064] The time length of the signal produced can be increased by repeating several times in a file a fragment or the totality of the sound file originally produced.

[0065] These processing means of characteristic electric signals can be used to improve performances of said characteristic electric signals. In the case of a first embodiment variant, a second transducer of the first type described above is envisaged. This second transducer transforms the excitation field into electric signals, in the absence of said substance. These electric signals are subtracted by an opposition series connection to the signals derived from the first transducer. Thus one obtains signals more representative of the interaction between the specific excitation field and the substance. In the case of a second embodiment variant, the processing means take into account the characteristics of the specific excitation field and reprocess the characteristic electric signals in the following way. First of all one proceeds by calculating the spread of the PSD. Then this power spectral density is contracted by conserving only the frequency band ranging for example from 140 Hz to 14 kHz, and reconstituting a signal from this PSD and randomly generated phases, and finally calibrating the power of the signal thus produced.

[0066] The characteristic electric signals available at the exit of the output from the device constituted by the combination of the emitter 4, the transducer 6 and if applicable the preamplifier 16 already themselves constitute products suitable for industrial applications. They can be amplified, processed, saved, stored, transferred remotely by implementing state of the art technologies in electronics, computers and telecommunications. The industrial applications for which they can in particular be implemented have been noted.

[0067] The file of characteristic electric signals, recorded under digital form as has just been described, possibly after processing, can be transferred remotely by a computer communication network. This network can comprise radio links 11. The file of characteristic electric signals thus transmitted is recorded by the mass storage of the microcomputer 18. For example, one can use a computer of the PC type, operating on a WINDOWS(R) 95 operating system from MICROSOFT. This microcomputer 18 can be of the same type as that used for generating the excitation field. The file of characteristic signals thus transmitted and recorded can be exploited, in known ways, to produce analog characteristic electric signals. The possibly processed file is transformed by a digital-analog converter 41 of the card 25 (or a separate card) of the computer 18. The digital-analog converter 41 delivers analog electric signals to its output 8 characteristic of the biological activity of the substance from which they are issued. These signals can be transformed, as described below, into electromagnetic fields and applied to biological systems.

[0068] Referring to FIG. 1c, a description is given of an embodiment of a system making it possible to apply characteristic electric signals to a biological system receiver and to modify its chemical behaviour. The flask 50 contains the biological system receiver. This is constituted, for example, of 10 ml distilled water for an injectable preparation (Biosédra or other brands) in a 15 ml tube in polypropylene (Falcon, Becton Dickinson 2097). This flask is set in an electromagnetic field radiated by a transducer 51, typically a bobbin. The bobbin, for example, has a length of 120 mm, an internal diameter of 25 mm, an external diameter of 28 mm, with 631 turns of wire of 0.5 mm diameter and a resistance of 4 ohms. The bobbin 51 is earthed. Without this representing any limiting character, the bobbin 51 of the transducer has a vertical axis making it possible to introduce the flask 50 containing the receptor biological system. The input terminals 8' of this bobbin 51 are linked, in the case of the embodiment variant described, to the output 8 of the digital-analog converter 41 of the microcomputer 18 via an amplifier 19 with the following specifications: passband from 10 Hz to 20 kHz, gain 1 to 20, input sensitivity 250 mV, output power RMS 60 W under 8 ohms, signal to noise ratio 80 dB. The voltage at the terminals of the bobbin 51 has an amplitude of 5 Veff and the signal is applied for 10 minutes. The input terminals 8' of the applicator can also be, in the case of certain embodiment variants, directly connected to the output of the preamplifier 16 or to the output 8 of the digital-analog converter 41 of the computer 17.

[0069] The invention also relates to the methods making i t possible to control the correlation between on the one hand, said signal derived from the transducer 6 and on the other hand, the biological and/or chemical activity or the biological and/or chemical behaviour of said substance or said active element contained in said substance. This control is carried out by applying, by means of a transducer of the type described in reference to FIG. 1c, signals derived from the transducer 6 to a biological control system and verifying that said biological control system reacts in a specific manner to the signals derived from said first transducer. In the case where said signals are processed, it is these processed signals which are applied to said biological control system. The reaction of said biological control system must be in relation to the nature of the biological and/or chemical activity or the biological and/or chemical behaviour of said substance or said active element contained in said substance from which are issued the signals derived from said first transducer.

[0070] As an example of a biological control system, an d referring to FIG. 2, a test will be described below derived from that known under the test name of a perfused isolated guinea-pig heart (or Langendorff experiment) and whose process is described in the work entitled: "Methods in Immunology and

Immunochemistry" published by Williams and Chase, Academic Press 1976, particularly page 68; or further in the work entitled: "L'experimentation animate en cardiologie" INSERM Médecine-Science-Coll. Flammarion-Author Bernard SWYNGHEDAUW-particularly Ch. 3.1 p.81 "Organe Isolé-Coceur Isolé selon Langendorff-Montage à pression coronaire constante"; or further in the work entitled "The isolated perfused Heart according to Langendorff" H. J. Döiring, H. Dehnart-Biomesstechnik-Verlag March GmbH, D-7806 March. In FIG. 2 one recognises the diagram known from the Langendorff experiment. The equipment described in these works has been completed by a transducer in the form of a bobbin 60 of a varnished copper wire of diameter 0.5 mm, with a diameter of 110 mm, a length of 40 mm and with an impedance of 4 ohms.

[0071] Three experiments were carried out with characteristic electric signals coming respectively from the following substances:

[0072] for the first, ionophoretic-calcium A 23187 (Sigma C-7522) (I) at a concentration of 10<-6>M in distilled water for injectable preparation (for example the Biosedra brand).

[0073] for the second, distilled water for injectable preparation (for example the Biosedra brand). (E)

[0074] for the third, caffeine (Sigma C-0750) (C) at a concentration of 10<-6>M in distilled water for injectable preparation (for example the Biosédra brand). (E)

[0075] For each of these three experiments, the substances were placed in the container 3 of the chamber 2 and their characteristic electric signals were acquired in conformity with the operating process described with reference to FIGS. 1, 1a and 1b.

[0076] The three characteristic electric signals produced as described above were applied to the guinea-pig heart, connecting the terminals 8' of the bobbin 60 to the output of the amplifier 19 of power 60 W. The three characteristic electric signals were applied for 2 minutes under a voltage of 5 Veff.

[0077] The fraction collector collected the tubes making it possible to measure the debit of the guinea-pig heart at the rate of 1 tube per minute. The buffer solution crossing the heart had the following composition: CaCl 2 mM, NaHCO3 25 mM, NaCl 118 mM, MgSO4 1.2 mM, KHPO4 1.2 mM, Glucose 11 mM, Pyruvate 2 mM.

[0078] The table below shows (in ml) the quantity of the buffer solution recuperated in the collector tubes during the time.

Time mins.	No signal	Signal ionophoretic-calcium	Signal water	Signal caffeine
1	4.4	4.4	4.5	4.3
2	4.3	4.3	4.5	4.4
3	4.3	4.4	4.4	4.4
4	4.4	4.3	4.5	4.5
5	4.4	4.2	4.5	4.2
6	4.3	4.9	4.4	4.0
7	4.3	5.2	4.4	3.6
8	4.4	5.4	4.5	3.4
9	4.3	5.4	4.5	3.2
10	4.4	5.2	4.4	3.0
11	4.3	5.0	4.5	3.0
12	4.4	5.0	4.4	3.2
13	4.3	4.8	4.4	3.4
14	4.4	4.8	4.5	3.6

15 4.3 4.6 4.4 3.8

20 4.3 4.5 4.4 4.0

25 4.3 4.5 4.5 4.1

30 4.3 4.5 4.4 4.0

[0079] This table shows that the guinea-pig heart reacted to the characteristic electric signals coming from ionophoretic-calcium, water and caffeine as it would have reacted to injections of each of these three substances (see table below).

Time ionophoretic-calcium caffeine

minutes Water 10<-6>M 10<-6>M

1 5.2 5.1 5.1

2 5.1 5.0 5.0

3 5.0 5.2 5.0

4 5.1 5.0 4.9

5 5.1 4.9 4.6

6 5.2 5.4 4.2

7 5.2 5.6 4.0

8 5.1 6.2 4.1

9 5.1 6.4 4.0

10 5.2 6.4 4.2

11 5.1 6.2 4.1

12 5.0 6.0 4.3

13 5.1 6.0 4.4

14 5.0 5.9 4.5

15 5.0 6.0 4.5

20 5.1 5.7 4.6

25 5.0 5.4 4.5

30 5.0 5.2 4.5

[0080] Next, as an example, a description follows with reference to FIGS. 3, 3a, 3c and 3d of a precipitation test between the polysaccharidic antigen of Escherichia coli K1 and an antibody against this antigen making it possible to control the characteristic electric signals of the biological activity of Escheria coli. This test is defined below under the name of precipitation test.

[0081] One tests the effects on a precipitation reaction between the polysaccharidic antigen of Escherichia coli K1 and an antibody directed against this antigen:

[0082] from the application of a characteristic electric signal of the biological activity of an antigenic substance foreign to this reaction such as the Streptococcus,

[0083] from the application of a characteristic electric signal of the biological activity of the polysaccharidic antigen of Escherichia coli,

[0084] from the simultaneous application of a characteristic electric signal of the biological activity of Streptococcus and the characteristic electric signal of the biological activity of an antibody directed against Escherichia coli,

[0085] from the simultaneous application of a characteristic electric signal of the biological activity of Escherichia coli and the characteristic electric signal of the biological activity of a n antibody directed against this antigen.

[0086] The acquisition of the characteristic electric signals of the biological activities of Escherichia coli, of its specific antibody and of the polysaccharidic antigen of Streptococcus was carried out by means of the device 10 described with reference to FIGS. 1, 1a, 1b.

[0087] The acquisition of the characteristic electric signal of the biological activity of Streptococcus was carried out by placing at the centre of the chamber 2 a container 3 holding 1 ml of an aqueous suspension of Streptococcus bacteria previously formalised (6.10<6 >cfu/ml).

[0088] The acquisition of the characteristic electric signals of the biological activity of the specific antibody of Escherichia coli and its specific antibody was carried out by operating in the same manner, but using respectively:

[0089] a container 3 holding 1 ml of an aqueous suspension of bacteria of Escherichia coli K1 previously formalised (6.10<6 >cfu/ml).

[0090] a container 3 holding 1 ml of a suspension of particles of a latex sensitised by a mouse monoclonal antibody specific of Escherichia coli K1, coming from a PASTOREX(R) MENINGITIS kit (Ref. 61709-SANOFI DIAGNOSTICS PASTEUR).

[0091] The tests were carried out using as reagents:

[0092] on the one hand, a solution of polysaccharidic antigen of Escherichia coli K1 prepared by dissolving an antigenic extract from a PASTOREX(R) MENINGITIS kit (Ref. 61709-SANOFI DIAGNOSTICS PASTEUR) in 1 ml of distilled and sterile water, then dilution to 1/7, 1/7.5 or 1/8 in physiological serum; and

[0093] on the other hand the latex sensitised by a mouse monoclonal antibody specific of Escherichia coli K1 present in this same kit, after dilution to 1/3 in physiological serum.

[0094] For each of these tests, the following protocol was used:

[0095] one places in an oven heated to 37[deg.] C. a transducer 151 constituted by a bobbin measuring 120 mm in length and 25 mm internal diameter, with 631 turns and a resistance of 4.7 ohms and linked by its input terminal 8' to the output 8 of the digital-analog converter of a Soundblaster card and a computer 17 (one could also use a computer 18 remotely) reinserting the recorded files constituted by the electric signals one wishes to apply for the time required to bring this transducer to the temperature of 37[deg.] C.;

[0096] one deposits on a slide 147 supplied with a capillary 149 in a serpentine shape (of the type of those provided in the PASTOREX MENINGITIS kits), at a small distance from the opening of the latter, a drop 145 (40 to 50 [mu]l) of the antigenic solution as described in point b) above, together with a drop 143 (also corresponding to a volume of 40 to 50 [mu]l), latex sensitised by the antibody, taking care that these drops do not mix.

[0097] one applies, to the two drops of reagents thus deposited, the electric signal or signals desired by placing the slide at the centre of the transducer 151 for about 2 minutes and reinserting a sound file with the aid of the computer 17 (or the remote computer 18),

[0098] one mixes the two drops of reagents 143, 145 for about 10 seconds and then leaves the reaction mixture in the oven for about 13 minutes to migrate into the capillary and the precipitation reaction to take place:

[0099] one takes the blade out of the oven and then proceeds to read this precipitation.

[0100] This reading is carried out by analysis, by means of analysis software and image processing on a PC type computer using the WINDOWS(R) 95 operating system (MICROSOFT), of an image acquired with the aid of a video camera positioned on an optical microscope and connected to said computer by a video acquisition card. The camera works in the grey shades. A first processing increases the contrast, the threshold being set so that the precipitates appear in black, while the zones without latex particles or precipitates appear white.

[0101] Based on the analysis of two-dimensional space spread of the dark zones of the image, the computer determines a precipitation index (I) calculated according to the formula: ***

[0102] The precipitation index is accordingly higher when the size of the precipitates formed during the precipitation reaction is greater. The control test for the presence of a characteristic signal of the biological activity of Escherichia coli is considered as positive when, during an experiment, the application of characteristic electric signals of the biological activity of Escherichia coli and/or the biological activity of its

specific antibody leads to obtaining a precipitation index significantly higher (by at least 40%) than the maximum of those obtained, under the same conditions, and over for example 3 experiments, after application of the characteristic electric signal of the biological activity of Streptococcus.

[0103] Table A below shows the precipitation indexes obtained in a first series of tests aimed at comparing the effects of the application of characteristic electric signals of the biological activity of Escherichia coli (E. coli) coming from a biological system containing Escherichia coli with those observed after application, under the same reaction conditions, of characteristic electric signals of the biological activity of Streptococcus (St) coming from a biological system containing Streptococcus and for 3 different dilutions (1/7, 1/7.5 and 1/8) of the polysaccharidic antigen of Escherichia coli K1 used as reagent in the precipitation reactions.

TABLE A

Dilution of the solution of E. coli K1 antigen	Precipitation index (I) Signal St	Signal E. coli
1/7	11	173
	6	52
	16	154
1/7.5	58	141
	32	117
	12	107
1/8	10	113
	6	37
	8	21

[0104] Moreover, FIGS. 3a and 3b show, as examples, images of the precipitates formed, on the one hand, after application of the characteristic electric signal of the biological activity of Streptococcus (FIG. 3a) and, on the other hand, after application of the characteristic electric signal of the biological activity of Escherichia coli (FIG. 3b). These images correspond respectively to the precipitation indexes of 32 and 117 which are recorded on line 5 of Table A.

[0105] As for Table B below, the precipitation indexes obtained in a second series of experiments within the framework of which the effects of simultaneous application of the characteristic electric signal of the biological activity of Escherichia coli and the characteristic electric signal of the biological activity of the antibody directed against Escherichia coli were compared to those of the simultaneous application, under the same reaction conditions, of the characteristic electric signal of the biological activity of Streptococcus and of the characteristic electric signal of the biological activity of the antibody directed against Escherichia coli, carried out for 2 different dilutions (1/7 and 1/7.5) of the polysaccharidic antigen of Escherichia coli K1 used as reagent.

TABLE B

Dilution of the solution of E. coli K1 antigen	Signal St + Signal antibody anti-E. coli	Signal E. coli + antibody anti-E. coli
1/7	18	94
	71	247
1/7.5	48	212
	93	1141

[0106] FIGS. 3c and 3d show, also as examples, images of precipitates corresponding respectively to the precipitation indexes 71 and 247 recorded on line 2 of Table B.

[0107] All these results demonstrate clearly the aptitude presented by a ligand/receptor couple for revealing and controlling the presence of a characteristic electric signal of the biological activity of a ligand and/or its receptor. In fact, in the presence of a specific characteristic signal of the ligand/receptor couple or one of the elements of this couple, the formation of complexes formed by the reaction between this ligand and this receptor is amplified. This amplification is very specific, since the characteristic electric signal of the biological activity of a biologically active element, but foreign to this reaction, does not itself produce this amplification effect.

[0108] In the meaning of the present invention, the "ligand/ receptor couple" means any couple formed by two substances able to recognise each other specifically, to link together and to act together to form complexes. Thus, it can concern an antigen/antibody couple, or hapten/antibody in which the ligand (the antigen or the hapten) can be a biological compound (protein, enzyme, hormone, toxin, tumour tag), a chemical compound (toxic or medicated active principle, for example), or a cell or particle antigen (cell, bacteria, virus, fungus, . . .), the receptor being able to be a soluble antibody or a membranous receptor. It can also be a couple formed by an enzyme and its specific substrate.

[0109] These results show clearly that it is possible to use ligand/receptor couples and, in general, test biological systems to constitute a correlation table between the characteristic signals issued from a determined substance or from an active element contained in a determined substance and the modifications they can induce on test biological systems, in particular such as a ligand/receptor couple.

[0110] These correlation tables can be used later for detecting active elements by analysing the effects of characteristic signals coming from them on test biological systems recorded in the correlation table.

[0111] As an example, with reference to FIG. 4, a presentation is given below of the test known under the name of guinea-pig cutaneous test and described in chapter 11 (p.346-351) in the second edition of "Immunology" edited by Jean-Francois Bach, coll. John Wiley & Sons; or further in the 3rd edition of "The handbook of Experimental Immunology" edited by D. M. Weir, coll. Blackwell, Ch. 21 "Passive cutaneous anaphylaxis (PCA)" by W. E. Brocklehurst; or further in the work edited by Williams & Chase entitled "Methods in IMMUNOLOGY and IMMUNOCHEMISTRY"-Vol. 5-Ch. 19 "Anaphylaxis".

[0112] The guinea-pig is used when still alive, and is given a n intravenous injection of a blue colorant (Evans blue-Sigma E 2129) which fixes on the blood albumin. The albumin does not leave the vessels, unless there is inflammation, and thus vasodilatation and permeability of the vessels, the typical example of such a reaction with man being urticaria.

[0113] The test is carried out by injecting under the skin of the animal prepared in this way, 0.1 ml of the solution whose activity is to be controlled. Nextone measures the diameter of the blue marks appearing around the points of injection. In order to do this the skin is scanned, and then the bitmap image file is recorded. Finally the sizes of the blue marks due to the reaction are evaluated.

[0114] In the example described, a control was carried out of the presence of signals characteristic of the biological activity of the acetylcholine neuromediator (ACh; Sigma A2661) in solution in a physiological solution, by analysing the effects o n the skin of a guinea-pig:

[0115] on the one hand, of an injection of 0.1 ml distilled water, after applying to this distilled water a characteristic electric signal of the biological activity of acetylcholine,

[0116] on the other hand, of an injection of 0.1 ml distilled water, after applying to this distilled water a characteristic electric signal of the biological activity of a product close to acetylcholine but inactive: the mixture acetate/choline (A-C) (A: Sigma S8625; C: Sigma C7017).

[0117] The acquisition of the characteristic electric signals of the biological activities of acetylcholine and the acetate/choline mixture was carried out by means of the device 10 described with reference to FIGS. 1, 1a, 1b.

[0118] The acquisition of the characteristic electric signal of the biological activity of acetylcholine was carried out by placing in the centre of the chamber 2 a container 3 holding 1 ml of a solution of acetylcholine in distilled water at the concentration of 10<-6>M.

[0119] The acquisition of characteristic electric signals of the biological activity of the mixture acetate/acetylcholine was carried out by operating in the same manner, but using a container 3 holding 1 ml of a solution of acetate/acetylcholine in distilled water at the concentration of 10<-6>M.

[0120] For each of the tests, the following protocol was used:

[0121] The bobbin 51 of FIG. 1c was used as applicator.

[0122] The numbers figuring in the first column of tables C, D and E below correspond to the references in FIG. 4.

TABLE C

No.	Distilled water solution injected	Dia. in mm
200	After application	12
201	of the ACh signal	6
202		7
203		
204		16
300	Without application	3
301	of the signal	2
302		4
303		3
304		1
305		0
306		1

[0123] Experiments numbered 200 to 204 show that the solutions of distilled water injected after application of the ACh signal setoff a significant cutaneous reaction (average 11 mm) compared with the same solutions of distilled water injected without application of the ACh signal. The latter d o not setoff a reaction as shown in experiments numbered 300 to 306 (3 mm).

TABLE D

No.	Solution injected	Dia. in mm
310	ACh in 10<-6>M solution	23
311		25
312		23
313		21
314		18

[0124] Comparison of the experiments in tables C and D shows that the injections of solutions of distilled water after application of the ACh signal (experiments 200 to 204) have effects which are less, but comparable, on the guinea-pig skin to those of injections of ponderal ACh solutions (experiments 310 to 314).

TABLE E

No.	Distilled water solution injected	Dia. in mm
400	After application	2
401	of the A-C signal	2
402		
403		3
404		1
410	A-C in solution at 10<-6>M	3
411		2
412		1

[0125] Experiments numbered 400 to 404 and 410 to 412 in Table E are carried out from a product close to acetylcholine but inactive: the acetate/choline (A-C) mixture.

[0126] Experiments 410, 411, 412, correspond to an injection of a ponderal solution of A-C 10<-6>M. One notes that an injection of distilled water solution after application of the A-C signal (exp. 400 to 404) and that a ponderal injection (exp. 410, 411, 412) do not provoke any effect (diameter between 1 and 3 mm). These injections show that the cutaneous reaction of the guinea-pig is really specific to the nature of the substance in solution because these injections, carried out under the same conditions as the injections numbered 200 to 202, have no effect.

[0127] The experiments of tables C to E make it evident that the guinea-pig skin test makes it possible to control the presence of a signal coming from a substance with a biological activity such as acetylcholine.

[0128] Below is described the method used for controlling the following homeopathic products: arnica 7CH, acetylcholinum 7CH.

[0129] First of all one has to produce the characteristic signals of the product to be tested. In the case where the homeopathic product to test is a solution, one proceeds by registering a sample of 1 ml as described in this patent. In the case where one wishes to test homeopathic granules, first of all a solution is prepared, for example 5 ml, by diluting 2 granules per ml of distilled water for injectable preparation (for example the Biosédra brand), and then one proceeds with the registering of a sample of 1 ml according to the method described in this patent.

[0130] Nextone uses for example one or several of the three methods described above (perfused isolated guinea-pig heart; precipitation test of a ligand/receptor couple and cutaneous test on a guinea-pig). Since the correlation between the reaction of these biological control systems and the biological and/or chemical activity of the

product having served to produce the homeopathic product has been demonstrated, a positive reaction of the biological control system will show the presence of the activity searched for in the homeopathic product tested. In the same way, a negative reaction of the biological control system will show the absence of the activity searched for in the homeopathic product tested.

Product to be tested	guinea-pig (diameter of marks in mm)	Detection of activity
Neutral granules	0.6 +- 0.5 (n = 5)	NO
Arnica 7CH granules	16.6 +- 2.9 (n = 5)	YES

WO0017637

METHOD AND SYSTEM FOR PRODUCING A SUBSTANCE OR A SIGNAL WITH COAGULATING OR ANTICOAGULANT EFFECT

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Abstract -- The invention concerns a method and a system for producing a signal, in particular an electric signal, or a substance having a coagulating or anticoagulant effect. The method is characterised in that it is based on a source substance with coagulating effect, in particular, Ca<++> ions, or an anticoagulant effect, in particular heparin. The method consists in: transforming (10) the electromagnetic field derived from said source substance located in the chamber (D), into a signal, in particular an electric signal, using a transducer-receiver sensing the electromagnetic field; applying (12) to a receiving substance located in the chamber (E), in particular water or a water-ethanol mixture or homeopathic granules, said signal derived from said transducer-receiver, using a transducer-transmitter. After said treatment, the receiving substance, initially inactive, has a coagulating or anticoagulant effect.

Present invention relates to a process and a system to produce a substance or a signal, especially an electrical signal, having a coagulant effect or anticoagulant. The invention also relates to such a therapeutic substance or such signal and their effects. The invention also relates to a process and a system to test 1 ' coagulant effect or anticoagulant of a substance or a signal.

One knows, since the workings of Research of Mr Jacques Benveniste, especially those described in patent application WO 94/17406 published on August 4, 1994, that one can collect starting from a biological and/or chemical element active such as a chemical compound, a cell or a microorganism, or starting from a substance containing this active element, a “electromagnetic signal characteristic of the biological activity and/or chemical or biological and/or chemical behavior " of the aforesaid substance and/or of the aforesaid active element contained in the aforementioned substance.

One knows also that it is possible to transform, especially by means of a transducer, such an electromagnetic signal in an electrical signal.

In the continuation of the text, one understands also by " electrical signal characteristic of the chemical biological activity and/or or the biological and/or chemical behavior of a substance or an active element contained in the aforementioned substance " any electrical signal derived by digitization and/or signal processing. In this expression, one employs " characteristic " in the direction where the physical parameters of the electrical signal are specific with substance or the active element contained in the aforementioned substance. In other words, the application of this electrical signal, via a transducer, a biological system of control allows: (I) to induce a biological activity and/or chemical on the aforementioned biological system of control in ratio with that of substance of origin or the active element which it contains, (II) to reveal a characteristic of substance or active element which it contains, with the origin of the aforesaid electrical signal.

Patent application WO 94/17406, published on August 4, 1994, described a process and an apparatus to collect " an electromagnetic signal characteristic of a biological activity and/or chemical or a biological and/or chemical behavior to start from a biological and/or chemical element active such as a chemical compound, a cell or a microorganism, or starting from a substance containing this active element such as a purified preparation, a biological taking away, a living being.

The inventors have since exposed that it is possible to improve the electromagnetic signal quality collected as well as the reliability of the production method of this signal and that it is consequently possible to produce a capable electrical signal characteristic of industrial applications.

These developments were described in the French application FR 98 12.058 deposited on September 23, 1998. As a requirement, the elements of this application, not yet published to date, useful with the comprehension of the present invention, will be extracted and inserted in the present application.

Process and system in accordance with the invention to produce a substance having a coagulant effect or

anticoagulant.

The process in accordance with the invention to produce a substance having a coagulant effect or anticoagulant, starting from a substance source having a coagulant effect, especially Ca++ ions, or anticoagulant, especially of heparin, comprises at least the following steps.

Step 1 has as an object to transform the electromagnetic field coming of the aforesaid the substance source into a signal, especially a signal electrical characteristic, by means of a collecting transducer-receptor the aforementioned electromagnetic field.

Step 2 has as an object to apply to a receiving substance, especially from 1 ' water or a homeopathic mixture water-ethanol or granules, the aforementioned coming signal of the aforesaid transducer-receptor, by means of one transducer-emitter.

It is noted that after the treatment above defined, receiving substance, initially inactive, present a coagulating or anticoagulant activity.

The receiving substance thus treated will be refer hereafter the " substance treated ".

Concentration of the active elements in the substance source, especially concentration of the Ca++ ions having a coagulant effect or heparin having an anticoagulant effect, can be about IpM. It can too to be very low and to reach 10 '4 Mr. The substance source could also be composed of homeopathic products, diluted if need in 1 ' water for injectable preparation.

Preferably, to transform the electromagnetic field coming from the aforementioned substance source in an electrical signal: one place the aforementioned substance source in a zone subjected to one excitation field of electrical, magnetic nature and/or electromagnetic and, one transforms the resulting fields of the interaction of the excitation field and the substance source into an electrical signal, with means of a transducer-receptor collecting the aforementioned resulting fields.

The system in accordance with the invention to produce a substance having an effect coagulant or anticoagulant, starting from a substance source having an effect coagulant, especially of the ions Ca++, or anticoagulant, especially of heparin, includes/understands at least the elements hereafter defined.

A transducer-receptor receives the electromagnetic field coming of the aforesaid the substance source. The aforementioned transducer-receptor transforms the aforementioned electromagnetic field into a signal, especially an electrical signal.

An transducer-emitter makes it possible to apply the coming signal of the aforesaid transducer-receptor to a receiving substance, especially of 1 ' a homeopathic water or mixture water-ethanol or granules.

After treatment implemented by the system above defined, receiving, initially inactive, present substance a coagulating or anticoagulant activity.

Preferably, the system in accordance with the invention includes/understands moreover an emitter generating excitation field of an electrical, magnetic and/or electromagnetic nature in a zone where the aforementioned substance source is located. A transducer-receptor, receiving the resulting fields of 1 ' interaction of the aforesaid excitation field and the substance source, transforms the aforementioned resulting fields into a signal, especially an electrical signal.

Substance in accordance with the invention having a coagulant effect or anticoagulant

The invention relates to a substance also having a coagulant effect or anticoagulant. The aforementioned substance, especially 1 ' a homeopathic water or mixture eauéthanol or granules, is characterized in what it was treated by means of an electrical signal or electromagnetic coming from a substance source having coagulants effects, especially Ca++ ions, or anticoagulant, especially of heparin.

The invention also relates to the therapeutic applications of such a substance. The substance in accordance with the invention can be used in the treatment of the embolic disease thrombo. It can be also used to proceed to tests of exploration of coagulation.

Process in accordance with the invention to test 1 ' coagulant effect or anticoagulant of a substance

The invention also relates to a process to test a substance having a coagulant effect, especially Cl++ ions, or anticoagulant, especially of heparin. The process comprises at least the following steps.

Step 1 has as an object to transform the electromagnetic field coming of the aforesaid substance, in a signal, especially an electrical signal, by means of a transducer-receptor collecting the aforementioned electromagnetic field.

Step 2 has as an object to apply to a sensitive biological system, directly or indirectly, the aforementioned coming signal of the aforesaid transducteurreceptor.

Preferably, according to 1 ' invention to transform the electromagnetic field coming of the aforesaid substance into an electrical signal:

one place the aforementioned substance in a zone subjected to an excitation field of electrical, magnetic and/or electromagnetic nature,

one transforms the resulting fields of the interaction of the excitation field and the substance source into an electrical signal, by means of a transducer-receptor collecting the aforementioned resulting fields.

Advantageously, the sensitive biological system can be blood or plasma to which one applique the aforementioned signal by means of a transducteuremettor. One can also use rich plasma in platelets advantageously.

Advantageously, according to another variant of performing, the sensitive biological system is an animal, especially a rabbit, to which one manages, especially under the language, a substance, especially of 1 ' water, treated by the aforementioned signal by means of an transducer-emitter.

The process in accordance with the invention to test the coagulant effect or anticoagulant of a substance can be applied with the control of production of homeopathic products.

Process and system in accordance with the invention to produce a signal having a coagulant effect or anticoagulant.

The process in accordance with the invention to produce a signal, especially an electrical signal or electromagnetic, having a coagulant effect or anticoagulant, starting from a substance source having a coagulant effect, especially Ca++ ions, or anticoagulant, especially of heparin, comprises at least the step to transform the electromagnetic field coming of the aforesaid the substance source, in a signal, especially an electrical signal, by means of a transducer-receptor collecting the aforementioned electromagnetic field.

Preferably, to transform the electromagnetic field coming of the aforesaid the substance source into an electrical signal:
one place the aforementioned substance source in a zone subjected to an excitation field of electrical, magnetic and/or electromagnetic nature,

one transforms the resulting fields of the interaction of the excitation field and the substance source, in a signal, especially an electrical signal, by means of a transducer-receptor collecting the aforementioned resulting fields.

Preferably also, the process in accordance with the invention to produce a signal, especially an electrical signal or electromagnetic, having a coagulant effect or anticoagulant, includes/understands moreover the step to control the correlations between on the one hand, the coming signal of the aforesaid transducteurreceptor and on the other hand, the coagulating or anticoagulant activity of the aforesaid the substance source, while applying, directly or indirectly, the aforementioned signal with a biological system of control and by checking that the aforementioned biological system of control reacts in accordance with the coagulating or anticoagulant activity substance source from which the signal results.

Advantageously, the biological system of control is blood or plasma to which one applique the aforementioned signal by means of a transducteuremettor. One can also use rich plasma in platelets advantageously.

Advantageously, in another variant of performing, the biological system of control is an animal, especially a rabbit, to which one manages, especially under the language, a substance, especially of 1 ' water, treated by the aforementioned signal by means of an transducer-emitter.

Present invention relates to also a system to produce a signal, especially an electrical signal or electromagnetic, having a coagulant effect or anticoagulant, starting from a substance source having an effect coagulant, especially ions Ca, or anticoagulant, especially of heparin. The aforementioned system includes/understands a transducer-receptor receiving the electromagnetic field coming of the aforesaid the substance source, the aforementioned transducer-receptor transformants the aforementioned field electromagnetic in a signal, especially an electrical signal.

Preferably, the system in accordance with the invention includes/understands moreover an emitter generating excitation field of an electrical, magnetic and/or electromagnetic nature in a zone where the aforementioned substance source is located.

The aforementioned transducer-receptor, receiving the resulting fields of the interaction of the aforesaid excitation field and the substance source, transforms the aforementioned resulting fields into a signal, especially

an electrical signal.

Preferably also, the system in accordance with the invention includes/understands moreover control means to control the correlations between on the one hand, the coming signal of the aforesaid transducer-receptor and on the other hand, the coagulating or anticoagulant activity of the aforesaid the substance source. The aforementioned control means include/understand an transducer-emitter applying, directly or indirectly, the aforementioned signal with a biological system of control. The aforementioned control means include/understand moreover verification means to check that the biological system of control reacts in accordance with the coagulating or anticoagulant activity substance source from which the signal results.

Advantageously, the biological system of control is blood or plasma to which one applique the aforementioned signal by means of the said transducteuremettor. One can also use rich plasma in platelets advantageously.

Advantageously in another variant of performing, the biological system of control is an animal, especially a rabbit, to which one manages, especially under the language, a substance, especially of 1 ' water, treated by the aforementioned signal by means of the said transducer-emitter.

Signal in accordance with the invention having a coagulant effect or anticoagulant

Present invention relates to also a signal itself, especially an electrical signal or electromagnetic, having a coagulant effect or anticoagulant. The aforementioned signal is obtained starting from a substance source having a coagulant effect, especially Cl++ ions, or anticoagulant, especially of heparin, by implementing the processes or the systems described above. The aforementioned signal is characterized in what a biological system of control reacts, after direct application or indirect of the aforesaid signal, in accordance with the coagulating or anticoagulant activity substance source from which the signal results.

Advantageously, the biological system of control is blood or plasma to which one applique the aforementioned signal by means of a transducteuremettor. One can also use rich plasma in platelets advantageously.

Advantageously in another variant of performing, the biological system of control is an animal, especially a rabbit, to which one manages, especially under the language, a substance, especially of 1 ' water, treated by the aforementioned signal by means of an transducer-emitter.

The invention also relates to the therapeutic applications of such a signal. The signal in accordance with the invention can be used, directly or indirectly via a receiving material, in the treatment of the embolic diseases thrombo. It can be also used, directly or indirectly via a receiving material, to proceed to tests of exploration of coagulation.

Process in accordance with the invention to test 1 ' coagulant effect or anticoagulant of a signal

The invention also relates to a process to test a signal having a coagulant effect or anticoagulant. The aforementioned signal is obtained starting from a substance source having a coagulant effect, especially Ca++ ions, or anticoagulant, especially of heparin, by implementing the processes or the systems previously described. The process in accordance with the invention includes/understands the step to apply the aforementioned signal, directly or indirectly, with a biological system test and to check that the biological system test reacts in accordance with the coagulating or anticoagulant activity substance source from which the signal results.

Advantageously, the biological system test is blood or plasma to which one applique the aforementioned signal by means of an transducer-emitter. One can also use rich plasma in platelets advantageously.

Advantageously, according to another variant of performing, the biological system test is an animal, especially a rabbit, to which one manages, especially under the language, a substance, especially of 1 ' water, treated by the aforementioned signal by means of an transducer-emitter.

The process in accordance with the invention to test 1 ' coagulant effect or anticoagulant of a signal can be applied with the control of production of homeopathic products.

Other characteristics and benefits of the invention will appear with the reading of the description of variants of performing of the invention, given as indicative and nonrestrictive example, like with the reading of the examples of experiments having made it possible to validate the production method of a substance or an electrical signal characteristic, having coagulants effects or anticoagulants. Description refers to the annexed drawings in which:

Figure 1 represents a scheme of an example of performing of a system making it possible to produce an electrical signal characteristic, and to thus apply the electrical signal characteristic product to a receiving substance or a biological system of control or a sensitive biological system, appear it represents it a detailed view in perspective of a part of the device of production of the electrical signal, showing the field emitter of excitation and the transducer-receptor receiving the resulting fields,

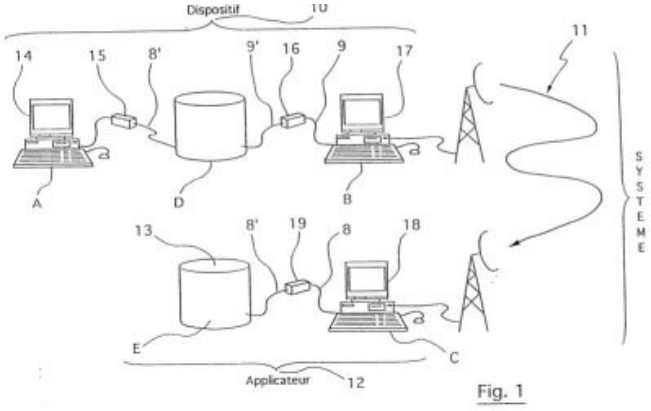

Fig. 1

Figure 1b represents in the form of block diagram the type of microcomputer used either to generate the excitation fields, or to record and transmit in digitized form the electrical signal characteristic,

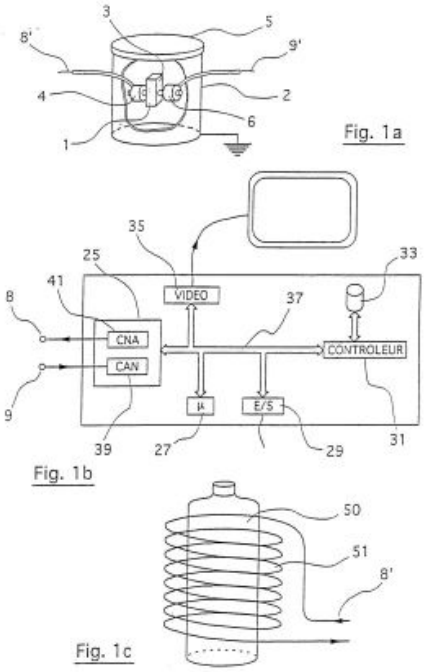

Fig. 1a

Fig. 1b

Fig. 1c

appear it represents it a detailed view in perspective of a part of an transducer-emitter intended to apply the electrical signal characteristic to a receiving substance or a biological system of control or a sensitive biological system.

General scheme of the system

One now will describe, while referring on figures 1, lb and LLC, an example of performing of an allowing system
(I) to produce

- to start from ions Ca an electrical signal characteristic having a coagulants effect, or

- to start from heparin an electrical signal characteristic having an anticoagulant effect and,

(II) to apply such a characteristic signal to a receiving substance or a biological system of control or a sensitive biological system.

The system includes/understands an apparatus 10 to produce an electrical signal characteristic of the biological activity and/or chemical or biological and/or chemical behavior of a substance 1 or an active element contained in the aforementioned substance. In the case of the variant described while referring on figures 1, 1 A, I B and LLC, the aforementioned substance 1 is:
maybe of the Ca++ ions in solution with 1 M in 1 ' water for injectable preparation (e.g. of Biosédra mark),

- is heparin with the concentration of 2. 5 U. I. /ml in the same water quality.

Apparatus 10, localized with Paris for example, product an electrical signal characteristic which is digitized after analog-to-digital conversion.

The signal thus digitized is, of known manner in oneself, transmitted remotely, for example by a computerized communication network of type Internet implementing microwave links 11. The digitized signal thus transmitted is received by an applicator 12, located in New York for example.

Applicator 12 comprises emitting means 13. Emitting means 13 make it possible to apply the characteristic signal (after digital-analogue conversion) to a receiving substance or a biological system of control or a sensitive biological system.

The means designed to remotely digitize and transmit the electrical signal characteristic of the ion Ca++ or heparin are not indispensable with the performing of the invention. They were described to put in evidence the technical and commercial benefits bound at the possibility produce an electrical signal characteristic of the ion Ca++ or heparin having, as the substances sources from which they result, of the coagulants effects or anticoagulants.

In the case of the variant described while referring on figures 1, 1 A, I B and LLC, it receiving substance is 1 ' water or a homeopathic mixture water-ethanol or granules, it biological system of control or the sensitive biological system is blood or plasma.

I The apparatus of production of the characteristic signal of the ion Cl++ or heparin

The enclosure

The apparatus of production of signal 10 includes/understands an enclosure D, 2 provided with an electrical shield and magnetic the insulating one of the parasitic fields coming from the environment. The shielded cylindrical enclosure is made up of three superimposed layers: copper, mild iron, mumétal, made out of sheet metal of 1 mm of thickness. The enclosure has an inner diameter of 65 mms and a height of 100 mms. The enclosure is closed by a shielded cover 5. In enclosure 2 is placed a tank 3 out of glass or plastic having as a dimension 10 mms X 10 mms X 45 mms. This tank 3 contains 1 ml of substance 1. I.e.:
maybe of the Ca++ ions in solution with 1ru in 1 ' water for injectable preparation (e.g. of Biosédra mark),
- is heparin with the concentration of 2. 5 U. I. /ml in the same water quality.

The emitter of the specific excitation field

An emitter 4 is located inside 1 ' enclosure. L generates a specific excitation field of electromagnetic nature. The emitter is supplied by a generator 14. Emitter 4 comprises a coil advantageously supplemented by a mild iron magnetic core. Transmitting coil 4 has an impedance of 300 ohms, an inner diameter of 6 mms, an outer diameter of 16 mm, a length of 6 mms. The mild iron magnetic core is placed in contact with the outer walls of tank 3. The aforementioned substance 1 is thus subjected to the emitted excitation field by emitter 4. The generator 14 is designed to generate a signal low frequency especially square or sinusoidal signals low frequency, pink noise or, advantageously, white noise. The signal spectrum of excitation feeding transmitting coil 4 corresponds substantially to the spectrum of the audible frequencies (20 Hz-20 000 Hz). The generator 14 can be a generator of analogue signal of known type, using for example a read-only memory (ROM, PROM, EPROM, EEPROM in Anglo-Saxon terminology) containing the digital signal of the desired noise.

This memory is connected of known manner in oneself to a digital-to-analog converter. One can also use a microcomputer 14, provided with a card his 25 comprising a digital-to-analog converter 41.

One can for example use a computer 14 of type PC, operative under operating system WINDOWSX 95 of Company MICROSOFT and comprising, in addition to the card his 25 a microprocessor 27, an interface of input/output 29, a controller 31 of a mass memory 33 and one interface video 35 connected by one or more bus 37. The analogue digital converter 41 of the card its 25 comprises an output terminal 8. Output terminal 8 of the card its of the microcomputer 14 is connected to input terminal 8 ' of emitter 4, via an amplifier 15 whose characteristics are the following ones: passband of 10 Hz with 20 Khz, profit 1 to 10, sensitivity of inlet +-1 V. Among the cards his 25 that one can use, one can quote for example the card Soundblaster 16 sold by CREATIVE Company LABS.

The transducer-receptor

Transducer-receptor 6, located inside 1 ' enclosure 2, receives the resulting fields of the interaction of the aforesaid specific excitation field and substance 1. Transducer-receptor 6 transforms the aforementioned resulting fields into an electrical signal. This electrical signal present, with output terminals 9 ' of transducer-receptor 6, in the shape of a difference in potential variable or a variable electrical current of intensity. Transducer-receptor 6 comprises a coil having a mild iron core. This coil has an impedance of 300 ohms, an inner diameter of 6 mms, an outer diameter of 16 mm, a length of 6 mms. The mild iron magnetic core is placed in contact with the outer walls of tank 3.

Advantageously, the electrical signal characteristic available with the outlet of transducer-receptor 6 is amplified by a amplificateurpreamplificator 16. The amplifier-preamplifier 16 present following features: passband of 10 Hz with 20 Khz, profit 10 to 100, sensitivity of inlet +-100 mV.

In the case of the variant of performing described while referring to figures 1, lb, LLC it is envisaged an emitter 4 of excitation field. The use of such an emitter 4 supports the production of an electrical signal characteristic of the ion Ca++ or heparin. However, one can also collect, by means of a transducer-receptor 6, a characteristic signal of the ion Ca++ or heparin, without implementing an excitation field and using of shielded enclosure.

The recording of the electrical signal analogue characteristic Recording

The recording of the electrical signal characteristic, or that of the electrical signal which in drift after amplification or treatment, can be carried out into analogue by a recorder of signal, especially on magnetic tape, adapt with the frequencies of the electrical signal characteristic to the outlet of transducer-receptor 6. As the passband used corresponds to the audio tape, one can especially use a tape recorder. Output terminal 9 ' of transducer-receptor 6 is connected to the inlet microphone or the inlet line of such a tape recorder. During the reading, the electrical signal characteristic recorded is collected with one output terminal, especially with the outlet line or the outlet loudspeaker of tape recorder.

Digital recording

Preferably, one carries out a digital recording of the signal electrical characteristic after analog-to-digital conversion of the aforesaid signal. For this purpose, one uses a microcomputer 17, provided with a card of signal acquisition 25. Microcomputer 17 comprises moreover one microprocessor 27, an interface of input/output 29, a controller 31 of one mass memory 33 and one interface video 35 connected by one or more bus 37. One can for example use a computer of type PC 17, operative under the operating system Windows 95 of Company MICROSOFT This microcomputer can be same type that that used to generate the excitation field. It can be the same microcomputer. Outlet 9 ' transducer-receptor 6 or amplifier-preamplifier 16 is connected to inlet 9 of the analog-to-digital converter 39 of card 25 of computer 17. Preferably, the analogue converter digital 39 A a great resolution with 12 bits. It is advantageously equal with 16 bits. Preferably also, the converter analog-to-digital 39 A a double frequency of sampling of maximum frequency that one wants to be able to digitize, for example 44 Khz.

One proceeds to an electrical signal acquisition characteristic pendent one duration for example ranging between 1 and 60 S (for example 6 dry) and one records the digital file in a mass memory 33, for example in the shape of a file its to format WAV.

All the connections are carried out in shielded cable. All the apparatuses are put with the mass.

Electrical signal processing characteristic

For the electrical signal processing characteristic or signal which in derives, one advantageously uses the Matlab software of the company " Tea MathWorks ".

The digital file, recorded like it was described above, can optionally undergo a digital processing, such as for example a digital amplification for calibration of the signal level, a filtering for the removing of nondesired frequencies, or be transformed into its spectrum by a discrete transform of FOURIER, preferably by the algorithm of rapid transform of FOURIER (FTT in Anglo-Saxon tenninology). The duration of the generated signal can be increased while repeating in a file several times a fragment or the whole of the fichierson product in an original manner.

Processing means of the electrical signal characteristic can be used to improve the performances of the electrical signal characteristic.

In the case of a first variant of performing, it is envisaged a second transducer-receptor of the same type that that previously described. In the absence of the aforementioned substance, this second transducer-receptor transforms the excitation field into an electrical signal. This electrical signal is withdrawn by a branch in series opposition with the signal coming from the first transducer-receptor. One thus obtains an electrical signal characteristic more representative of the interaction of the specific excitation field and substance.

In the case of a second variant of performing, the processing means take into account the characteristics of the specific excitation field and reprocess the electrical signal characteristic in the following way. One proceeds first of all to the calculating of the distribution of spectral power (PSD). Then one truncates this spectral power while not preserving that the strip of the frequencies going for example of 140 Hz at 14 Khz, one reconstitutes a signal starting from this spectral power and of neutral phases, for example generated by chance, finally one gauge the signal power thus product. By neutral phases, one indicates phases not coming from a substance source presenting a biological activity.

In the case of the variant of performing described while referring to figures 1, lb, LLC, it is envisaged to

digitize, record and treat the electrical signal characteristic before applying it to a receiving substance or a biological system of control or to a sensitive biological system.

These operations are not indispensable to 1 ' exploitation of the electrical signal characteristic of the ion Ca++ or heparin, same if they support the bringing in work of it.

The electrical signal characteristic available with the outlet of transducteurreceptor 6 and if necessary of preamplifier 16 already constitutes in oneself a capable product of industrial applications. One will see hereafter for which applications it can be especially implemented by means of an applicator 12 making it possible to apply them to a receiving substance or a biological system of control or a sensitive biological system.

II. Remote transmission of the electrical signal characteristic

The file of the electrical signal characteristic of the ion Ca++ or heparin, recorded in digital form as it has been just described, optionally after treatment, can be transferred remotely by a computerized communication network. This array can comprise microwave links 11. The file of the electrical signal characteristic of the ion Ca++ or heparin, thus transmitted, is recorded by the mass memory of a microcomputer 18. One can for example use a computer of type PC, operative under the operating system Windows 95 of Company MICROSOFT. This microcomputer 18 can be same type that that used to generate the excitation field. The file of the electrical signal characteristic digitized, thus recorded by remote microcomputer 18, can be exploited, of known manner in oneself, to produce an analogue electrical signal characteristic. The file, optionally treated, is transformed by a digital-to-analog converter 41 of card 25 (or a separate card) of computer 18. The digital-to-analog converter 41 delivers on its outlet 8 an analogue electrical signal characteristic of the biological activity of the ion Ca++ or heparin from which it results. This analogue electrical signal can be transformed, as it will be described hereafter, in electromagnetic and applied field with a receiving substance or a biological system of control or a sensitive biological system. in. The applicator of the characteristic signal of the ion Cl++ or heparin

One now will describe, while referring to figure LLC, an example of performing of a system allowing to apply the electrical signal characteristic of the ion Ca++ or heparin to a biological system receptor and to modify the chemical behavior of it.

50 récipent contains the biological system receptor. In the case of the variant described while referring on figures 1, lb and LLC, container 50 contain:

- a receiving substance such as 1 ' water or a homeopathic mixture eauéthanol or granules, or

- a biological system of control or a sensitive biological system such of the blood or plasma.

Container 50 is laid out in a radiated electromagnetic field by an transducer-emitter 51, typically a coil. The coil has for example a length of 80 mms, an inner diameter of 50 mms, an outer diameter of 55 mms. It present 300 turns of a wire of diameter 0, 5 mms. Its impedance is of 4 ohms. Coil 51 is connected to the mass. Without that representing any restrictive character, coil 51 of the transducer-emitter has a vertical axis allowing the introduction of container 50 container the biological system receptor. Input terminals 8 ' of this coil 51 are connected, in the case of the variant of described performing, with outlet 8 of the analog-to-digital converter 41 of microcomputer 18 via an amplifier 19 having the following features: passband of 10 Hz with 20 Khz, profit 1 to 20, sensitivity of inlet 250 mV, output power RMS 60W under 8 ohms, ratio signal on noise 80 dB. The tension with the terminals of coil 51 has an amplitude of 10 effective Volts and the signal is applied pendent 10 min.

Input terminals 8 ' of the applicator can be also, in the case of certain variants of performing, directly connected to the outlet of preamplifier 16 or outlet 8 of the digital-to-analog converter 41 of computer 17.

Experiments

In order to illustrate a variant of performing,

- of a process and a system in accordance with the invention to produce a substance having a coagulant effect or anticoagulant,

- of a substance in accordance with the invention having a coagulant effect or anticoagulant,

- of a process in accordance with the invention to test 1 ' coagulant effect or anticoagulant of a substance and its application to the production of homeopathic products,

- of a process and a system in accordance with the invention to produce a signal having a coagulant effect or anticoagulant,

- of a signal in accordance with the invention having a coagulant effect or anticoagulant

- of a process in accordance with the invention to test 1 ' anticoagulant coagulant effect of a signal and its application to the production of homeopathic products, following experiments one carried out.

Return of the effects of heparin and the Ca' ions on the coagulation of the human plasma or rabbit

Heparin (25 000 U. I. /5 ml, Choay Laboratory, Sanofi Winthrop) is an acting anticoagulant by inhibition of the transforming of thrombin prothrombin. At the site of action, 1 ' effect of heparin is immediate. It acts via a natural inhibitor called cofactor, or antithrombin III.

Sulfate of protamine (10 000 U. I. /10 N it Laboratory Choay, Sanofi
Winthrop) form a salt with heparin and involves a suppression unit for unit of 1 ' anticoagulant effect of this one. 1 ml of solution of protamine neutralizes the anticoagulant activity of 1000 units of heparin.

The ion Cl++ calcium is an indispensable ion with coagulation.

Substances sources and hardwares used

The electrical signals characteristics were recorded starting from samples of 1 ml of the following solutions: - Ca++ in solution with 1RM in 1 ' water for injectable preparation (for example of Biosédra mark) - Mg++ in solution with read. M in the same water quality, - heparin in solution with the concentration of 2. 5U. I. /ml in the same water quality, - complex heparin + protamine (respectively 2. 5 U. I. /ml and 0. 025 mg/ml), in solution in the same water quality.

The used hardware was described while referring to the figures, lb, LLC. The transducer-receptor 6 present described characteristics. Transducer-emitter 51, making it possible to apply the electrical signal characteristic to a receiving substance or a biological system of control or a sensitive biological system, is an electromagnetic coil having the following features:
- length: 80 mms,
- inner diameter: 50 min,
- number of turns: 300 turns,
- impedance: 4 ohms.

An evaluating of coagulation was made by using the following notation:
- substantial coagulation: 2
- moderate coagulation: 1
- no coagulation: 0
Protocol N L. " In vitro " experiment: Action coagulating or anticoagulant electrical signals characteristics on Plasma
Rich in Platelets (PRP).

This protocol has as an object to put in evidence that:
- of an hand, the process and the system described make it possible to produce an electrical signal characteristic of the ion Ca++ and heparin having respectively a coagulant effect or anticoagulant, and
- of another hand, the process and the system described make it possible to test an electrical signal having respectively a coagulant effect or anticoagulant.

Like biological system of control making it possible to reveal the electrical signal characteristic of the ion Ca++ and heparin, or like sensitive biological system making it possible to test 1 ' coagulant effect or anticoagulant of an electrical signal, one uses rabbit plasma (or human).

The blood of a rabbit " New-Zealand White " is taken with the artery of the ear and is collected on an anticoagulant ACD (9 flight. sang/1 flight. ACD) whose composition is the following one: citric acid 0. 8%, sodium citrate 2. 2%, anhydrous glucose 2. 23%.

After centrifugation (180 G, 15 minutes) at ambient temperature, the blood is divided into 3 layers: of high into low, Rich plasma in Platelets (PRP), the leucocytic layer and the base of red globules. The PRP is taken with the pipette by mild suction.

Anticoagulant effect of a signal, anticoagulant effect of the electrical signal characteristic of heparin 5 ml of PRP are placed in a tube 50 at the center of an electromagnetic coil 51 to be exposed to the pendent applied signal 10 mn with a tension of 10V to the terminals of the coil.

Samples of 1 ml of PRP thus treated are placed in four tubes.

One delivers in each tube 20gel Ca (50, 100, 150 and 200 mms) to obtain final calcium concentrations in the PRP of (1, 2, 3 and 4 mms). Then one lets incubate 15 to 20 minutes.

The results obtained are presented in the table hereafter:
<Tb> N: number of values; Moy: average; SD: deviation standard

It is observed that an application of the signal of heparin has an effect of inhibition of the coagulation of the PRP. In the same conditions, the nonexposed PRP with a signal or the PRP exposed to a signal of control, such as for example that of complex the héparine+protamine, present step of effect of inhibition. This effect of inhibition of coagulation is particularly substantial for a concentration in Ca++ ranging between 2 and 3 mms.

Thus thus, the biological system of control consisted rich plasma in platelets makes it possible to control that the characteristic signal of heparin has an anticoagulant effect.

Thus thus, the sensitive biological system consisted rich plasma in platelets makes it possible to test if a characteristic signal has an anticoagulant effect.

Coagulant effect of a signal, coagulant effect of the electrical signal characteristic of the ion calcium (Ca++) 1 ml of PRP is placed in a tube at the center of an electromagnetic coil to be exposed to the pendent applied signal 10 mn with a tension of 10 V to the terminals of the coil.

The results obtained are presented in the table hereafter

Interpretation

It is observed that an application of the signal of the Ca++ calcium has an effect of coagulation of the PRP comparable with that of the Ca++ calcium itself.

It is observed that an application of the signal of induced the Mg++ magnesium no effect of coagulation of the PRP.

Thus thus, the biological system of control consisted rich plasma in platelets makes it possible to control that the characteristic signal of calcium Ca++ for a coagulant purpose.

Thus thus, the sensitive biological system consisted rich plasma in platelets makes it possible to test if a characteristic signal has a coagulant effect.

Protocol N2. Experiment " in vivo ": Coagulating or anticoagulant action electrical signals characteristics.

This protocol has as an object to put in evidence that:
- of an hand, the described process and the system allow
* to produce an electrical signal characteristic of the ion
Ca++ and of heparin, and
* to after apply this electrical signal to a receiving substance presenting treatment respectively a coagulant effect or anticoagulant, and
- of another hand, the process and the system described make it possible to test a substance having respectively a coagulant effect or anticoagulant.

Like biological system of control making it possible to reveal 1 ' coagulant effect or anticoagulant of treated substance, or like sensitive biological system making it possible to test 1 ' coagulant effect or anticoagulant of a substance, one uses a rabbit to which one manages, by sublingual path, of 1 ' water treated by means of an electrical signal characteristic of the substance source.

Water used is water for injectable preparation Biosédra out of ampoules of 10 ml.

1. Water (10 ml) is placed in a tube 50 at the center of a coil electromagnetic 51. Water is exposed to the characteristic signal
considered pendent 10 mn with a tension with the terminals of the coil of 10 V.

2. One agitates then 1 ' water pendent 15 seconds with the maximum rate of vortex.

3. One manages with rabbit by sublingual path 1 ml of 1 ' water thus treated by the characteristic signal considered.

Blood samples (1 ml) are taken on glass tubes with the artery of the ear, before administration, then 1, 5, 10, 15 and 30 minutes after administration of 1 ' treated water.

The results obtained are presented in the table hereafter

Interpretation

It is observed that a water administration treated by the characteristic signal of heparin has an effect of inhibition on blood coagulation pendent fifteen minutes. On the other hand, a water administration treated by the signal of complex the héparine+protamine product no effect of inhibition.

Thus thus, the biological system of control consisted an animal makes it possible to control that a receiving substance treated by the characteristic signal of heparin, especially of 1 ' water, has an anticoagulant effect.

Thus thus, the sensitive biological system consisted an animal makes it possible to test, by controlling the characteristic signal of a substance (for example complex the héparine+protamine), if this present substance a coagulant effect or anticoagulant.

It is thus thus established that one can control the production of homeopathic products by the use of substances with 1 ' known effect (like heparin) and while controlling that homeopathic products (granules, solutions,…) products starting from this substance also present them, at the end of the chain, the corresponding activity (in 1 ' example described, the activity of anticoagulation).
The characteristic signal of a drug or a receiving substance treated by the characteristic signal of a drug has the same biological effects as the drug source of the signal considered.

Same manner, similar anticoagulant effects are obtained with the hirudien on blood or plasma of rabbit or human. The signals coming from 'hirudine present an anticoagulant effect more substantial than those coming from heparin.

One will find hereafter the résulats obtained with hirudine and blood of rabbit:

One will find hereafter the résulats obtained with 'hirudine and human blood:

WO0204958
METHOD FOR DETERMINING POTENTIAL ALTERATIONS OF A SUBSTANCE HAVING BIOLOGICAL ACTIVITIES

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The invention concerns a method applied to a substance treated to exhibit a biological activity, for example a coagulating or anticoagulation activity. The treated substance has been obtained, from a source substance having the biological activity, after a treatment such that the treated substance does not contain any molecule of the source substance in significant amount. The treatment may consist in carrying out a high dilution process of the type used for producing homeopathic solutions or granules. The method is designed to diagnose potential alterations of the treated substance by external factors. It comprises the step which consists in: placing a reference substance sample in a zone (19) protected from external influence; subjecting a sample of the treated substance to external influence (20); comparing the results of the tests carried out using a biological control system respectively with the reference substance sample and the treated substance sample. Thus, if the results of the tests are different, the alterations of the treated substance by external influence (20) are demonstrated.

In the patent NR WO 00/17638 publish on March 30, 2000 and have for title " Method, system and apparatus to produce, starting from a substance of signal, especially of electrical signal, characteristic of biological activity and/or chemical of the aforesaid substance ", that one can treat a substance receiving, present initially no biological activity particular, especially of 1 ' water, so that it present after processing a biological activity. The receiving substance after processing is called hereafter the " Substance Treated " (or Informed Material). When the receiving substance is water, the Treated Substance is called L " 'Water Treated " (or of Informed Water). The substance having a biological activity can be also appeared as preparation or homeopathic granules.

It will be pointed out, later in the description of the present invention, how one can produce of 1 ' Informed Water, starting from a substance parent, especially a substance parent having an anticoagulant effect such as heparin. This return will be carried out by reference with the patent NR WO 00/17637 published on March 30, 2000 and having for titre " Method and system to produce a substance or a signal having a coagulant effect or anticoagulant. Therapeutic applications of the aforesaid substance or of the aforesaid signal. “

Like that was described in patent NR WO 00/17637, to test a Treated Substance, especially of Informed Water, one it applique with a sensitive biological system to observe the effects of them. For example, if one tests Informed Water having a coagulant effect, or anticoagulant, one mixture respectively in the following proportions (1/3,2/3) of Informed Water and the plasma. Then one measuring times of coagulation.

Such tests are particularly sensitive with interfering phenomena. The inventors noted of manner surprising and not explained that certain individuals have an inhibiting effect or potentialisator on Informed Water. For example, it is enough for them to approach Water Informed to deteriorate the properties of them. Other individuals on the other hand amplify the effects of the properties of Informed Water.

It would be desirable to know, a priori, the individuals having such capacities since, by their presence, they can act of positive or negative manner on the properties of Informed Water. They can thus compromise the implementation of the industrial applications of this one. For example, the transport and handling, by such persons, of Substances Traitées constitutes an obstacle (the activity is faded) with the industrial development of

these substances. Such is thus the problem posed.

How to diagnose the persons having such effects of inhibition or potentiating?

There is no state of the known art proposing a solution with the problem posed. The reason in is single: on the one hand, until the workings of search of the inventors, it was not known that one could modify the activity of 1 ' water, on the other hand, one was unaware of that unknown phenomena could remotely deteriorate the biological properties of Informed Water.

The invention relates to a method to diagnose the potential alterations of a substance having biological activities of a substance source not being more present in significant amount.

The method is applied with a substance presenting a biological activity, for example a coagulating or anticoagulant activity. The substance was obtained, starting from a substance source possessing the biological activity, at the end of a processing such as substance does not contain a molecule of the substance source in significant amount (the substance is called hereafter the treated substance). The processing can especially consist in implementing a method of high dilution of Mrs. nature that that used to produce homeopathic solutions or granules. The processing can also especially consist in implementing the method of Jacques Benveniste, comprising several steps, as described in the patent NR WO 00/17637 published on March 30, 2000 and having for titre " Method and system to produce a substance or a signal having a coagulant effect or anticoagulant. Therapeutic applications of the aforesaid substance or of the aforesaid signal. “It includes/understands the step to transform the electromagnetic field coming from the substance source having a biological activity, in a signal, especially an electrical signal, by means of a transducer-receptor collecting the electromagnetic field. The substance source has, for example, an effect on coagulation of the blood and can, for example, to contain Ca++ ions or heparin. The method includes/understands moreover the step to apply to a receiving substance, not presenting initially any particular biological activity, the signal coming from the transducer-receptor, by means of a transducteuremettor. Thus, for example, after processing above defined, receiving substance, not presenting any particular, present biological activity initially then a coagulating or anticoagulant activity.

The method is conceived to diagnose the potential alterations of substance treated by outer influences and is characterized in what it includes/understands several steps.

It includes/understands the step to place a sample of a reference substance in a zone at the shelter of the outer influence making the object of the diagnosis.

It includes/understands moreover the step to subject a sample of substance treated with the outer influence making the object of the diagnosis.

It includes/understands moreover the step to respectively compare the results of the tests carried out by means of a biological system of control with the sample of reference substance and the sample of treated substance subjected to the outer influence.

Thus, if the results of the tests are different, the potential alterations of substance treated by the outer influence are put in evidence.

Preferably, the reference substance is the treated substance.

Preferably, the sample of reference substance is calibrated by means of the biological system of control, in the absence of the outer influence.

Preferably, to place a sample of reference substance in a zone at the shelter of the outer influence making the object of the diagnosis, one protects it from the effects of the electromagnetic fields.

Preferably, to protect the sample from reference substance of the effects of the electromagnetic fields, one it place in an enclosure surrounded by a magnetic shield carried out especially out of soft iron or mumétal.

Preferably, the biological system of control is characterized in what it reacts, in the presence of treated substance, in accordance with the biological activity of the substance source.

Preferably, the biological system of control is blood or blood plasma.

Preferably, the outer influences can tre induced by the persons located near treated substance.

The invention also relates to a system to diagnose the potential alterations of a substance having biological activities of a substance source not being more present in significant amount.

The system is applied with a substance presenting a biological activity, for example a coagulating or anticoagulant activity. The substance was obtained, starting from a substance source possessing the biological

activity, at the end of a processing such as substance does not contain a molecule of the substance source in significant amount (the substance is called hereafter the treated substance). The processing can especially consist in implementing a method of high dilution of Mrs. nature that that used to produce homeopathic solutions or granules. The processing can also especially consist in implementing the method of Jacques Benveniste, comprising several steps, as described in the patent NR WO 00/17637 published on March 30, 2000 and having for titre " Method and system to produce a substance or a signal having a coagulant effect or anticoagulant. Therapeutic applications of the aforesaid substance or of the aforesaid signal includes/understands the step to transform the electromagnetic field coming from the substance source having a biological activity, in a signal, especially an electrical signal, by means of a transducer-receptor collecting the electromagnetic field. The substance source has, for example, an effect on coagulation of the blood and can, for example, to contain Ca++ ions or heparin. The method includes/understands moreover the step to apply to a receiving substance, not presenting initially any particular biological activity, the signal coming from the transducer-receptor, by means of a transducteuremettor. Thus, for example, after processing above defined, receiving substance, not presenting any particular, present biological activity initially then a coagulating or anticoagulant activity.

The system is conceived to diagnose the potential alterations of substance treated by outer influences and is characterized in what it includes/understands several elements.

It includes a sample of a reference substance placed in a zone with the shelter of the outer influence making the object of the diagnosis.

It includes moreover a sample of treated substance subjected to the outer influence making the object of the diagnosis.

It includes moreover a biological system of control to respectively carry out comparative tests with the sample of reference substance and the sample of treated substance subjected to the outer influence.

Thus, if the results of the tests are different, the potential alterations of substance treated by the outer influence are put in evidence.

Preferably, the reference substance is the treated substance.

Preferably, the sample of reference substance is placed in a protected zone of the effects of the electromagnetic fields.

Preferably, the sample of reference substance is placed in an enclosure surrounded by a magnetic shield carried out especially out of soft iron or mumétal.

Preferably, the biological system of control is characterized in what it reacts, in the presence of treated substance, in accordance with the biological activity of the substance source.

Preferably, the biological system of control is blood or blood plasma.

Other characteristics and advantages of the invention will appear with the reading of the description of the variants of performing given as indicative and nonrestrictive example and of figures 1 and 2 presenting a system making it possible to produce of 1 " informed water ", starting from a substance parent having an anticoagulant effect, especially of heparin, figure 3 presenting a schematic view of the method and system, in accordance with the invention, making it possible to diagnose the potential alterations of 1 " water informed " starting from a substance parent having an anticoagulant effect, especially of heparin.

One first of all will recall how one can produce of 1 " informed water ", starting from a substance parent having an anticoagulant effect, especially of heparin.

Figures 1 and 2 represent a system in conformity with that described in the patent NR WO 00/17637 published on March 30, 2000 and having for titre " Method and system to produce a substance or a signal having a coagulant effect or anticoagulant. Therapeutic applications of the aforesaid substance or of the aforesaid signal. “The system makes it possible to produce, starting from a substance parent, electrical signals, characteristics of its biological activity. In the present case, the substance parent is heparin 1 having an anticoagulant effect. The system includes/understands a cylindrical enclosure 2, provided with an insulating shield 1 ' enclosure 2 of the parasitic fields coming from the environment.

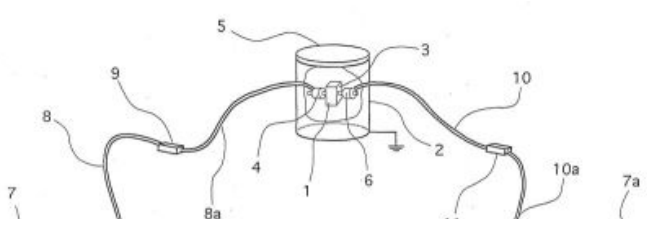


Fig. 1

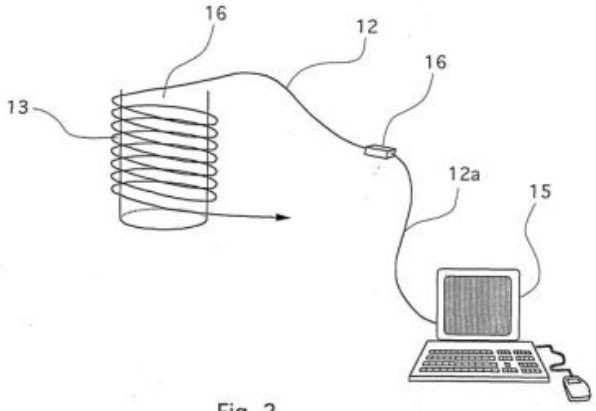


Fig. 2

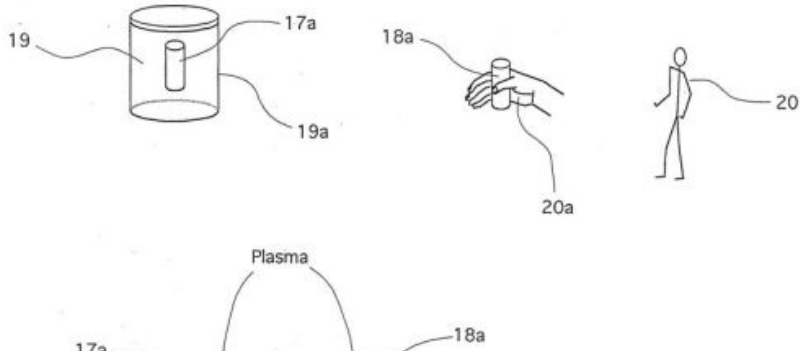
Enclosure 2 is closed by an also shielded cover 5. A transmitter 4 is located inside the enclosure. It generates a determined excitation field of electromagnetic nature. The transmitter is supplied by a generator 7 designed to generate a signal low frequency, especially signals square or sinusoidal low frequency, pink noise or, advantageously, white noise. Like one represented it, generator 7 takes the shape of a microcomputer 7, provided with a card its comprising an analog-to-digital converter. The converter comprises an output terminal 8. Output terminal 8 of the card its of microcomputer 7 is connected to the input terminal 8a transmitter 4, via an amplifier 9.

In enclosure 2 are placed a tank 3 into plastic containing lml heparin as well as a transducer-receptor 6. Transducteurreceptor 6 receives the resulting fields of the interaction of the specific excitation field and heparin 1. Transducer-receptor 6 transforms these fields into electrical signals. These electrical signals are presented, to outputted the 10 of transducer-receptor 6, in the shape of a difference in potential variable or a variable electric current of intensity. The electrical signals available to outputted the 10 of transducer-receptor 6 are amplified by a preamplifier 11. The electrical signals, after analog-to-digital conversion, make the object of a digital recording. For this purpose, one uses a microcomputer 7a, of Mrs. type that microcomputer 7, comprising an analog-to-digital converter. Outputted the 10 of transducer-receptor 6 is connected to the input 10a analog-to-digital converter of the microcomputer 7a, via preamplifier 11. Moreover, like one represented it on figure 2, the system allows to produce of 1 " informed water ", starting from the electrical signals, obtained thus that one described it above. In a tank 14 into plastic is placed a receiving substance, not presenting any particular biological activity initially, especially of water.

This tank 14 is laid out in a radiated electromagnetic field by a transducer-transmitter 13, in the present case a coil 13. Input terminals 12 of coil 13 are connected to outputted the 12a of an analog-to-digital converter of a microcomputer 15, via an amplifier 16.

Microcomputer 15 contains in memory the electrical signals digitized by means of microcomputer 7. The comprising microcomputer the 15 analog-to-digital converter similar with microcomputer 7, is previously described. The analog-to-digital converter product of the electrical signals starting from the digital recording.

Thus, by applying the electrical signals to receiving substance, contained in the tank 14, by means of transducer-transmitter 13, one obtains from 1 ' informed water presenting an anticoagulant activity. Informed water thus obtained is capable industrial applications, in so far as its properties are not faded. Like one exposed it in the preamble of present description, certain individuals have an inhibiting effect or, contrary, potentialisator on 1 ' informed water, especially when they handle a tube containing of 1 ' informed water.



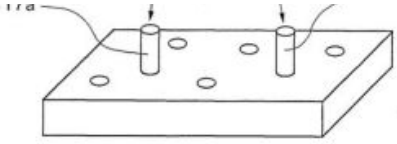


Fig. 3

Figure 3 presents a schematic view of the method and system, in accordance with the invention, allowing to diagnose the potential alterations of 1 " informed water ". By potential alterations one indicates 1 ' inhibiting effect or potentialisator whom an individual 20 can produce out of 1 ' informed water, by handling the tube which contains it. A tube 17a and a tube 18a contain of 1 ' informed water presenting an anticoagulant activity.

Informed water was previously produced like one described it cidessus. The tube 17a is placed in an enclosure 19. Enclosure 19 is surrounded by a magnetic shield 19a, carried out out of soft iron or mumétal.

Thus, the tube 17a is located at the shelter of the potential alterations of individual 20. The tube 18a is subjected to the influence of individual 20. For this making, individual 20 seizes in his hand 20a the tube 18a, pendent one determined duration, for example thirty seconds.

Like that was described in the patent NR WO 00/17637 published on March 30, 2000 and having for titre " Method and system to produce a substance or a signal having a coagulant effect or anticoagulant.

Therapeutic applications of the aforesaid substance or of the aforesaid signal ", one uses a biological system of control, consisted plasma, to check that 1 ' water informed starting from the heparin, contained in the tubes 17a and 18a, reacts in accordance with the biological activity of heparin. To arrive to this confirmation, one mixture plasma with 1 ' water informed in the tubes 17a and 18a, then one lets incubate pendent 15 to 20 minutes.

One measuring then the coagulation of the plasma in the tubes 17a and 18a. If the speeds of coagulation observed differ, one deduces from it that individual 20 deteriorated the properties of 1 ' informed water. Accurately, if the coagulation observed in the tube 18a is carried out substantially rapidly than in the tube 17a, one deduces from it that the individual has a capacity of inhibition of 1 ' informed water. Contrary, if the coagulation observed in the tube 18a is carried out substantially slowly than in the tube 17a, one deduces from it that the individual has a capacity of potentiating of 1 ' informed water.

WO9954731

METHOD FOR AMPLIFYING THE FORMATION OF LIGAND-RECEPTOR COMPLEXES AND USES

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The invention concerns a method for amplifying the formation of complexes between the two elements of a ligand/receptor pair and its uses for detecting the presence of a substance corresponding to one of the two elements of a ligand/receptor pair in a sample and the electromagnetic signal characteristic of a substance biological activity corresponding to one of the two elements of a ligand/receptor pair in an electromagnetic signal. Said amplification method consists in: contacting the two elements of the ligand/receptor pair in conditions ensuring their reaction; prior to, simultaneously with or subsequent to said contacting, applying to one and/or the other of said elements an electromagnetic signal characteristic of the biological activity of one and/or the other of said elements. The invention is applicable to biological diagnosis in human and veterinary medicine, bacteriological control in the pharmaceutical, cosmetic and food industry.

The present invention refers to a method amplification of the formation the complex ones between the two elements of a couple ligand/receptor, with a method and with a detecting apparatus of the presence, in a sample (hereafter “analytic sample”), of a substance corresponding one to the one of the two elements of a couple ligand/receptor, implementing this method of amplification, with the applications of this detecting method, like a detecting method of the presence has, in an electromagnetic signal, electromagnetic signal characteristic of the biological activity of a substance corresponding one to the one of the two elements of a couple ligand/receptor, putting also in work the aforementioned method of amplification.

To detect the presence of an analytic substance in a sample, one proposed very numerous based methods on the capacity of this substance specifically to be bound to another substance and to react with it.

In particular, the properties of affinity which the antibodies with respect to the antigens present are at the base of a large number of immunologic methods of detection which have jointly to use the formation of complex antigènesanticorps it sought substance being able tre either the antigen, or it antibody-and to detect, to even quantify, complex the thus formed ones.

As immunologic examples of methods of detection which are very frequently used, one can quote the immunoprecipitation, the agglutination reactions, dialysis with the equilibrium, the extinction of fluorescence,

the fluorescence polarization, the immunoelectrophoresis, counterimmunoelectrophoresis or électrosynérèse, proportionings radioimmunological (RIA), enzymatic proportionings immuno- (ELISA) or the immunofluorescence.

These immunologic methods of detection, if they have large qualities incontestably, do not give however completely satisfaction.

Initially, their sensitivity (which is defined by the minimum concentration of sought substance that these methods detect) is, in the majority of the cases, insufficient. Thus, BERZOFSKY and BERKOWER (Antigen-Antibody Interaction, In: WE Paul, Fundamental Immunology, RAVEN NEAR, New York, 1984,595) showed that, concerning for example the detection of the antibodies, except for the tests of neutralizing of the phages with which it is possible to detect the presence of only one antibody molecule but of which the use extrmement is extrmement limited, very sparingly of methods have a low sensitivity with 10 ng of antibody per ml of sample.

It is thus desirable to develop the new technical ones which makes it possible to lower the detection threshold of a sought substance.

In addition, all the immunologic detection methods suggested to date include/understand a step which consists in incubating a volume determined-which is generally at least 500 SSL-of the taking away to be analyzed with a specific reagent and this, for each sought substance. So they present the disadvantage of requiring, as soon as the analysis of a door taking away on several substance-like that is very often the case of the medical analyses with diagnostic sight, a taking away of a relatively substantial volume, which is not always well supported by the patients, especially in the case of blood taking away.

Moreover, the fact that these detection methods requires, for their implementation, to have of the taking away to analyze or, at the very least, a sample of this one, is not without presenting a certain number of constrained. Indeed:
- of an hand, it is frequent that taking away taking place given with preserved analyses owe tre so that the reliability of these analyses can tre subsequently controlled or that complementary analyses can tre carried out. Thus, for example, the centers of blood transfusion, the services of legal medicine and the tissue centers of taking away preserve samples of all the biological taking away which they are brought to carry out. This conservation, which is carried out by congelation of the aforesaid samples, in addition to having a nonnegligible cost, requires equipments and the local adapt ones.

- of another hand, it is also frequent that taking away cannot tre analyzed on the place where they were carried out and which it is necessary to convey them to the loaded laboratory to carry out the analysis of it. Gold, the routing of biological taking away, in addition to that it is never very easy to implement because of the low shelf life of biological substances in the absence of congelation, poses a certain number of difficulties when these taking away are potentially contaminating. Moreover, the duration of such a routing differs from as much the obtaining of the results of the analysis.

The problem is posed, consequently, to provide a method which makes it possible to detect the presence of a substance in a sample with, at the same time, a very large sensitivity and an high specificity, while offering the possibility as many carry out analyses as necessary starting from microsamples, free, moreover, of constrained of conservation, forwarding and transport of the taking away which present the methods currently used for detection of a substance, and which can, moreover, tre implemented readily and rapidly without requiring an heavy and expensive equipment.

Gold, in the frame of their workings on the transmission of a biological activity in the shape of an electromagnetic signal, the Inventors noted that the application, with the one and/or the other of the elements of a couple ligand/receptor such as a couple antigen/antibody, electromagnetic signal characteristic of the biological activity of the one and/or the other of these elements has, in a way completely surprising, for effect to amplify the formation of complex between the two elements of this couple when these last is put to react together and this, of very specific manner, and had the idea to put at profit this effect to detect on the one hand, presence of an analytic substance in a sample and, in addition, the presence of the electromagnetic signal characteristic of the biological activity of a substance in an electromagnetic radiation.

The present invention has, therefore, for object a method of amplification of the formation the complex ones between the two elements of a couple ligand/receptor by reaction of these two elements, which method is characterized in what it includes/understands:

put it in contact of the two elements of the couple ligand/receptor under conditions suitable to allow their reaction, and previously, simultaneously or subsequently to this setting in contact, the application with the one and/or the other of these elements of the electromagnetic signal characteristic of the biological activity of the one and/or other of the aforesaid elements.

Within the meaning of the present invention, one understands by " couple ligand/receptor ", any formed couple by two substances capable to be recognized specifically, to bind and react together by forming the complex

ones. Thus, it can be a question of a couple antigen/antibody or hapten/antibody in which the ligand (antigen or hapten) can tre a biological compound (protein, enzyme, hormone, toxin, tumorous marker,…), a chemical compound (medicinal active principle for example), or a cellular or particulate antigen (cell, bacterium, virus, mushroom,…), the receptor which can tre a soluble antibody or a membrane receptor. It can also be a question of a formed couple by an enzyme and its specific substrate.

In addition, one understands by " electromagnetic signal characteristic of the biological activity " of an element, the electromagnetic signal collected starting from a biologically active element such as a substance, a cell or a microorganism,…, or of a material containing this element such as a purified preparation, a biological taking away, a body or a living tre, like that was described in International application WO 94/17406 in the name of J. BENVENISTE. One understands also by " electromagnetic signal characteristic of the biological activity " of an element, the derived signals of a signal such as defined above by digitization and/or signal processing. In addition, in this expression, one employs the term “characteristic " in the direction where the collected electromagnetic signal contains information characterizing the fact that the material from which is collected this signal present the biological activity in question. The electromagnetic signal collected starting from an hardware containing a plurality of biologically active elements present the biological activity of each element which it contains.

According to a prefered first mode of implementation of the method of amplification in conformity with the Invention, the reaction between the ligand and the receptor is carried out by using two reagents respectively containing the ligand and the receptor, and one applique, with the one and/or the other of these reagents, an electromagnetic signal to test and suspect to include/understand the electromagnetic signal characteristic of the biological activity of this ligand and/or this receptor.

In what precedes and in what follows, one indicates under the term of “réactif', any preparation whose composition is known, which contains the ligand or the receptor in an amount also known and present either in a dry form such as one was freeze-dried to reconstitute in a solvent, or in a liquid form such as a solution or a suspension, the ligand or the attached receptor being able tre on a solid phase (particles or balls of latex, glass or polystyrene,…).

According to a first advantageous provision of this first mode of implementation, the application, with the one and/or the other of reagents, electromagnetic signal to be tested is carried out by exposure of a solution or a suspension containing one and/or the other of these reagents, with this electromagnetic signal.

In variant, the application, with the one and/or the other of reagents, electromagnetic signal to be tested is carried out by dilution of a solution or a suspension comprising one and/or the other of these reagents, in a solvent having been previously exposed to this electromagnetic signal.

Thus, for example, when the reagents which one wishes to use are in solution or suspension in a liquid phase, it is possible to apply the electromagnetic signal to them to be tested:

* is previously with their use: by exposing one and/or the other of these reagents or aliquot of the one and/or the other of
these reagents with this electromagnetic signal, or in diluent one and/or other of the aforesaid reagents or their aliquot in a volume of one solvent having been previously exposed to the said electromagnetic signal,

* is at the time of the implementation of the method of amplification in conformity with the Invention: Zen exposing to this electromagnetic signal aliquot of each one of these reagents, after deposit of these aliquot on a support (blade for example) but previously with their setting in contact, or mixing aliquot first reagent with aliquot of second reagent on a support or in a tube, and by exposing this mixture to the signal electromagnetic, or by mixing aliquot first reagent with aliquot of second reagent on a support or in a tube and into diluent this mixture in a volume of one solvent having been previously exposed to that the electromagnetic signal.

According to another advantageous provision of this first mode of implementation, the application, with the one and/or the other of reagents, electromagnetic signal to be tested is carried out by dissolution or setting in suspension of this or these reagents in a solvent having been previously exposed to this electromagnetic signal. This present provision a very particular intért when the reagents which one wishes to use, are in a form dehydrated such as a lyophilisate, since it is then possible to apply the electromagnetic signal to them to be tested simply by dissolving them or by putting them in suspension according to case's, in a volume of a solvent having been previously exposed to this electromagnetic signal.

Advantageously, the electromagnetic signal to be tested is an electromagnetic signal collected starting from a test sample and suspect to contain this ligand and/or this receptor, this sample being able tre as well resulting from a biological taking away (blood, urine, milk,…) that of a nonbiological taking away (water, food product, pharmaceutical product, cosmetic product,…).

In variant, the electromagnetic signal to be tested also can tre a radiated electromagnetic signal by a source of electromagnetic radiation, especially a source suspectée to emit an harmful radiation for the living tre of the type line high-tension, transformer, electric motor, furnace with microwaves, particle accelerator, ray source X,…

From Mrs., the electromagnetic signal to be tested can come from the acquisition of a mechanical signal like vibrations, of an electrostatic or different signal.

According to a second mode of implementation prefered of the method of amplification in conformity with the Invention, the reaction between the ligand and the receptor are produced by putting in contact a test sample and suspect to contain the ligand and/or the receptor, with a reagent containing either the receptor, or the ligand (according to present substance suspectée tre in the analytic sample with which one wishes to make react this reagent), and one applique, with this sample and/or this reagent, the of the aforesaid electromagnetic signal characteristic of the biological activity ligand and/or of the aforesaid receptor.

According to a first advantageous provision of this second mode of implementation, the application, with the test sample, electromagnetic signal characteristic of the biological activity of the ligand and/or receptor is carried out by exposure of this sample to this or these electromagnetic signals, or by dilution of this sample in a solvent having been previously exposed to (X) said (S) the signal (with) electromagnetic (S).

According to another advantageous provision of this second mode of implementation, the application, with reagent intended to react with the test sample, of the electromagnetic signal characteristic of the biological activity of the ligand and/or the receptor is carried out by exposure of a solution or a suspension containing this reagent to this or these electromagnetic signals, or by dilution of such a solution or suspension in a solvent having been previously exposed to this or these electromagnetic signals, or by dissolution or setting in suspension of this reagent in a solvent having been previously exposed to (X) said (S) the signal (with) electromagnetic (S).

In variant, one applique, with the test sample and reagent intended to react with him, the electromagnetic signal characteristic of the biological activity of the ligand and/or the receptor, by exposure of a solution or a suspension containing this sample and this reagent to this or these electro signals magnetic, or by dilution of such a solution or suspension in a solvent having been previously exposed to (X) said (S) the signal (with) electromagnetic (S).

According to a provision particularly prefered of this second mode of implementation, one applique, with the test sample and/or reagent intended to react with him, at the same time the electromagnetic signal characteristic of the biological activity of the ligand and the electromagnetic signal characteristic of the biological activity of the receptor. Indeed, the Inventors noted that, if it is enough to apply, with the elements of the couple ligand/receptor, the electromagnetic signal characteristic of the biological activity of only one of these elements to obtain an amplification of complex formed by their reaction, this amplification is higher when one applique with these elements simultaneously the electromagnetic signals characteristics of the biological activity of each one of them.

Whatever the mode of setting of work of the method of amplification in conformity with the Invention, the solvent having been previously exposed to (X) the signal (with) electromagnetic (S) is advantageously of 1 ' water or the physiological solute.

Capable reagents of tre used in the method of amplification in conformity with the Invention and containing the ligand on the one hand, and the receptor on the other hand, can as well tre of the available reagents prts to employment in the trade as of reagents especially designed and prepared for the implementation of this method. In addition to the fact that, as mentioned herebefore, these reagents can be presented in different forms (dry, liquid,…), they can, in addition, tre coupled to a marker such as a radioactive isotope, an enzyme, a fluorescent substance, a coloured particle, biotin or a compound organometallic, suitable to allow the detection and/or the measuring of the complex ligands-receptors resulting of the reaction between the ligand and the receptor.

The method of amplification advantageously includes/understands, moreover, one acquisition step of the electromagnetic signal characteristic of the biological activity of the one and/or the other of the elements of the couple ligand/receptor.

As previously indicated, the electromagnetic signal characteristic of the biological activity of the one and/or the other of the elements of the couple ligand/receptor can come either from a test sample and suspect to contain this or these elements, or of a source of electromagnetic radiation or acquisition of a mechanical signal (vibrations), electrostatic or different, or still of reagents containing the ligand or the receptor in solution or suspension in a solvent, according to modes' of implementations of the method of amplification in conformity with the Invention.

Of manner particularly advantageous, the method of amplification in conformity with the Invention includes/understands, also, a step of recording and of playback of information representative of the electromagnetic signal characteristic of the biological activity of the one and/or the other of the elements of the couple ligand/receptor. Thus, the electromagnetic signal characteristic of the biological activity of an analytic sample, once recorded, can tre preserved indefinitely and used as many time as necessary. Of similar manner, the electromagnetic signals characteristics of the biological activity of the ligand and biological activity of the receptor collected starting from reagents, can tre once recorded for all and tre used to carry out a plurality of reactions bringing into play this ligand and this receptor.

The method of amplification includes/understands advantageously, moreover, a detecting step of complex resulting of the reaction between the ligand and the receptor and, optionally, of measuring of these complex. This step can be advantageously supplemented by the comparing of the results obtained with those observed for a reaction serving of " witness ", i.e. a reaction led with Mrs. couples ligand/receptor and in reactional conditions Mrs., but without application of an electromagnetic signal with the elements of this couple, that it is previously, simultaneously or subsequently to their setting in contact.

The detection and/or the measuring of the complex ligands-receptors are capable of tre carried out by all the methods conventionally used to reveal and quantify the formation of such complex. Thus, in the case of complex antigen-antibodies, it is possible to as well use a revelation by agglutination, immunoprecipitation, extinction of fluorescence, fluorescence polarization that a radioimmunological, immuno-enzymatic test or a test of immuno-fluorescence.

According to a mode of implementation particularly prefered of the method of amplification in conformity with the Invention, the ligand is an antigen or a hapten, while the receptor is an antibody or a membrane receptor directed specifically against this ligand.

Of manner particularly advantageous, the reaction between this ligand and this receptor is a reaction revealed by agglutination, because of its simplicity and of its speed of execution.

The present invention has, also, for object a detecting method of the presence of a substance corresponding one to the one of the two elements of a couple ligand/receptor in an analytic, characterized sample in what it includes/understands the implementation of a method of amplification such as defined herebefore.

According to a first mode of implementation particularly prefered of this detecting method, this one includes/understands:
put it in contact of two reagents respectively containing the ligand and the receptor, under conditions suitable to allow their reaction, previously, simultaneously or subsequently to this setting in contact, the application, with the one and/or the other of these reagents, of the electromagnetic signal characteristic of the biological activity of the analytic sample, and
it detection and/or the measuring of the complex formed ligands-receptors during the reaction enters the two reagents.

Thus, obtaining of an amplification of the formation of complex ligands-receptors between two reagents compared to a " pilot " reaction (such as previously defined) translated the presence, in the electromagnetic signal of the biological activity of the test sample, the electromagnetic signal characteristic of the biological activity of sought substance and, by way of consequence, translated the presence, in this sample, of sought substance.

If such an amplification is obtained and where the analytic sample is capable to not contain only one of the two elements of the couple ligand/receptor, but these two elements, the presence, in this sample, of sought substance can be confirmed by the comparing of the results obtained with:

- is those observed for a reaction led in reactional conditions Mrs. but with an application at the same time of the electromagnetic signal characteristic of the biological activity of the test sample and electromagnetic signal characteristic of the biological activity of the ligand,

- is those observed for a reaction led in reactional conditions Mrs. but with an application at the same time of the electromagnetic signal characteristic of the biological activity of the test sample and characteristic signal of the biological activity of the receptor.

Thus, if the simultaneous application of the electromagnetic signal characteristic of the biological activity of the analytic sample and the electromagnetic signal characteristic of the biological activity of the ligand results in an amplification of the formation of the complex ligands-receptors compared to the application of the single electromagnetic signal characteristic of the biological activity of the aforesaid analytic sample, then that means that this sample does not contain a ligand and thus only the receptor contains. The absence of increase of the formation of the complex ligands-receptors signing, it, the presence of the ligand in the analytic sample.

Of similar manner, if the simultaneous application of the electromagnetic signal characteristic of the biological activity of the analytic sample and electromagnetic signal characteristic of the biological activity of the receptor results in an amplification of the formation of the complex ligands-receptors compared to the application of the single electromagnetic signal characteristic of the biological activity of the aforesaid analytic sample, then it can in tre deduces that this sample does not contain a receptor and thus only the ligand contains. The absence of increase of the formation of the complex ligands-receptors signing, it, the presence of the receptor in the sample.

In order to avoid obtaining wrongfully negative results, i.e. results which would not make it possible to reveal an effect of amplification of the application of the electromagnetic signal characteristic of the activity of the analytic sample and this, although this last contains actually sought substance, the concentrations of the ligand

and the receptor put to react are advantageously selected so as to tre sufficient lead to the obtaining of complex the detectable in the absence of the application of the electromagnetic signal characteristic of biological activity of the aforesaid sample, but low ligands-receptors with the concentrations capable to lead to a saturation of the reaction between this ligand and this receptor.

According to a second mode of implementation prefered of this detecting method, this one understands: put it in contact of the analytic sample with a reagent containing is the receptor, if the sought substance in the sample is the ligand, that is to say the ligand, if the sought substance in the sample is the receptor, under conditions suitable to allow their reaction, previously, simultaneously or subsequently to this setting in contact, the application, with this sample and/or this reagent, of the electromagnetic signal characteristic of the biological activity of the ligand and/or the receptor, and it detection and/or the measuring of the complex optionally formed ligands-receptors, in which case, the obtaining of complex ligands-receptors translates the presence of sought substance in the analytic sample.

This second mode of present implementation prefered a very particular intért to detect the substance in a sample presence, which one knows that they are not detectable or that very sparingly by the other available detection methods, because of what these substances are generally present with very low concentrations, even with the state of traces.

The detecting method of the presence of an analytic substance in a sample conforms to the present Invention of numerous advantages.

Indeed, on the one hand, it makes it possible to detect the presence of a sought substance with a very large sensitivity and an high specificity. So in the case, for example, of a bacteriological analysis, it makes it possible to remove the need to insulate the different germs, to cultivate them, proceed to a antibiogramme and to identify these germs by their biochemical, morphologic and immunologic characters, and authorizes the obtaining of results much rapidly than the immunologic detection methods currently used in bacteriology.

In addition, insofar as it is enough to have a sample to the size of a drop to acquire and record the electromagnetic signal characteristic of the biological activity of this sample and where, this signal, once recorded can be restored with the application, this method offer the possibility carry out analyses as many as one wishes starting from a microsample.

Lastly, 1 ' recording of an electromagnetic signal which can tre preserved indefinitely, for example in the shape of a capable computerized file of tre preserved on a single diskette or a CD-Rom, and of tre transmitted of a place to another by any transmission means the given digital ones, this method makes it possible, moreover, to remove all constrained conservation, of forwarding and transport of the taking away which present the methods currently used for detection of a substance.

This method capable of being used to detect any substance capable to bind specifically with another substance and to react with it, being understood that the term " substance " such as it is used here, a biological compound, a chemical compound indicates as well, a cell that a microorganism of the type bacterium, virus or mushroom, knowing especially that for any hapten, protein or complex proteinaceous, it is possible to find on the market or to make manufacture the corresponding antibodies. For this reason, this method finds, especially, application in the biological diagnosis, that it is in human or veterinary medicine, or for the electromagnetic control characteristic of the biological activity of a substance corresponding one to the one of the two elements of a couple ligand/receptor, which method is characterized in what it includes the implementation of a method of amplification such as defined herebefore.

According to a mode of implementation prefered of this detecting method, the electromagnetic signal to be tested is the radiated electromagnetic signal by a source of electromagnetic radiation.

The Invention has, also, for object a detecting apparatus of the presence of a substance corresponding one to the one of the two elements of a couple ligand/receptor in an analytic sample, which apparatus is characterized in what it in accordance with the invention implements a method and in what it comprises:

a) receiving means of the analytic sample and a reagent containing either the receptor, or the ligand, allowing their setting in contact under conditions suitable to allow their reaction;

b) an electromagnetic signal source characteristic of the activity of the ligand and/or receptor;

c) application means of the signal delivered by the aforementioned electromagnetic signal source with the sample and/or the reagent; and

d) detection means and/or of measuring of the complex formed ligands-receptors during the reaction enters the sample and the reagent.

The Invention has, moreover, for object a detecting apparatus of the presence of a substance corresponding one to the one of the two elements of a couple ligand/receptor in an analytic sample, which apparatus is

characterized in what it in accordance with the invention implements a method and in what it comprises:

a) receiving means of two reagents respectively containing the ligand and the receptor, allowing their setting in contact under the conditions suitable to allow their reaction;

b) acquisition means of an electromagnetic signal of the analytic sample;

c) application means of the signal delivered by the aforementioned acquisition means of electromagnetic signal to the one and/or the other of reagents; and

d) detection means and/or of measuring of the complex formed ligands-receptors in the course of the reaction enters the two reagents.

According to an advantageous embodiment of these apparatuses, the detection means comprise detection means optical.

Of prefered manner, these apparatuses comprise an enclosure provided with an electrical shield and magnetic surrounding the aforementioned receiving means.

In addition to the provisions which precede, the Invention still includes/understands other provisions which will arise from the complement of description which follows, which refers to examples of performing of apparatuses of acquisition, recording and signal supplying capable of tre used in accordance with the invention like with examples of experiments having made it possible to validate the method of amplification object of the present invention, and which refers to the drawings annexed in which:

Figure 1 represents a scheme of a first example of performing of a capable apparatus of signal acquisition of tre used according to the present invention;

Figure 2 represents a scheme of a second example of performing of a capable apparatus of signal acquisition of tre used according to the present invention;

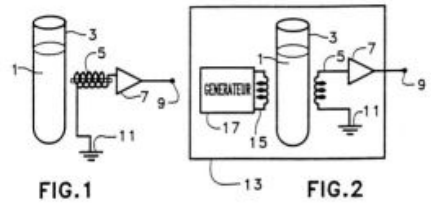


FIG.1 FIG.2

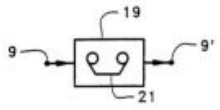


FIG.3

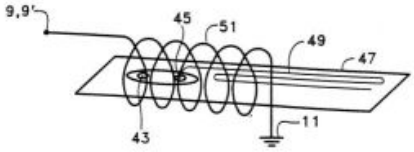
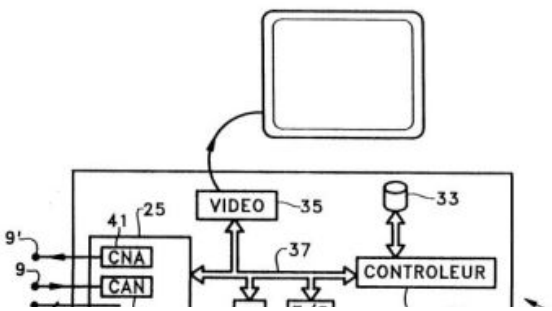


FIG.5

Figure 3 represents a scheme of a first example of performing of an apparatus of capable recording of signal of tre used according to the present invention;

Figure 4 represents a scheme of a second example of performing of an apparatus of capable recording of signal of tre used according to the present invention;



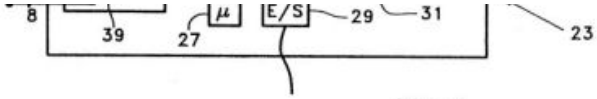


FIG. 4

Figure 5 represents a scheme of an example of performing of a capable apparatus of signal supplying of tre used in accordance with the Invention;

Figure 6 watch an image black and white of 320 pixels X 240 pixels of the formed agglutinats during an agglutination reaction enters the antigen polysaccharidic of Escherichia coli Kl and an antibody directed against this antigen, after application of the electromagnetic signal characteristic of the biological activity of Streptococcus;

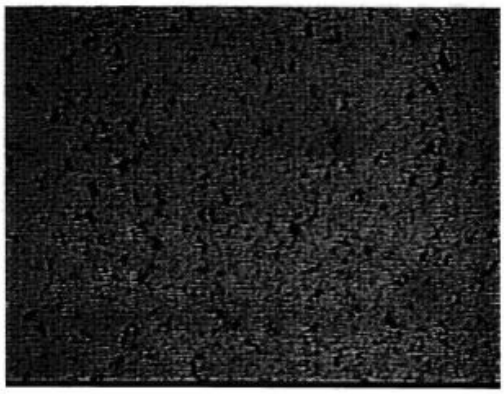


Fig. 6

Figure 7 watch an image black and white of 320 pixels X 240 pixels of the formed agglutinats during an agglutination reaction enters the antigen polysaccharidic of Escherichia coli Kl and an antibody directed against this antigen, after application of the electromagnetic signal characteristic of the biological activity of Escherichia coli;

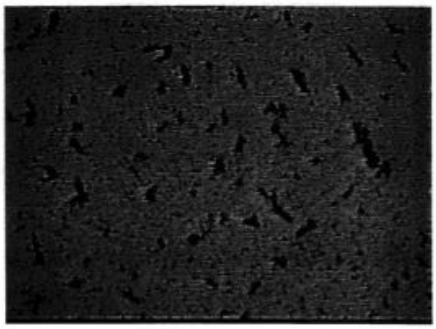


Fig. 7

Figure 8 watch an image black and white of 320 pixels X 240 pixels of the formed agglutinats during an agglutination reaction enters the antigen polysaccharidic of Escherichia K1 coli and an antibody directed against this antigen, after simultaneous application of the electromagnetic signals characteristics of the biological activity of Streptococcus and the biological activity of an antibody directed against Escherichia coli;

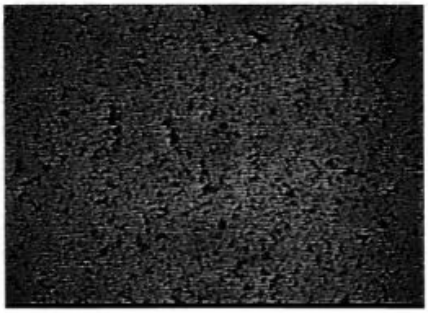


Fig . 8

Figure 9 watch an image black and white of 320 pixels X 240 pixels of the formed agglutinats also during an agglutination reaction enters the antigen polysaccharidic of Escherichia Kl coli and an antibody directed against this antigen, after simultaneous application of the electromagnetic signals characteristics of the biological activity of Escherichia coli and the biological activity of its specific antibody; and

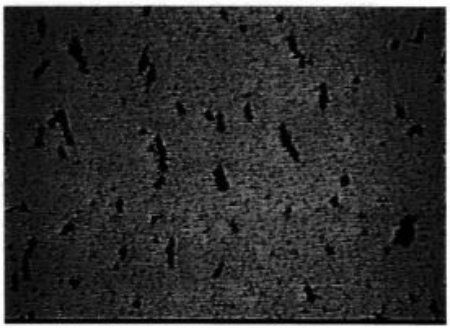


Fig. 9

Figure 10 represents a scheme of an example of performing of an apparatus of detection and/or measuring of the complex ligands-receptors capable of tre used according to the present invention.

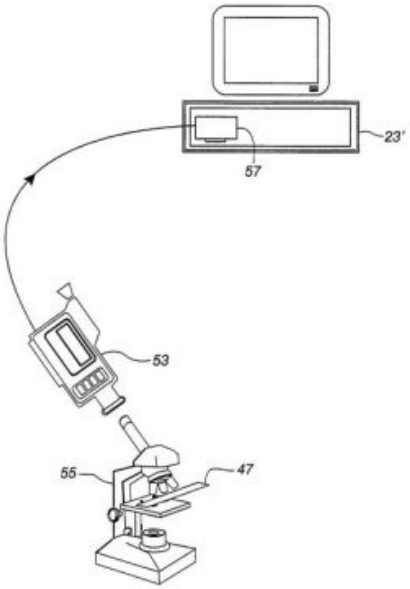


Fig. 10

In Figures 1 to 5 and 10, one used references Mrs. to designate elements Mrs.

In addition, each image of Figures 6 to 9 corresponds to a surface of approximately 2 mms X 1,5 mms of the support on which the agglutination reactions were carried out.

It owes of course, however, that these examples are given only as illustrations of the object of the Invention and do not constitute in any manner a limitation of it.

One refers first of all on Figures 1 to 5.

On Figure 1, one schematically represented a first example of performing of an apparatus of acquisition of the electromagnetic signal characteristic of the biological activity of a substance 1 laid out in a container 3, for example a test tube. A sensor 5, typically a coil of type " telephonic sensor " marketed for tre applied on an earphone telephonic and connected to a tape recorder, is applied against container 3. Container 3 can tre also consisted a biological wall, especially the skin of a living tre.

In such a case, the acquisition of the electromagnetic signal is carried out of noninvasive manner.

The signal collected by coil 5, advantageously, is amplified by an amplifier 7 and is available with an output

terminal 9. Without that present any restrictive character of 1 ' illustrated example, a first end of coil 5 being connected to the input of amplifier-preamplifier 7, the opposite end being connected to a mass 11. In an example of performing, coil 5 is a telephonic sensor of the trade having a length of 6 mms, an internal diameter of 6 mms containing a metal core, an outer diameter of 16 mm and an impedance of 300 Q.

On Figure 2, one accounted for 1 schematically ' prefered example of performing of an apparatus of acquisition of the electromagnetic signal characteristic of the biological activity of a substance 1 contained in a container 3, in which the apparatus includes/understands, preferably, in an enclosure 13 provided with an electrical shield and magnetic, a transducer 15 of irradiation of the aforesaid substance 1 supplied with a generator 17. Transducer 15 comprises, for example, a coil, advantageously supplemented by guides of waves, for example, an air-gap (not represented) placed in contact with the outer walls of container 3.

Generator 17 generates a sinusoidal signal low frequency, signals square low frequency, pink noise or, advantageously, white noise. The signal spectrum of excitation feeding coil 15 corresponds substantially to the spectrum of the audible frequencies (20 Hz-20 000 Hz). Generator 17 can tre a generator of analogue signal of known type or, for example, a read-only memory (ROM, PROM, EPROM, EEPROM in Anglo-Saxon terminology) containing the digital signal of the desired noise and which is connected to a converter numeriqueanalogic, or the outputted line of a card sounds of a microcomputer multimedia.

However, the implementation of upper frequencies does not leave the frame of the present invention.

The sensor of acquisition 5 can comprise a like coil with coil 5 of the apparatus of Figure 1 or, advantageously, a coil of small diameter connected by a guide of electromagnetic waves to the wall of container 3.

Advantageously, the signal collected by sensor 5 is available with an output terminal 9 of a amplifier-preamplifier 7.

The available signal on terminal 9 can tre directly applied with or substances to be irradiated, especially with the ligand, the receptor or the couple ligand/receptor (especially using the apparatus illustrated on Figure 5 and described ciaprès).

The recording of the signal can tre carried out into analogue by a recorder of signal 19 (Figure 3), especially on magnetic tape 21 adapt with the frequencies of the collected signal. For the acoustic frequencies, one can especially use a tape recorder. Output terminal 9 of the apparatus of signal acquisition of Figures 1 or 2 is connected to the input microphone or the input line of such a tape recorder. During the reading, the signal is collected with an output terminal 9 ', especially with the outputted line or outputted the loudspeaker of tape recorder 19.

Advantageously, one carries out a digital recording after analog-to-digital conversion of the signal. One uses, for example, a microcomputer 23 illustrated on Figure 4, provided with a card of signal acquisition 25. It is for example about a computer of type PC, rotating under operating system WINDOWSX 95 of Company MICROSOFT and comprising, in addition to the card of acquisition 25, a microprocessor 27, an interface of input/output 29, a controller 31 of a mass memory 33 and one interface video 35 connected by one or more bus 37. The card of acquisition 25 comprises an analogue converter 39 having, preferably, an upper resolution with 10 bits, for example equal with 12 bits, as well as a frequency of double sampling of the maximum frequency which one wants to be able to digitize for the signal processing. In the acoustic frequencies, the frequency of sampling is advantageously substantially equal to 44 Khz. For the processing of these signal types, one advantageously uses a card its for microcomputer, for example the card Soundblaster 16 or the card Soundblaster 32 sold by CREATIVE Company LABS. The computer 23 provided with the card of acquisition of playback 25, especially of a card Soundblaster 32 can advantageously replace the generator of signal 17 of Figure 2.

One connects outputted the 9 of the apparatuses of signal acquisition of Figures 1 with input 9 of the analog-to-digital converter 39 of card 25 of the computer 23; one proceeds to an acquisition of the pendent signal one duration for example ranging between 1 and 60 S and one record the digital file in a mass memory 33, for example in the shape of a file its to the format. WAV. This file can optionally undergo a digital processing, such as for example a digital amplification for calibration of the signal level, a filtering for the removing of nondesired frequencies, or tre transformed into its spectrum by a transform of FOURIER discrete, preferably by the algorithm of rapid transform of FOURIER (FTT in Anglo-Saxon terminology).

The time of sound reproduction can tre increased while repeating in a file several times a fragment or the whole of the file its original.

On order, the optionally treated file is transformed by a digital-to-analog converter 41 of the card 25 (or of a separate card), which delivers on outputted the 9 ' the analogue electromagnetic signal characteristic of the biological activity to be applied, according to the method of amplification in conformity with the Invention, for example to aliquot 43 of a first reagent and to aliquot 45 of a second reagent, as illustrated on Figure 5.

Advantageously, the application of the signal with these aliquot is carried out previously with their mixture. The

support on which these aliquot are deposited, for example, a blade 47 provided with capillary 49 in the shape of serpentine, is laid out in a radiated electromagnetic field by a transducer 51, typically a coil whose first end 9,9 ' is connected to outputted the 9 of an apparatus of acquisition of

Figures 1 or 2 or to outputted the 9 ' of an apparatus of recording of Figures 3 or 4.

The end of the coil opposed to connecting terminal 9,9 ', for example, is connected to mass 11.

Without that representing any restrictive character, transducer 51 comprises a coil advantageously, of horizontal axis allowing the introduction of blade 47. The coil has, for example, a length of 120 mms, an internal diameter of 25 mms, an outer diameter of 28 mms, present 631 revolutions of a wire of diameter 0,5mm and a resistance of 4,7 Q.

Advantageously, the applied electrical signal on this coil 51 will have an amplitude of 2 effective volts.

EXAMPLE 1: AMPLIFICATION OF the FORMATION Of AGGLUTINATS BETWEEN ANTIGEN POLYSACCHARIDIQUE Of ESCHERICHIA Kl COLI AND AN ANTIBODY DIRECTS AGAINST THIS ANTIGEN

The method of amplification in conformity with the Invention was validated by testing the effects, on an agglutination reaction between the antigen polysaccharidic of Escherichia coli Kl and an antibody directed against this antigen:

- of the application of the electromagnetic signal characteristic of the biological activity of a foreign antigenic substance to this reaction such as Streptococcus,

- of the application of the electromagnetic signal characteristic of the biological activity of Escherichia coli,

- of the simultaneous application of the electromagnetic signal characteristic of the biological activity of Streptococcus and the electromagnetic signal characteristic of the biological activity of an antibody directed against Escherichia coli, and finally

- of the simultaneous application of the electromagnetic signal characteristic of the biological activity of Escherichia coli and the electromagnetic signal characteristic of the biological activity of an antibody directed against this antigen.

1) Performing of the tests: a) Acquisition of the electromagnetic signals:

The acquisition of the electromagnetic signals characteristics of the biological activities of Streptococcus, Escherichia coli and its specific antibody was carried out by means of the hardware of recording of Figure 2.

The acquisition of the electromagnetic signal characteristic of the biological activity of Streptococcus was carried out while placing at the center of 1 ' enclosure a 13 tube containing 1 ml of an aqueous suspension of previously formolized Streptococcus bacteria (6.106 bactéries/ml).

The acquisition of the electromagnetic signals characteristics of the biological activity of Escherichia coli and its specific antibody was carried out into operative of Mrs. manner, but while using respectively:

- a tube containing 1 ml of an aqueous suspension of bacteria
Escherichia coli previously formolized (6.106bactéries/ml); and

- a tube containing 1 ml of a particle suspension of a latex sensitized by a specific monoclonal antibody of mouse of Escherichia Kl coli, coming from a kit PASTOREXs MENINGITIS (Reference 61709-SANOFI DIAGNOSES PASTEUR). b) Preparation of reagents of the agglutination reaction:

The tests were carried out while using as reagents:
- of an hand, a prepared antigen solution polysaccharidic of Escherichia K1 coli by dissolution of an antigenic extract coming from a kit PASTOREXX MENINGITIS (Reference 61709-SANOFI
DIAGNOSES PASTEUR) in 1 ml of water distilled and sterile, then dilution to the 1/7, 1/7,5 or 1/8 in physiological serum; and
- of another hand, the latex sensitized by a specific monoclonal antibody of mouse of the antibody of Escherichia present K1 coli in this Mrs. kit, after dilution to the 1/3 in physiological serum. c) Application of the electromagnetic signals with the agglutination reaction:
For each test, one used the following protocol:
one place in a drying oven heated at 37 C a transducer consisted a coil measuring 120 mms of long and 25 mms internal diameter, presenting 631 revolutions and a resistance of 4,7 Q, and connected to outputted the 9 ' of the digital-to-analog converter 41 of a Soundblaster card of a computer 23 restoring the files of recording consisted the electromagnetic signals which one wishes to apply, time necessary to bring this transducer to the temperature of 37 C;

one deposits on a blade provided with capillary in the shape of serpentine (of type of those supplied in kits PASTOREX MENINGITIS), at low distance from the opening of this last, a drop (either 40 to 50 J. l) antigenic solution as described at the foregoing point b), as well as a drop (corresponding one also with a volume from 40 to 50, ul) of latex sensitized by the antibody, by taking guard so that these drops do not mix;
one applique, with the two drops of reagents thus deposited, the electromagnetic signals wished while placing the blade at the center of the pendent transducer approximately 2 mn and by restoring a file its using the computer 23 of
Figure 4;
one mixture the two reagent drops pendent approximately 10 seconds and one lets pendent approximately 13 minutes in the drying oven the reaction mixture migrate in the capillary one and the agglutination reaction to occur;
one then leaves the blade the drying oven and one carries out a reading of this agglutination.

Like visible on Figure 10, this reading is carried out by analysis, by means of a software of analysis and image processing implemented on a computer of rotating type PC 23 ' under operating system VJNDOWSO 95 (MICROSOFT), of an acquired image using a video camera 53 positioned on an optical microscope 55 and connected to that the computer by a card of acquisition video 57. Camera 53 works in grey levels. A first processing increasing contrast, the threshold being controlled so that the agglutinats appear into black, while the zones deprived of latex particles or agglutinats appear into white.

From the analysis of the two-dimensional spatial distribution of the dark areas of the image, the computer determines an index of agglutination (I) calculated according to the formula:
Surface occupied by the agglutinats of upper size to 60 pixels
Surface occupied by the agglutinats of equal or low size to 60 pixels
This index of agglutination is all the more high as the size of the formed agglutinats during the agglutination reaction is more substantial.

The amplification is regarded as positive when, during an experiment, the application of the electromagnetic signals characteristics of the biological activity of Escherichia coli and/or biological activity of its specific antibody led to the obtaining of at least upper indices of agglutination of 40% to the maximum index of agglutination obtained, in conditions Mrs., and on for example 3 experiments, after application of the electromagnetic signal characteristic of the biological activity of Streptococcus.

2) Results:
Table 1 hereafter present indices of agglutination (I) obtained in first series of tests aiming at comparing the effects of the application of the electromagnetic signal characteristic of the biological activity of Escherichia coli with those observed after application, in reactional conditions Mrs., from the electromagnetic signal characteristic of the biological activity of Streptococcus, and this, for 3 different dilutions (1/7,1/7,5 and 1/8) of the polysaccharidic antigen solution of Escherichia K1 coli used like reagent in the agglutination reactions.

TABLE 1

In addition, Figures 6 and 7 show, as examples, of the images of the formed agglutinats on the one hand, after application of the electromagnetic signal characteristic of the biological activity of Streptococcus (Figure 6) and, on the other hand, after application of the electromagnetic signal characteristic of the biological activity of Escherichia coli (Figure 7). These images correspond respectively to the indices of agglutination of 32 and 117 which are brought back to the 5th line of results of Table 1.

Table 2 below present, as for him, the indices of agglutination (I) obtained in a second series of experiments in the frame of which effects of the simultaneous application of the electromagnetic signal characteristic of the biological activity of Escherichia coli and the electromagnetic signal characteristic of the biological activity of the antibody directed against
Escherichia coli, were compared with those of the simultaneous application, in reactional conditions Mrs., of the electromagnetic signal characteristic of the biological activity of Streptococcus and the electromagnetic signal characteristic of the biological activity of the antibody directed against Escherichia coli and this, for 2 different dilutions (1/7 and 1/7,5) of the polysaccharidic antigen solution of Escherichia coli
K1 used as reagent.

TABLE 2
Figures 8 and 9 show, also as examples, of the images of the agglutinats which corresponding respectively with the indices of agglutination of 71 and 247 brought back to the 2nd line of results of Table 2.

All these results clearly put in evidence the ability that present the electromagnetic signal characteristic of the biological activity of an element of a couple ligand/receptor, to amplify the formation of complex formed by the reaction between this ligand and this receptor and this, of very specific manner, since the electromagnetic signal characteristic of the biological activity of a biologically active, but foreign element with this reaction product, him, not of effect of amplification.

They show also that this amplification is all the more marked as one applique, with the two elements of the

couple ligand/receptor, at the same time the electromagnetic signal characteristic of the biological activity of this ligand and the electromagnetic signal characteristic of the biological activity of this receptor.

EXAMPLE 2: DETECTION OF the PRESENCE Of ESCHERICHIA COLI
IN A SAMPLE
The intért of the use of the method of amplification conforms to the Invention for the detection of a present substance in an analytic sample was checked by carrying out a series of tests aiming at comparing the effects of the application, on an agglutination reaction between the antigen polysaccharidic of Escherichia K1 coli and a monoclonal antibody of specific mouse of this antigen identical with that implemented in 1 ' example 1 herebefore, of the electromagnetic signal collected starting from a sample of a food product, in the species an apple compote, previously contaminated by bacteria Escherichia coli, with those obtained at the time of the application, in reactional conditions Mrs., of the collected electromagnetic signal starting from a control sample, i.e. not contaminated, of Mrs. food product.

1) Performing of the tests:
The acquisition of the electromagnetic signals of the apple compote samples (contaminated control samples and samples) was carried out by means of the hardware of recording of Figure 2, while placing at the center of 1 ' enclosure 13:
- in the case of the control samples, a tube containing 1 ml of compote previously diluted to the 1/2 with physiological serum, and
- in the case of the contaminated samples, a tube containing 1 ml of compote previously diluted to the 1/2 with physiological serum and contaminated, by adding of bacteria previously formolized Escherichia coli, at a rate of 3.106 bacteria per ml of diluted compote.

The tests were carried out while using as reagents:
- of an hand, a suspension containing of the bacteria Escherichia coli previously formolized in physiological serum, at a rate of
10 ' bactéries/ml, and
- of another hand, the latex sensitized by a specific monoclonal antibody of mouse of the antibody of Escherichia present K1 coli in this Mrs. kit, after dilution to the 1/3 in physiological serum, and into following an operational protocol identical with that described with the paragraph c) of 1 ' example 1 herebefore.

2) Results:
The Table 3 hereafter present indices of agglutination (I) obtained in three series of tests.

TABLE 3
Like visible on Table 3, the size of the formed agglutinats during the reaction between the antigen polysaccharidic of Escherichia Kl coli and its specific antibody is substantially higher if the applied electromagnetic signal during this reaction were collected starting from an apple compote sample contaminated by bacteria Escherichia coli.

These results show that the method of amplification conforms to the Invention can tre advantageously used to detect the presence, in an analytic sample, of a biologically active susbtance such as a bacterium, Mrs. when this sample present a complex composition, i.e. it contains, as in the case of the apple compote samples, of numerous others susbstances biologically active.

As that spring by what precedes, the Invention is limited by no means to the forms of performing which come from tre described in a more explicit way; it embraces of them on the contrary all the variants which can come to mind from the technician in the matter, without deviating from the frame, nor of the span of the present one Invention.

WO0001412
METHOD FOR ACTIVATING AN INACTIVE SOLUTION

The invention concerns a method for activating an inactive solution with very low concentration of a specific biological and/or chemical substance. The method comprises a step which consists in subjecting said solution to a mechanical excitation field, in particular generated by a vortex.

Present invention relates to a process of activation of an inactive solution and to very low concentration of a biological and/or chemical determined substance in a solvent. Present invention relates to also applications of the aforesaid process of activation.

One indicates under the term of " very low concentration ", of the concentrations ranging between 10 - 6 and zero moles per liter (M).

One knows methods of preparation of solution " highly diluted " by dilution and successive agitation. One of the methods of preparation employed traditionally in homeopathy (method of Hannemahn) consists, starting from a

relatively concentrated solution carried out using a dyeing parent (of great concentration with 10 - 6 M), to carry out a dilution of a factor 10 or 100, then with a mechanical agitation (refer " dynamization "). Each operation after is noted that the solution remained active in the sense that it starts a reaction within a sensitive biological system.

As examples of solutions which were prepared by the described method cidessus, one can quote all the homeopathic preparations. As sensitive examples of biological systems allowing to test the active character such solutions one can quote: the isolated heart of guinea-pig (experiment of Langendorff) or the cutaneous test carried out on the skin of a guinea-pig or a living rabbit.

The need to start from an active solution before carrying out a dilution then with an agitation appeared impossible to circumvent to obtain active diluted solutions. The dilutions carried out up to 10 - 6 M and beyond that, without implementing this process of successive agitation and dilution, did not allow until present obtaining active solutions. L is not without interest to recall either that technical agitation and of dilution bringing in work by Hannemann was extrapolated without one being able until present showing the vertues such high dilutions beyond factor 5 CH.

Gold the inventors, who are known for their workings on high dilutions (Nature 1988: “Dégranulation of Basophilic human started by a solution with high dilution of anti-IgE antibody”), noted of surprising manner that it was of possible to obtain an active solution starting from an inactive solution and with very low concentration of a biological and/or chemical determined substance in a solvent. They showed that a solution with very low concentration whose activity is non-existent at the beginning, can be made active by processes particularly single to implement. Thus, they conceived a process which makes it possible to make active of the solutions to very low concentration without it being necessary to previously prepare them by technical traditional successive dilutions and agitations.

They have thus resolved a problem whose industrial implications are considerable. Indeed:

- it is from now on possible to detect biological and/or chemical determined substances in solution with very low concentration in a solvent,

- it is from now on possible to design and carry out medicaments implementing biological and/or chemical determined substances in solution at very low concentration in a solvent,

- it is from now on possible to control the production of products with very low concentration especially of the homeopathic products.

The present invention thus has as an object a process to activate a solution with very low concentration of a biological and/or chemical determined substance in a solvent. The aforementioned process includes/understands the step to place the aforementioned solution in a mechanical excitation field. The mechanical excitation field could be created, for example, by one shock wave being propagated in the solution, by ultrasounds or sound waves diffused in the solution, by vibrations transmitted by the container containing the solution. Preferably, the mechanical excitation field results from an agitation forces especially obtained by means of a stirrer of Vortex type made up of a disc which turns rapidly until a vortex is formed in the liquid column in the course of agitation. Preferably, the aforementioned solution is subjected to a pendent mechanical excitation field at least 15 seconds.

As examples of substances in solution which were activated by the method described above, one can quote: arnica, ovalbumin, acetylcholine, the calcium ionophore.

The process in accordance with the invention present of interest only when the concentration of the aforesaid determined substance in the aforementioned solution is low to 10-6 moles per liter. Indeed with the top of 10-6 moles per liter the solution is already active (traditional pharmacology). Preferably, the concentration of the aforesaid determined substance in the aforementioned solution is included/understood in a low with 10-6 moles per liter and great fork with
10-l6 moles per liter.

Preferably also the aforementioned solvent contains at least water 5%. It indeed appeared that in on this side certain proportion of water in solvent the solution subjected to a mechanical excitation field residue inactive.

It also appeared that an alcohol percentage from at least 2% in the solvent causes that the solution pendent residue active a longer period.

The process in accordance with the invention includes/understands moreover the step to control the active state of the aforesaid the solution by implementing an experimental protocol identical or similar with that which one would implement to account for the presence of the aforesaid determined substance in a medium which would contain it.

As sensitive example of biological system allowing to test the active character of the solutions of following

determined substances: calcium-ionophore, acetylcholine, histamine, ovalbumin (in the animal made sensitive), arnica, bradykinin, anti-IgE antibody, one can quote: the experiment of Langendorff (heart of isolated guinea-pig) as well as the cutaneous test carried out on a skin of guinea-pig or living rabbit. Thus for example, to control the active state of the acetylcholine solution it is checked that an injection of this one, under the skin of a guinea-pig especially, causes cutaneous reactions.

Present invention relates to also the application of the process with the detection of a determined substance, diluted in very low concentration.

Indeed since the solution is activated, it is possible to proceed to tests of identification by implementing an experimental protocol identical or similar with that which one would implement to account for the presence of the aforesaid determined substance in a medium which would contain it. As example of determined substances that one can detect in solution, one can quote following substances: calciumionophore, caffeine, nicotine, toxins and endotoxines bacterial; and following tests of identification: the experiment of Langendorff (heart of isolated guinea-pig) as well as the cutaneous test carried out on a skin of guinea-pig or living rabbit.

Present invention relates to also the application of the process with the production of medicaments implementing biological materials and/or chemical at very low concentration. Indeed, since it is possible to prepare active solutions with very low biological and/or chemical determined substance concentration, new therapeutic applications of these substances become possible.

As example of medicaments which one can thus produce, one can quote the following medicaments: coronary vasodilators (ex: trinitroglycérinej, bétabloquant (propranolol), caffeine, nicotine.

Present invention relates to also the application of the process with the control of the production of products with very low concentration, especially of the homeopathic products. Indeed, one of the problems to be solved when homeopathic products are manufactured is that of control in production of successive dilutions. The process in accordance with the invention makes it possible to test the activity of the homeopathic products at the time of the different phases of their manufacturing process. As example of homeopathic products which one can thus control the production one can quote the following medicaments: arnica 5 to 30 CH, histaminum 5 to 30 CH, acétylcolinum 5 to 30 CH, apis mellifica 5 to 30 CH.

Other characteristics and benefits of the invention will appear with the reading of the description of variants of performing of the invention, given as indicative and nonrestrictive example, like with the reading of the examples of experiments having made it possible to validate the process of activation, object of the present invention, and which refer to the drawings annexed in which: -

Figure 1 represents a perspective view of a variant of performing of the system of agitation.

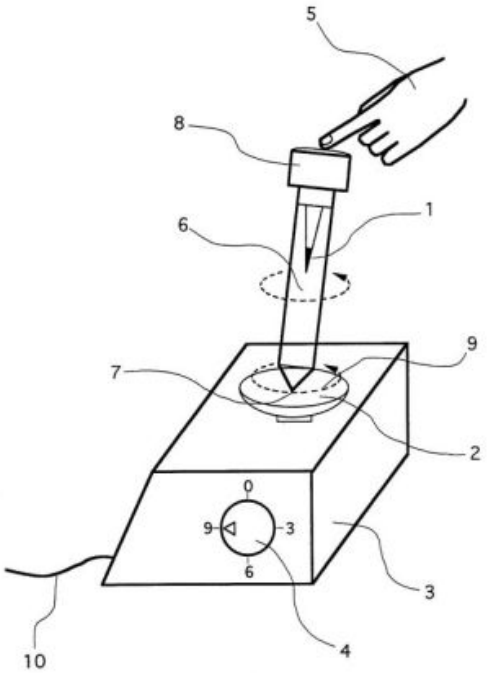


Fig. 1

Figure 2 represents a perspective view of a system making it possible to test the activity of the solution (experiment of Langendorff).

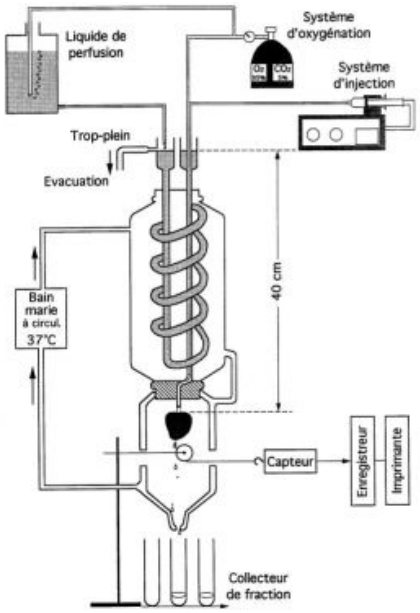


Fig. 2

Figure 3 represents an image of the skin of a guinea-pig

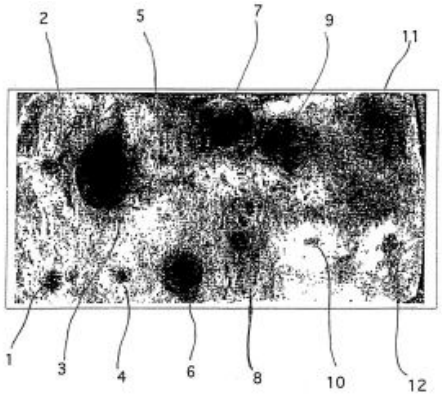


Fig. 3

One now will describe figure 1 which represents a perspective view of a variant of performing of the system of agitation. A rubber 2 roller is mounted pivotal around a vertical axis. The rubber roller is put in rotation around the vertical axis by an electric motor (not represented), located inside case 3. The electric motor is supplied by a cable 10. The rotational speed of the roller is controlled by a potentiometer 4.

Tube 1 contains solution 6 of acetylcholine to the concentration of lpM, having to be agitated violently. Operator 5 maintains the end low 7 of the tube applied against roller 2 while supporting on upper part 8 of tube 1. The low end 7 of tube 1 described a circle 9. It results from it that a vortex product within solution 6. This vortex agitates and mixture violently the solution.

As example, one now will describe by referring on figure 2 the test realized starting from a heart of isolated guinea-pig perfusé, known since 1897 (under the name of 1 ' Experiment of Langendorff) and described in the books of traditional pharmacology, especially in The animal experimentation in cardiology - Medicine - Science Inserm - Flammarion, Bernard SWYNGHEDAUW, Chapter 3.1 P. 81 Isolated body: isolated heart according to Langendorff. The collecting one of collecting fraction tubes at a rate of a tube per minute and measurement thus flow of the heart of guinea-pig minute per minute.

Here results coming from the experiments carried out with following substances, after they vortexées (agitated) like it was described while referring on figure 1, in a tube of 15 ml containing 10 ml of solution: - for the first, a mixture of acetate-choline (AC) lpM (sodium acetate 1 pM + choline 1 chloride pM) vortexée pendent 15 seconds, - for the second, of the acetylcholine (ACh) pendent IpM vortexée 15 seconds, - for the third, of the acetylcholine (ACh) 1pM vortexée pendent 5 seconds - for the third, of the acetylcholine (ACh) 1pM vortexée pendent 2 seconds - for the third, of the acetylcholine (ACh) 1pM vortexée pendent 1 seconds

The buffer solution had the following composition: Ca 2+ 2mM, NaHC03 25 mms.

The solvent employed in the five cases was water.

The table hereafter indicates (in ml) the amount of buffer solution recovered in the header tubes in the course of time.

This table puts in evidence several things: a) in the experimental protocol the solutions are tested before being vortexées in order to check that they are inactive. The results of experiment 5 illustrate one of these tests. The solution is else inactive before being vortexée. Indeed variation of flow of 0, 2ml/min (min=3,6; max=3,8) corresponds to uncertainties of measurement and the normal variations of flow of the biological system which is the heart of perfusé isolated guinea-pig. b) the comparing of the results of experiment 1 with the experiment 2 watch which the action to agitate is not sufficient in itself: still it is necessary that molecules to which the biological system is sensitive are present. Indeed a very adjacent product of acetylcholine, acetate-choline, prepared in the same conditions which the acetylcholine, does not start of reaction (Exp 1). c) the series of experiments 2,3, and 4 was carried out on the same heart of guinea-pig and watch the influence of the time of agitation using the Vortex on the activity produced in substance. Thus for a 2 seconds agitation one obtains a maximum variation of flow of 0,3 ml/min (min=3,5; max=3, 8) whereas for a 15 seconds agitation one obtains a maximum variation of flow of 0,6 ml/min (min=2, 8; max=3, 4)

As other examples here results coming from the experiments carried out with like substance of the acetylcholine lpM, vortexée 15 seconds for different contents ethanol in solvent. The experiments were carried out 9 days after the agitation of the solution.

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This table puts in evidence that the content ethanol supports the conservation of the activity in water.

One now will describe by referring on figure 3 the cutaneous test in the guinea-pig

We use a living guinea-pig, to which one injects by venous path a blue dye (blue of Evans) which fixed on blood albumin. The albumin does not leave the vessels, except if there is ignition, therefore vasodilation and permeation of the vessels, the typical example of such a reaction at the man being urticaria.

The test is carried out by injecting under the skin of the animal thus prepared 0.1 ml of the solution of which it is advisable to control the activity. One measurement then the diameter of the blue stains appeared around the injection points. For this purpose one scanne skin, then the file bitmap is recorded. Then one measurement dimension in pixels of the blue stains due to the reaction.

The numbers 3,4, 10,11 which appear in the first column of the table hereafter correspond to the references of figure 3.

<Tb> - The injection number 3 watch that the vortexée solution with very low concentration (1pM) of the neurotransmitter acetylcholine (ACh) starts a substantial cutaneous reaction (1 949.103 pixels) compared to the same not vortexée solution which does not start reaction like the watch the injection number 4 (43 103 pixels).

- The comparing of the injection number 3 (1 949.103 pixels) and of the injection number 11 (1 154.103 pixels) watch that the activity of a solution with very low vortexée concentration is very with fact comparable with that of a not vortexée solution with higher concentration (L, uM, usual concentration in traditional pharmacology) - the injection number 10 is carried out with a vortexée solution picomolaire (lpM) of a product near of acetylcholine but inactive: the mixture acetate/choline (AC). This injection watch that cutaneous reaction of guinea-pig is else specific of the nature of substance in solution bus this solution of acetate/choline vortexée in the same conditions that the present injection number 1 no effect (25 103 pixels).

EP1112748
Method and device for transmitting the biological activity of a carrier material as a signal to another carrier material, and for processing said signal, and product thereby obtained

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WO9113611
PROCESS FOR MAKING HIGHLY DILUTED HOMEOPATHIC COMPOSITIONS OR PREPARATIONS FROM AN INITIAL SOLUTION CONTAINING AN ACTIVE SUBSTANCE

The invention has as an object a new process of preparation of homeopathic compositions starting from an initial solution containing an active substance.

The invention also has as an object an automatic apparatus for the bringing in work of the aforementioned process.

Until this day, the technical ones of manufacture used for the preparation of composition homeopathic take as a starting point those recommended by Hannemann and it act primarily of the triturate, the impregnation and dilution (for example, centesimal or decimal).

The technical one of dilution makes it possible to prepare homeopathic dilutions which are capable to be used such as they are or which are embedded with one excipient neutral for the Galenic shaper which constitutes the finished drug.

With regard to the technical one of manufacture by centesimal dilution, one can proceed in the following way
- one lays out a series of bottles and stoppers washed with water and dried, from of corresponding number with the number of centesimal dilution to obtain;
- one puts in the first bottle a part by weight of basic substance supplemented at 100 parts by weight in volume by means of the suitable carrier;
- 100 times at least are shaken; dilution obtained is the first CH; one takes a part by volume of this first CH and one pours in the second bottle containing 99 parts of the vehicle already;
- 100 times also are shaken; dilution obtained thus is the second CH.

For decimal dilutions, one operates in an identical way, but according to the decimal series.

The step of shake previously evoked constitutes dynamization.

This step of shake is also refer agitating or succussion, and it is of fundamental importance, for the obtaining of active homeopathic compositions.

To date, in an industrial way, the process of preparation of homeopathic drugs rests on the use of two apparatuses: one is a conventional apparatus of dilution and the second an apparatus of agitating. Between each step, the solution to be diluted is extracted from the apparatus of dilution and is bringing in the apparatus of succussion, then again in the apparatus of dilution for the following step.

The bringing in work of this process implies manual manipulating of the tubes, which constitutes a loss of time and a possibility of error.

One of the appearances of the invention is to provide a process of preparation of homeopathic compositions in which the step of succussion is simplified.

One of the appearances of the invention is to provide a process of preparation which can be automated by reducing the losses of time and the sources of error.

One still of the appearances of the invention is to propose a machine wholly automated.

The process of the invention of homeopathic preparation of composition starting from solutions of given dilution, themselves coming from an initial solution containing an active substance, in which the initial solution containing active substance undergoes a series of steps of successive dilution, the first dilution of the initial solution being obtained by taking away of a fraction or whole of the initial solution, and the mixing of this fraction or whole of the initial solution in a solution of dilution, which gives the solution of the first dilution, the second dilution of the initial solution being obtained by taking away of a fraction or whole of the solution of the first dilution, and the mixing of this fraction or whole of the solution of the first dilution in a solution of dilution, to give the solution of the second dilution and so on, until the solution of last dilution, each solution going of the solution of the first dilution to the solution of last dilution, constituting a solution of given dilution, is characterized in what one proceeds to a step of agitating of the solution of given dilution at least after all ten dilutions, preferably after all three dilutions, advantageously after each dilution, agitating being consisted a creating step of bubbles in the solution of given dilution using blowing degaz ata sufficient rate of agitating to create the formation ofa vortex in the solution of given dilution to agitate.

By “vortex”, one indicates a vortex created in an intermediate solution, by gas insufflated under a sufficient pressure.

By simplification, the expression “creation of bubbles” will be indicated by the term “bullage”.

By “homeopathic compositions”, it is necessary to include/understand the compositions obtained starting from solutions of given dilution containing of active substances to very low amounts, even to infinitesimal amounts and compositions in which there is no more active substance.

Preferably, the first dilution is carried out by taking a part of the initial solution, and each given dilution is carried out by taking a part of the solution of previous dilution.

To fix the ideas, the homeopathic compositions coming from solutions of given dilution obtained by the process of the invention are such as active substance is low with 10-10 moles/l, advantageously low with 10-12 moles/l, and preferably low with 10-14 moles/l, or do not contain any more active substance.

In the process of the invention, one simplified the step of agitating by a step of bullage using gas blowing ata sufficient rate of agitating to create the formation of a vortex in the solution to be agitated.

According to a beneficial embodiment of the process of the invention, the bullage is carried out after each dilution.

According to another embodiment of the invention, some of the steps of agitating can be carried out manually for example while placing the container containing the solution to be agitated on an apparatus with agitating by eccentric.

One after can for example the first dilution, to manually agitate the solution of the first dilution like indicated above, then to use the bullage, in accordance with the invention.

The insufflated gas can be consisted nitrogen, advantageously by air.

To create the formation of a vortex in the solution to be agitated via the bullage, the responsible gas of the agitating can be insufflated with a pressure from approximately 5 with approximately 6 bars.

According to an embodiment prefered of the process of the invention, the appropriate rate of agitating is obtained by blowing of air of an active volume of approximately of the third to approximately once the volume of the solution to be agitated, the insufflated volume of air being advantageously a volume from approximately 100 1 to approximately 10 ml, particularly 500A1, under a sufficient pressure, and such as the temperature approximately 45 " C do not exceed.

According to another embodiment of the process of the invention, one carries out, between two successive dilutions, a rinsing step of the apparatus which allows the taking away of a fraction of the whole of the initial solution and each intermediate solution and the mixing of this fraction or whole in a solution of dilution.

The rinsing step between two successive dilutions (respectively indicated by “dilution coming initially” and “dilution coming in second place”) can be carried out between dilution coming initially and the bullage of the solution of corresponding given dilution, or can take place after the bullage, or can be concomitant with each dilution.

In this last case, for this making, and as example relative with the concomitant rinsing with dilution coming in second place, one takes dilution initially coming an amount of this one with the rinsed apparatus (in a concomitant way to dilution coming initially), amount which one introduces into the tube intended for dilution coming in second place, then one takes with the same device approximately the half of the volume necessary to dilution coming in second place, and one rejects it into the tube intended for dilution coming in second place and one takes still approximately the half of the volume necessary to dilution coming in second place and one rejects it into the intended tube with dilution coming in second place, which has for effect to twice rinse the apparatus which has tracking the serving taking away to carry out dilution coming in second place, and so on.

In what follows, the process such as defined cidessus will be indicated by “process comprising the step of bullage”.

The invention relates to an automatic apparatus for the bringing in work of the defined process above, comprising: - means to carry out successive dilutions of an initial solution containing an active substance, which dilutions lead to solutions of given dilution, - means of bullage programmed to carry out the bullage at least after all ten dilutions, advantageously after all three dilutions and preferably after each dilution, - optionally of the means of rinsing between each dilution.

The step of dilution is made up by what was indicated above, but can be carried out in any other way for example according to the method of
Korsakow out of single bottle, or else according to the method of the 50 millésimales or according to the method by continuous fluxion.

The initial solution and the solution of dilution can be alcoholic solutions or of glycerin and are advantageously aqueous solutions.

The process of the invention of preparation of composition homeopathic can also comprise a control of dilution and contamination of solutions of given dilution.

The process of preparation of homeopathic compositions starting from solutions of given dilution coming from an initial solution containing an active substance, which process includes/understands the control of the dilution and the contamination of the aforesaid solution of given dilution, and is such as the initial solution

- sudden of successive dilutions, the first dilution of the initial solution being obtained by taking away of a fraction or whole of the initial solution, and the mixing of this fraction or whole of the initial solution in a

solution of dilution, which gives the solution of the first dilution, the second dilution of the initial solution being obtained by taking away of a fraction or whole of the solution of the first dilution, and the mixing of this fraction or whole of the solution of the first dilution in a solution of dilution, to give the solution of the second dilution and so on, until the solution of last dilution, each solution going of the solution of the first dilution with the solution of last dilution constituting a solution of given dilution, - successive dilutions being such as at least one of the solutions of given dilution contains active substance in amount low with 10-10 moles, advantageously low with 10-12 moles/l and preferably low with 10-14 moles/l, or does not contain any more active substance, the aforementioned solution of given dilution still presenting an activity at great dilutions with that to which the active substance disappeared, is characterised in that - one introduces, before at least any of dilutions N, N being a great integer with 0, in the solution of dilution n-l a soluble pilot substance in the aforementioned solution and not interfering with the solution of dilution n-l, and the pilot substance presenting the property to disappear between dilution N - 1 + m and N + m, m being the number of dilutions where is present the pilot substance, and being included/understood particularly from 5 to 8, - one proportions pilot substance at least once after dilution N, preferably at least once in the interval going from dilution N at dilution N - 1 + m and at least once in the going interval of dilution N + m at dilution N + m + y, y being the range of dilution libr

In this embodiment of the process of the invention, one uses like pilot substance, of a substance which is easily detectable and which is detectable until it disappears. In a beneficial way, one uses a detectable enzyme by his chromogenic activity.

Presence of substance pilot in first the dilutions and its absence in dilutions extreme, (i.e. great dilutions with the quatorzième dilution and particularly with the twenty-third dilution) which is nevertheless active, affirm the relation between the phenomenon observed and the mechanism of high dilutions. Hand of an initial solution containing an active substance and one it is diluted a first time using a solution of dilution, which leads to a solution of the first dilution, which with his revolution is diluted, to give a solution of the second dilution, and so on to give successive dilutions, until the solution of last dilution. Each dilution leads to a solution of given dilution.

The process of the invention is such as it makes it possible to control with each dilution (which gives place to a solution of given dilution), if dilution were correctly made and if there is no contamination. An improper dilution could, for example, to consist of the forgetting of a front dilution or of the introduction of a drop, container for example the active substance with last detected dilution, or the use of a badly rinsed pipette, which with such dilutions risk all to change.

The process is such as it makes it possible to control each dilution and when the pilot substance is still present, to quantify dilution by comparing the calculated theoretical amount of pilot substance and the actually found amount. When there is no more pilot substance, there is no more either of active substance since the pilot substance disappears after the active substance. After a certain number of dilutions one must expect not only the active substance absence, but also the pilot substance absence.

Like indicated above, dilutions will be located by the first dilution, the second dilution, the third dilution, nth dilution or dilution 1, 2, 3, 4,… N. In an usual way, last dilutions are dilutions 30 or 40 (decimal).

These dilutions can be made according to any scale, particularly decimal or centesimal.

In all that precedes and all that follows, the quantified values correspond, except opposite indications, with decimal dilutions.

But, the term “dilution” can apply to centesimal dilutions. Progressively with dilutions, the diluted solution will be such as it contains 10-10 moles/l, then 10-12 moles/l, then a low amount with 10-14 moles/l. Generally audelà of this molar concentration per liter it has there no more molecules in the solution, which does not prevent the aforementioned diluted solution from still presenting, and in a completely unexpected way, an activity.

Into the process of the invention, one can introduce pilot substance before any dilution, i.e. in any solution of given dilution.

By “pilot substance not interfering with the solution of dilution N - 1” one defines a pilot substance such as its presence does not affect any the optional activity of the solution of dilution N - 1.

When one introduced pilot substance with dilution N - 1 and that one carries out the first dilution of the solution of dilution N - 1 container the pilot substance, one continuous then to dilute until one reaches a dilution to which pilot substance disappears. The process of the invention implies at least a proportioning of pilot substance after dilution N. This proportioning preferably takes place at least once in the interval going from dilution N at dilution N - 1 + m, i.e. at least once of the first dilution of pilot substance to the dilution to which the pilot substance disappears.

On the interval going from dilution N at dilution N - 1 + m of pilot substance, one can make in theory a

quantitative proportioning since one after can each dilution of N with N - 1 + m to proportion pilot substance and compare it with the calculated theoretical corresponding value.

When the pilot substance is such as it is not present any more in any solution of given dilution, it is also beneficial to make a proportioning for example on three dilutions which follow that from which the pilot substance disappeared, dilution pendant which one does not add pilot substance.

This makes it possible to confirm that there no was contamination compared to the dilution to which one notes that there is no more pilot substance. In this case, it does not act more than one quantitative control but of a qualitative control permitted by the absence of pilot substance, the pilot subtance behaving then like a negative control.

According to another embodiment prefered of the invention, one at least twice introduces pilot substance, one of the two introductions of pilot substance being carried out into the solution of dilution N - 1, pilot substance disappearing between dilution N - 1 + m and N + m, the other introduction is carried out to more - early into the solution of dilution N + m, and preferably into the solution of dilution N + m + y, N being a great integer with 0, m being advantageously included/understood from 5 to 8, y being advantageously included/understood from 3 to 5.

This process corresponds to the fact that one introduces a first time the pilot substance into the solution of dilution N - 1, one dilutes until the obtaining of a solution of dilution in which the pilot substance disappeared and without adding pilot substance again before this one did not disappear, if not the proportioning carried out on each dilution of the solution of dilution N to the dilution in which the pilot substance disappeared would not have any more a smell.

It is thus waited until the pilot substance disappeared to add some again. One can again add pilot substance to the dilution which immediately follows that for which the pilot substance disappeared, but in a beneficial way, one again adds pilot substance from 2 to 10 dilutions which follow the dilution to which the pilot substance disappeared for the first time, and advantageously starting from the third, fourth or fifth dilution which follows dilution corresponding one to the disappearance of pilot substance.

One can thus repeat the process of introduction of pilot substance all the times that this one disappeared or while waiting for 3 dilutions with 5 dilutions after the dilution from which the pilot substance disappeared.

In practice, according to the process of the invention, one can reintroduce pilot substance has regular interval, and it is enough for example to be able to detect pilot substance on 3 or 4 dilutions, to show that the reduction in pilot substance is regular (thus that dilutions are correct) and that apart from the zones where the pilot substance is detectable, there is no contamination.

According to an embodiment of the process of the invention, one introduces pilot substance for the first time into the solution of dilution N - 1, then one reintroduces pilot substance in the solution of dilution which follows that which corresponds to the disappearance of pilot substance introduced into dilution N - 1, then one reintroduces second once the pilot substance in the solution of dilution which follows that which corresponds to the disappearance of reintroduced pilot substance and so on.

According to another embodiment of the process of the invention, one introduces pilot substance for the first time into the solution of dilution N - 1, pilot substance disappearing between dilution N - 1 + m and N + m, m being included/understood particularly from 5 to 8, one reintroduces pilot substance in the solution of dilution N + m + y, y being included/understood from 2 to 10, advantageously from 3 to 5, and so on.

According to another embodiment one introduces pilot substance for the first time into the solution of dilution N - 1, then every m dilutions, m being included/understood from 5 to 15, advantageously from 10 to 15, and advantageously still 10 or 15.

According to a beneficial embodiment, the process of the invention is such as - the solution initial sudden of successive dilutions, the first dilution of the initial solution being obtained by taking away of a fraction or whole of the initial solution, and the mixing of this fraction or whole of the initial solution in a solution of dilution, which gives the solution of the first dilution, the second dilution of the initial solution being obtained by taking away of a fraction or whole of the solution of the first dilution, and the mixing of this fraction or whole of the solution of the first dilution in a solution of dilution, to give the solution of the second dilution and thus of continuation, until the solution of last dilution, - each solution going of the solution of the first dilution to the solution of last dilution constituting a solution of given dilution, - each solution of given dilution undergoes a vigorous agitating, - successive dilutions being such as at least one of the solutions of given dilution contains active substance in amount low with 10-10 moles, advantageously low with 10-12 moles/l and preferably low with 10-14 moles/l, or does not contain any more active substance, the aforementioned solution of given dilution still presenting an activity at great dilutions with that to which the active substance disappeared, characterised in that:: - one introduces, before at least any of dilutions N, N being a great integer with 0, in the solution of dilution n-l a soluble pilot substance in the aforementioned solution and not interfering with the solution of dilution n-l,

* pilot substance presenting the property to be detectable at great dilutions with that from which the active substance is not detectable any more, and

* the pilot substance presenting the property also to disappear between dilution N - 1 + m and N + m, m being the number of dilutions where is present the pilot substance and being included/understood particularly from 5 to 8, - one proportions pilot substance at least once after dilution N, preferably at least once in the interval going from dilution N at dilution N - 1 + m and at least once in the going interval of dilution N + m at dilution N + m + y, y being the range of free dilution of pilot substance and being advantageously included/understood from 3 to 5, - and of dilution N to dilution N - 1 + m one compares the value of the concentration of pilot substance obtained and the value of the concentration of pilot substance calculated according to dilution, which makes it possible to control dilutions quantitatively, and - dilution N + m with dilution N + m + y, one checks that there is no more pilot substance, which makes it possible to control on the one hand the quality of dilutions and on the other hand the absence of contamination.

The pilot substance is endowed with the property to be detectable to great dilutions with that from which the active substance is not detectable any more, i.e. if it were introduced into the initial solution before the first dilution of the starting solution it disappears with a great dilution with that to which the active substance is not detectable any more.

To fix the ideas, taking into account the available technical means to date, a substance is detectable by the usual biochemical means until an amount of approximately 106 moles/l.

In certain cases, the active substances can be detectable up to 10-12 moles/l. But, this implies very responsive detection systems.

With regard to pilot substance, in a general way, it is detectable until approximately 3x10-10-3x10-11 moles/l, which corresponds to dilution 7 when the initial concentration is approximately 0,1 approximately 10 mg/l, particularly from approximately 1 mg/ml. Amount in moles/l, until which the pilot substance is detectable corresponds to the limit from which one considers that there is no more pilot substance.

Gold, the usable active substances in the process of the invention are detectable with concentrations of approximately lx10-3 with 1x10-6 moles/l, i.e. until approximately the third decimal dilution, for an initial concentration of approximately lx10-3 moles/l.

According to a beneficial embodiment of the process of the invention, the pilot substance is introduced with dilution N - 1, and disappearing between dilution N - 1 + m and N + m, it is reintroduced with dilution N + m + there the interval m + y + 1 being such as, - on approximately one of the two halves of this interval dilutions are such as the corresponding solutions of dilution are not active and - on approximately the other half of this interval, one at least of the corresponding solutions of dilution is active.

Represented on **Figure 1**
- in dotted lines variation of the activity of the solution of dilution according to dilution,
- in strokes full variation of the initial concentration of pilot substance added at the beginning according to dilution, and
- in short dotted lines - long dotted lines, variation of initial concentration of active substance according to the solution of dilution.

To fix the ideas, the initial concentration of pilot substance is approximately 1 mg/ml, and that of active substance is approximately 1 mg/ml.

On this figure 1, given with illustrative and nonrestrictive titre, the pilot substance disappears between dilution 7 and 8, the active substance disappears between dilution 4 and 5, the interval of dilution on which the pilot substance is present is 0 to 8 dilutions. On the half of this interval it be-to saying from 0 to 4 dilutions, one notes that the present solution a certain activity whereas on the other half of the interval, i.e. fourth with eighth dilutions, the present solution more activity. On the interval from 0 to 4 dilutions, the pilot substance is such as there is an activity in the solution of corresponding dilution and on the interval from 4 to 8 dilutions the pilot substance is such as it does not have there only an activity of the solutions of corresponding dilutions.

According to another embodiment, the present pilot substance the property according to which if it is introduced into the initial solution before the first dilution of the starting solution and if the active substance disappears (is not detectable any more) between dilution p and dilution p + 1, the pilot substance disappears between dilution p + X and dilution p + X + 1, X being included/understood particularly from 2 to 4, p being included/understood particularly from 3 to 6.

In the know-indicated definitions, m corresponds to p + X, when the pilot substance is introduced into the initial solution before the first dilution of the starting solution.

According to another embodiment of the invention, the process includes/understands the following steps: - one

introduces pilot substance into the initial solution containing active substance before the first dilution of the aforesaid the initial solution, - one carries out successive dilutions of the initial solution containing active substance and pilot substance, the first dilution of the initial solution being obtained by taking away of a fraction or whole of the initial solution, and the mixing of this fraction or whole of the initial solution in a solution of dilution, which gives the solution of the first dilution, the second dilution of the initial solution being obtained by taking away of a fraction or whole of the solution of the first dilution, and the mixing of this fraction or whole of the solution of the first dilution in a solution of dilution, to give the solution of the second dilution and so on, until the solution of last dilution, the active substance disappears between dilution p and dilution p + 1 and the pilot substance disappears between dilution p + X and dilution p + X + 1, p being included/understood from 3 to 6, X being included/understood from 2 to 4, the solution still presenting an activity for at least a dilution great or equal with dilution p + 1, - after the first dilution, one takes for at least a given dilution, starting from the obtained solution with the exit of the aforesaid dilution an amount sufficient of solution to proportion pilot substance, and preferably one proportions pilot substance with each dilution of dilution 1 with dilution p + X and preferably at least once of dilution p + X + 1 with dilution 1 + p + X + y, y being advantageously included/understood from 2 to 10, advantageously from 3 to 5, and advantageously still with each dilution of dilution p + X + 1 with dilution 1 + p + X + y, - and of dilution 1 with dilution p + X, one compares the value of the concentration of pilot substance obtained and the value of the concentration of pilot substance calculated according to dilution, what makes it possible to control quantitativemen

According to another embodiment one introduces for the first time pilot substance before the first dilution, then one introduces pilot substance for the second time into the solution of dilution which follows that to which the pilot substance introduced for the second time disappears, then one introduces third once the pilot substance into the solution of dilution which follows that which corresponds to the disappearance of introduced pilot substance the second time, and so on.

In a beneficial way, each introduction of pilot substance takes place from 2 to 10, advantageously 3 to 5 dilutions after that which corresponds to the disappearance of pilot substance previously introduced.

According to another embodiment one introduces pilot substance before the first dilution and every m dilutions, m being included/understood from 5 to 15, advantageously from 10 to 15, and advantageously still 10 or 15.

According to another embodiment, one introduces pilot substance for the first time into the initial solution, then all ten dilutions starting from the initial solution.

According to another embodiment one proportions pilot substance advantageously all 10 dilutions, and preferably with each dilution.

According to another embodiment the initial solution contains an active substance at a rate of approximately lx10-3 with approximately lx10-6 moles/l.

According to another embodiment, when the pilot substance is consisted an enzyme, this one is selected among peroxidase, is particularly the horseradish peroxidase, detectable by its reactivity with the substrate D-phenylene diamine in medium H2O2.

The initial solution and the solution of dilution are aqueous solutions, of pure alcohol, or glycerin, and a beneficial way of the aqueous solutions.

The invention also relates to an automatic apparatus for the bringing in work of the defined process above, comprising: - means to carry out successive dilutions of an initial solution containing an active substance, which dilutions lead to solutions of given dilution, - means of bullage programmed to carry out the bullage at least after all ten dilutions, advantageously after all three dilutions and preferably after each dilution, - optionally of the means of rinsing between each dilution, - means to carry out the control of dilution and contamination of the solutions of given dilution obtained repsectivement at the end of successive dilutions.

The solutions of dilution given, obtained in accordance with the defined process above comprising the step of bullage and optionally controlled as for their dilution and with their contamination can be used such as they are like finished drug, or can be used as active solutions, for the preparation of composition homeopathic Galenic solid.

With regard to the solid homeopathic Galenic compositions, one can quote the granules or globules, which are made active by impregnation in a solution of given dilution obtained according to the defined process above comprising the step of bullage and optionally controlled as for its purity and its contamination like indicated above.

EXAMPLE I:

This example is relative with the control of the activation and the inhibition of the achromasy of basophilic human, by using the comprising process the steps of bullage and verification of dilution and the contamination in accordance with the invention.

Accurately, in this example, one checks the effect of high dilutions of an anti-IgE antibody on the basophilic human ones, the solutions of high dilution prepared and being controlled as for their dilution and with their contamination according to the process of the invention.

I- BLOOD TAKING AWAY

I1 is carried out at subjects not presenting any recognized allergy, neither hiv-positive individuals nor hepatitis-positive.

Twenty ml of blood of these donors are collected in two glycerol-coated glass tubes containing each one 250 1 of anticoagulant thus prepared
- To mix (1: 1) two solutions of EDTA-Na2 and EDTA-Na4 (Merck, Darmstadt, FRG) 0,2 M, pH 7,40.

* EDTA-NaCl2 (PM=372,24): 3,7 G dissolved in 50 ml of water distilled heated,
* EDTA-Na4 (PM=452,24): 4,5 G dissolved in 50 ml of water distilled cold.

- A 100 ml of the mixing, one adds heparin without phenol (Choay, Paris, France) to the final concentration of 40 U/ml (c.a.d. 4000 U in 100 EDTA ml of solution).

The tubes containing the anticoagulant (250 pl/tube) prepared with the advance and are preserved at +4 " C.

II PREPARATION OF THE PLUGS

One has two plugs
- a plug of washing, not containing calcium, necessary with the preparation of the cells;
- a plug of dilution, container of calcium, necessary with the preparation of dilutions.

These two plugs are prepared extemporanément starting from the plug of Tyrode following stock 1. Plug of plugged Tyrode with 1 ' HEPES: “Tyrode
HEPES "

The products are dissolved hot by agitating in ultrapure water (obtained after processing by a machine with reverse osmosis and filtration). The pH is adjusted to 7,40 with NaOH 5N and 1N.Le plug then is filtered (filter 2 clam, Costar, Cambridge, the USA) under sterile hood and is preserved at +4 " C during 10 days maximum.

Source of the products
KC1, Nazi, Glucose, EDTA-Na4 are reagents for cellular culture, Sigma Chemical Company, Saint Louis, Missouri, the USA.

HEPES, Seromed S, Biochrom KG, Berlin, GDR.

2. The plug of washing
It is the plug of Tyrode-HEPES brought back to the temperature ambient and adjusted with pH 7,40 extemporanément.

3. The plug of dilution
It is the plug of previous Tyrode, carried at temperature ambient and adjusted extemporanément with pH 7,40 after addition of calcium.

a) Solution stock of CaCl, 220 mms
1,62 G of CaCl2 2H20 in 50 ml of plug of
Tyrode-HEPES with pH 7.40. The conservation takes place at +4 " C.

b) Plug of dilution
The final concentration in dilutions is of llmM: 5 ml of CaCl2 220 mms q.s.p. 100 ml of plug of Tyrode-HEPES. To adjust with pH 7,40 with NaOH 1N or 0, lN.

III PREPARATION OF the RANGES OF DILUTIONS Of ANTI-I9E (TEST), Anti-IgG AND ULTRAPURE WATER DISTILLEE (CONTROLS)
Dilutions of ultrapure distilled water prepared and are not tested systematically for all the experiments.

1. Anti-IgE antisérums and anti-IgG
I1 acts of a antisérum of human goat anti-IgG (specific Fc, GAHu/IgG (Fc)) and of a antisérum of human anti-IgE goat (specific Fc,
GAHu/IgE (Fc))(Nordic Immunology, Tilburg, Tea
Netherlands) whose concentration in antibody is 1 mg/ml.

The antisérums freeze-dried are taken again by 1 aliquot ml of water distilled ultrapure then out of tubes

eppendorf (15 Al/tube) and preserved at -200C.

The concentration in antibody is 1 mg/ml.

2. Dilution of the antisérums and ultrapure distilled water
Dilutions are done under the control of a seeker foreign at the laboratory and responsible of the random processing of results (INSERM U292).

They are carried out under hood with laminar flow on a Programmable controller 222-401E Gilson (Gilson Medical Electronics, France) by using sterile tubes new pulleds up with the fate of 5 ml out of polypropylene (Greiner). The plug of dilution used is plug of Tyrode-HEPES containing of calcium 11 mms and adjusted with pH 7,40.

One dilutes initially ultrapure distilled water, then the anti-IgG, then the antione. A cycle of rinsing is programmed at the beginning and fine of each range of dilutions. Those comprise 29 tubes going from dilution 1 X 102 with dilution 1 X 1030 of distilled water ultrapure or the solution of antisérum anti-IgG or anti-IgE starting.

As enzymatic tracer allowing to judge good practice of dilutions, one adds peroxidase (Sigma) at the same time as water distilled or the antisérum anti-IgG or anti-IgE. The peroxidase solution prepared to 1 mg/ml, aliquotée out of tubes eppendorf (15 Al/tube) and was preserved at -20 C.

Performing of a range of dilutions
The 29 tubes of the range, voids and supporting the corresponding number with their dilution labeled with the felt, are placed on portoir it automatic apparatus
Gilson.

In the first tube, corresponding one with dilution 1 X 102, 10 1 of distilled water or anti-IgG or the antione (1 mg/ml) and 10 peroxidase p1 (1 mg/ml) are added to 980 p1 plug of Tyrode containing of calcium 11 mms (plug of dilution). The tube is stopped and agitated pendent 30 dry on a Vortex.

Dilution 1 X 102 made manually is replaced on portoir it and the automatic programme of dilution is then committed. After a cycle of rinsing with the plug of dilution, a syringe of 500 1 (piston stainless) takes 100 pl dilution 1 X 102 and aspires 400 it plug of dilution. The whole is rejected into the following, corresponding tube with dilution 1 X 103. Five hundred lil of plug of dilution are still aspired and rejected into the tube of dilution 1 X 103, which ensures the rinsing of the syringe. The agitating of dilution is ensured by a suction-delivery of 500 Tl of air, 5 times of continuation, with maximum of rate of delivery.

Does the needle take then 100 p1 dilution 1 X 103, aspires 400? L then 500 pl of plug of dilution according to the same process that previously to obtain dilution 1 X 104 and so on de.

With dilution 1 X 1030, the apparatus stops automatically and engages a cycle of rinsing. A new range can then be bringing in work.

Scheme of the process of dilution automatic of the anti-IgE antisérum
This scheme makes the object of figure 2, on which one represented the automatic process of dilution in which the steps of agitating mentioned by “bullage” are made in accordance with the process of the invention and in which the step of agitating which follows the first dilution is made manually on an apparatus with agitating by eccentric.

3. Coding of the tubes of dilutions of distilled water and antisérums
During an experiment “activation”, one tests
- ponderal dilutions 1 X 102 to 1 X 104 the antiones and control anti-IgG and/or distilled water;
- high dilutions 1 X 1021 to 1 X 1030 (audelà of the limited number of molecules calculated thanks to the number of Avogadro) the antione and control anti-IgG and/or distilled water. Any part of the range of dilution beyond the given limit by the number of Avogadro can be used;
- inner witnesses controlling the sensitivity with calcium of basophilic and corresponding one to the plugs of Tyrode without calcium and Tyrode with calcium.

a) Performing of the code
In this protocol “activation”, all the tubes are tested into blind.

The foreign seeker at the laboratory and controlling the performing of the experiments allots to each tube, following a table of randomization
- a number ranging between 1 and 30 when the experiment compares the effectiveness of dilutions the antione and anti-IgG;
- a number ranging between 1 and 43 when the experiment compares the effectiveness of dilutions of distilled water, anti-IgG and anti-IgE.

For this making, the numbers corresponding one with dilutions and labeled with the felt on the tubes are unobtrusive with alcohol and of the labels supporting a code number are stuck on the tubes.

Example: in the case of a code ranging between 1 and 30, the coded tubes will be
- tubes of dilutions lx102 to 1x104 the anti one
IgG (3 tubes) and of anti-IgE (3 tubes);
- tubes of dilutions 1 X 1021 to 1 X 1030 of anti-IgG (10 tubes) and anti-IgE (10 tubes);
- inner tubes of controls: 1) plug of dilution, Tyrode with calcium (2 tubes); 2) plug of washing, Tyrode without calcium (2 tubes).

b) Diluting of the coded range from 1 to 30 or 1 to 43
Of each coded tube, one takes 200 iil (Pipetman 200) which are deposited in tubes of agregameter of 1 ml. The tubes are stopped and carried by the person having made the code for a proportioning of optional, subsequent and independent control, immunoglobulines being able to be contained in dilutions (proportioning by polyacrylamide gel electrophoresis) or any other appropriate control (mass spectrometry etc…).

IV CELL PREPARATION
Before proceeding to the obtaining of an enriched suspension into basophilic starting from the blood collected on the anticoagulant heparin-EDTA (paragraph I), one determines the number of basophilic present per mm3 of total blood of the subject.

1. Coloring and counting of basophilic on total blood
The basophilic ones are counted thanks to their property of metachromatic coloring with the blue one of toluidine.

a) Solution of coloring: the blue one of toluidine
Hundred mg of blue of toluidine (Toluidine Blue, HEREINAFTER N0 52040 C15 H16 CIN3 S, PM=305,84, Fluka, Mulhouse,
France) are dissolved in 100 ml of ethanol 25% and adjusted with pH 3,20-3,40 with 80-100 p1 of glacial acetic acid. The solution is preserved at ambient temperature out of hermetically closed bottle and at the shelter of the light.

b) Coloring
It is carried out by mixing 90 1 of colorant and 10 p1 of total blood in the well with round bottom of a plate of microtitration (Costar). The mixing is immediately and gently agitated by 5 to 6 suctions and deliveries using the pipette (Pipetman 200) having been used to deposit the colorant.

c) Counting of the basophilic ones
Five to 10 minutes after the mixing of the blood and the colorant, this one again gently is agitated and immediately deposited in a chamber of Fuchs
Rosenthal (3,2 mm3) using a pipette (Pipetman 200).

The blade is deposited in moist atmosphere (in one limps closed and humidified) to avoid its drying. After 3 to 5 minutes corresponding one at time necessary so that the suspension coloured deposited in the chamber of the hemocytometer, one carries out counting on a Olympus microscope with the enlargement
G X 10 X 20.
The basophilic ones are the single cells having a coloured cytoplasm. They appear red and are very easily identified on a pale bottom. In case of doubt, it is necessary to modify the focus in order to else distinguish the cytoplasms coloured into pink-red from basophilic from those of the other cells which remain transparent. The cores of the other leucocytes are slightly coloured into blue.

Generally, on total blood, one counts on average 7 to 15 basophilic per chamber of Fuchs
Rosenthal, their number which can go from 2-3 to 30-35.

2. Obtaining of an enriched suspension into basophilic
When 10 the 1 necessary ones with the counting of basophilic on total blood are taken, of the Dextran T500 4, % (Pharmacia, Uppsala, Sweden) is added at a rate of a volume for five blood volumes. The tubes are inclined and progressively sedimentation (20 min approximately with 1 X G at ambient temperature), one collects the rich plasma in leucocytes as well as red globules in suspension. The presence of these last reinforce the coloring of basophilic by the blue one of toluidine (viewing empirically made at the laboratory during the experimental tests).

The sedimentation is collected in 2 plastic tubes of 10 ml to which one adds plug of washing (Tyrode-HEPES without calcium, pH 7,40). After centrifuging (150 X G, 10 min), the bases of leucocytes (+ red globules) are joined together in only one tube, suspended in 10 ml of plug of washing and again centrifuged (150 X G, 10 min). The rich plasma in leucocytes is initially separate in 2 tubes in order to better wash the cells.

The base is finally included in aliquot of the same plug, between 400 and 900 p1 approximately, according to the number of wells to be deposited (10 p1 suspension X time the number of wells + 40 to 60 1 additional for

the losses on the walls of the tube).

One represented on figure 3 the scheme of the cell preparation.

V- PROTOCOL OF THE ACTIVATION OF BASOPHILIC HUMAN BY the ANTI-IGE (ANTI-IGG OR WATER DISTILLEE As Control)
After the preparation of dilutions of antisérums anti-IgG and anti-IgE (and, for about fifteen experiments, dilutions of distilled water) and their coding, after obtaining of the enriched suspension into basophilic, one proceeds to the test itself, under hood with laminar flow. The process is identical in the case of the code with 30 tubes and the code with 43 tubes.

1. Protocol
- Ten iil of plug of washing (Tyrode without calcium) are deposited at the bottom of 30 (or 43) wells at round bottom of a plate of sterile microtitration (Costar), by avoiding the peripheral wells where the risks of contamination and evaporation are larger,
- twenty 1 of each coded dilution (code from 1 to 30 or 1 to 43) is then deposited at the bottom of these same wells,
- the plate is preincubated 5 min at 37 " C, under Scotch tape and lid to avoid the evaporation of the contents of the wells,
- ten iil of enriched suspension are then added,
- the plate is then gently agitated by slow rotary motions in order to homogenize the contents of each well; it is substantial that this agitating is mild in order to avoid any contamination from one well to another,
- the plate is then incubated 15 min at 37 C, under adhesive tape and lid to avoid any evaporation,
- after incubation, 90 1 of blue of toluidine are added to each well and immediately agitated by 5 to 6 suctions and deliveries with a pipette multichannel. One changes cones between each row of well,
- after coloring, the plate is ribbon adhésifée and preserved one night at +4 " C before carrying out the reading. On cells, washed, the coloring is more homogeneous after several hours.

2. Scheme of plate

10 L plug of washing (Tyrode without Ca++)
+ 20 p1 coded dilutions (1 to 30)
PREINCUBATION 5 min at 37 " C
+ 10 y1 suspension rich into basophilic (+
red globules)
INCUBATION 15 min at 37 " C
+ 90 toluidine p1 blue
CONSERVATION one night at +4 " C then READING
3. Reading with the optical microscope. Counting of the basophilic ones
The basophilic ones are counted the following day the experimentation consequently person who has prepared the cells the sleep. The technical one is identical with that of the counting of basophilic in total blood (IV-l-c paragraph).

When the number of basophilic is substantial (great to 100 on a whole chamber of Fuchs
Rosenthal), it is possible not to read (for all the wells) only one half-chamber. So more than 150 basophilic appear on a half-chamber of Fuchs-Rosenthal, it is preferable to deposit the contents of the wells in the chamber of a hemocytometer of Malassez (1 mm3).

Three to five minutes are necessary to count the contents into basophilic of a chamber of hemocytometer. A trained experimenter can prepare 3 to 4 hemocytometers at the same time with the proviso of storing those in one limps humidified in order to avoid their drying. In case of doubt about an account, it is possible to redeposit the contents of a well in a chamber of Fuchs-Rosenthal to proceed on a new account. Be born it is recommended not to renew this operation more twice for a same well because it seems that the taking away repeated with agitating can damage the sample and involve erratic results then.

The numbers of basophilic are deferred in a table to the image of the scheme of the plate.

4. Control quality of dilutions of antisérums anti-IgG and anti-IgE: Proportioning of peroxidase
The peroxidase is proportioned day-same experimentation, independently, by the second person taking hand with the protocol and not having made the experiment this day. The purpose of I1 is controlling the process of dilution and detecting an optional contamination of high dilutions by ponderal concentrations of antisérum, contamination which would be then responsible biological activity observed with high dilution.

It is a proportioning by spectrocolorimetry with 490 Nm based on the reactivity of peroxidase with the substrate O-Phenylene-Diamine in medium H2 2
a) Preparation of the plugs and solutions
- Plug citrate pH 5,0
It is a mixing of 20,5 ml of a solution 0,1M of citric acid (PM=210,1) and 29,5 ml of a solution 0,1M of sodium citrate (PM=294,1).

- Solution d10-Phenylene-Diamine (OPD)
Eight mg of OPD (Sigma) are dissolved extemporanément in 10 ml of plug citrate pH 5,0.

The solution is preserved at the shelter of the light under aluminium sheet.

b) Proportioning
In the wells of a plate of flat-bottomed microtitration 96 wells (Costar), one deposit successively
- 50 1 of each coded dilution (from 1 to 30 or 1 to 43) and of uncoded dilutions (1 X 105 to 1 X 1020) of the ranges of distilled water, anti-IgG and anti-IgE (Pipetman 200).

- 50 1 of OPD with 8 mg/10 ml (pipette saddle jib crane eppendorf).

- 10 H2 iil 2 30 flight. (pipette saddle jib crane eppendorf).

One observes a coloring yellow-orange of the corresponding well to the most concentrated dilutions.

To let the reaction be made pendent 10 minutes with the shelter of the light, under aluminium.

To add 50 A1 (pipette saddle jib crane eppendorf) of H2 S04 9% (H2 concentrated S04, diluted 10 times, 3% final in the well). The coloring previously observed is accentuated (coloring ochre-orange).

To see immediately to 490 Nm with a spectrophotometer-reader of automatic plate (Dynatech Laboratories). The results are automatically recorded and printed.

Scheme of plate
Example in the case of a code from 1 to 30

5) Results “activation”
The results (numbers of basophilic + proportioning peroxidase) are given each day to the person having made the codes.

The tubes corresponding one with each experiment are locked up in an envelope sealed and dated and preserved at +4 " C, with fine of subsequent controls.

a) Interpretation of the results
It will be made after an independent statistical analysis, on the experiments selected according to following criteria's
1. Number of basophilic in the witnesses great with 35. The witnesses correspond to dilutions of distilled water, anti-IgG and with the inner witnesses (plug of Tyrode with and without Ca++).

2. Anti-IgE activity with ponderal amount great to 40% of achromasy compared to respective ponderal dilutions of distilled water or anti-IgG.

(The anti-IgG with ponderal amount can theoretically involve a achromasy of basophilic compared to same dilutions of distilled water or the witnesses “Tyrode with calcium”. One can call upon a recognizing of the slight chains of IgE by the anti-IgG or a anaphylactic reaction IgGdépendante).

3. In the presence of single calcium, i.e. without addition the antione, the number of basophilic should not vary of more than 25%. This is checked by comparing the number of basophilic put in the presence of Tyrode-calcium plug with that of basophilic put in the presence of plug of Tyrode without calcium.

The achromasy is thus given
Nb basos of the pilot well - Nb basos of the well test00
Nb basos of the pilot well basos = basophilic
For each experiment selected according to criteria's
1) calculating of the difference between the average of the number of basophilic counted in the wells containing the anti-IgE solution and the average of the number of basophilic counted in the wells containing the pilot solution (distilled water or anti-IgG), for all the dilutions ranging between 1 X 1021 and 1 X 1030.

2) Research, also, for high dilutions the antione, presence of at least a peak of achromasy from 3 to 4 significant successive points according to given abacus (paragraph VI-2).

b) Representation of the results
It is made after opening of the codes, at the end of 18 interpretable experiments of activation (c.a.d. answering the criteria of selection).

The number of experiments necessary was given after statistical analysis of the supplied results by preliminary experiments.

The results are represented in the following way
Dilutions (anti-IgE, anti-IgG or distilled water) logarithmic are spans in abscissae while the number of basophilic is carried in ordinates.

Each graphic corresponds to an experiment. I1 comprises
1) a curve which represents the variations of the number of basophilic according to dilutions the antione;
2) one (or two) curve (S) control (S) appearing the variations of the number of basophilic according to dilutions of distilled water and/or anti-IgG.

According to the average number of basophilic obtained for pilot dilutions of distilled water and for the witness "Tyrode with calcium", one defines a limit of significativity corresponding one in the number below which the effect of 1 ' anti-IgE (or the anti-IgG) will be regarded as significant. This limit generally corresponds to approximately 20% of achromasy. It is given by an abacus (cf. figure 4) and is represented in dotted line on the graphic ones.

The lower the number of basophilic is by comparing with the average of the witnesses, the more the effect observed is significant.

One represented on figure 4 the abacus to determine the significativity of the achromasy of basophilic human.

The abacus indicates the significativity ($p < 0,05$) of the achromasy observed for the basophilic ones. Example when 70 basophilic is counted in the well controls, 56 basophilic, at most, must be counted in the well test so that the achromasy is significant.

VI EXPERIMENTAL PROTOCOL OF THE MODULATING OF
The ACHROMASIE OF BASOPHILIC BY APIS MELLIFICA
This protocol is practised either at the same time as the protocol "activation" when the number of basophilic in the total blood of the donor is sufficient (great to 15 on a chamber of Fuchs-Rosenthal), or independently of the protocol "activation", on the blood of another donor.

1) Principle
The modulator effect of dilutions of Apis mellifica of 15 with 20CH (Centesimal Hahnemannienne) is tested comparatively with corresponding control, NaCl 137 mms 20CH on the achromasy of basophilic in the presence of ponderal dilutions the antione. The effect Apis mellifica and of NaCl 137 mms is tested, as control, on the basophilic ones put in the presence of the single plug of dilution of anti-IgE, without anti-IgE.

2) Dilutions of Apis mellifica and NaCl 137 mms
They are supplied by the Boiron laboratories
L.H.F. (Lyon, France) out of sterile ampoules of îml, in NaCl 137 mms. The contents of the ampoules are transvased in new sterile tubes of 5 ml out of polypropylene, under hood with laminar flow. The tubes are progressively stopped and agitated pendent 30 seconds on a Vortex.

Identical ampoules are addressed to an outer laboratory in order to control the quality of the products by mass spectrometry.

3) Coding of dilutions of Apis mellifica and of
NaCl 137 mms
In this protocol, we studied the modulator effect of dilutions 15 with 20CH d'Apis mellifica comparatively with a control, dilution 2OCH of
NaCl 137 mms.

All these dilutions are tested into blind. An arbitrary code number ranging between 1 and 8 is allotted randomly to each dilution (6 dilutions 15 with 20CH d'Apis mellifica and 2 dilutions 20CH of NaCl 137 mms) by the foreign seeker at the laboratory which controls the process and is responsible random interpretation of the results.

For this making, a label supporting a code number is stuck on each tube containing corresponding dilution. The code is changed with each new experiment, of new labels supporting a new number replacing the previous ones.

The coded tubes are preserved of one experiment at the other at +4 " C under pendent aluminium sheet 2 weeks. After this time, a new procedure (transfer of the ampoules in the tubes and coding) is carried out and this, pendant all the duration of the experimental protocol.

4) Ponderal dilutions the antione
Dilutions (1 X 102 to 1 X 104) are prepared manually out of plug of dilution (plug of Tyrode
HEPES + final Ca++ 11 mms, pH 7,40), under hood with laminar flow, out of sterile tubes of polypropylene 5ml, starting from antisérum of anti-IgE goat human (1 mg/ml of antibody) aliquot out of tubes eppendorf and preserved at -20 C.

Ten p1 the antione (1 mg/ml) are added to 990 1 of plug of dilution. The tube is stopped and agitated pendent 30 seconds on a Vortex: dilution 1 X 102 is obtained.

Hundred 1 is taken and added by it to 900 p1 of plug of dilution contained in a second tube.

This one is stopped and agitated with its pendent revolution 30 seconds on the Vortex. One obtains dilution 1 thus X 103 and one proceeds in the same way for dilution 1 X 104
5) Protocol itself
Initially were thus prepared
- dilutions d1Apis mellifica,
- dilutions the antione,
- the suspense cellular ion enriched into basophilic (paragraph IV).

The test
- Ten 1 of each of 8 coded dilutions are deposited at the bottom of the wells at round bottom of a plate of sterile microtitration (Costar). Each dilution is as many deposited once as of amounts the antione to inhibit and once for the plug of dilution. In this protocol, coded dilutions of Apis mellifica and NaCl 137 mms are thus deposited 4 times (anti-IgE 1 X 102, 1 X 103, 1 X 104; plug of dilution).

- Ten Ctl of suspension rich into basophilic are then deposited in each well.

- The plate is delicately agitated by very mild rotation in order to homogenize the contents of the wells and is left 30 minutes at ambient temperature, under adhesive tape and lid in order to avoid any evaporation.

- After this time of preincubation of basophilic with products 20 p1 the antiones with dilutions 1x102 lx103, lux104 and 20 it of plug of Tyrode-HEPES containing of calcium 11 mms (and without anti-IgE) are added to the wells for each coded dilution of Apis mellifica and NaCl 137 mms.

- The plate is gently agitated to homogenize the contents of the wells and is placed 15 minutes at 37 " C under adhesive tape and lid in order to avoid the evaporation in the wells.

- After incubation, 90 Cl of blue of toluidine are added to each well and immediately agitated by suctions and deliveries, using a pipette multichannel. One changes the cones between each row of well.

- After coloring, the plate is covered with an adhesive tape and is preserved one night at +4iC before carrying out the reading. The coloring of the washed cells is more homogeneous after several hours.
Scheme of plate
The witnesses “Tyrode without Ca++” (*) are added, as well as in the protocol “activation”, to control the sensitivity of basophilic with calcium.

6) Reading with the microscope and account of the basophilic ones:
The principle identical with that is described for the activation of basophilic (V-3 paragraph).

7) Results
The accounts the basophilic ones are deferred in a table to the image of the scheme of the plate and are given for each experiment to the person having made the code.

a) Interpretation of the results
The experiments, after decoding, are retained for random interpretation only if they answer 3 criteria
1. Number of basophilic in the witnesses great with 35 (basophilic preincubated with of NaC1 137 mms or Apis mellifica and not having been put in the presence of anti-IgE).

2. Spontaneous sensitivity of basophilic with single calcium low with 25% of achromasy by comparing on the one hand the basophilic ones which, preincubated with NaCl 137 mms, are, in the absence of very anti IgE, put in the presence of Tyrode-calcium plug with, in addition, those put in the presence of plug of Tyrode without calcium.

3. Presence of at least an amount the antione presenting a achromasy ranging between 40 and 60% of basophilic which, preincubated with NaCl 137 mms, are put in the presence of anti-IgE 1x102 at 1x104 compared to those put in the presence of plug of Tyrode-Ca++ without anti-IgE. This is based on preliminary studies which showed that below 40%, the achromasy of basophilic is too low so that the study of inhibition can be carried out. Above 60%, it is too strong to be able significantly to be modulated by agonists with high dilution.

The achromasy of basophilic is thus given:
Nb basos of the pilot well - Nb basos of the well tests100
Nb basos of the pilot well basos = basophilic
b) Representation of the results
Three types of results (of number of basophilic) are obtained and must be compared
- the corresponding wells with basophilic put in the presence of Apis mellifica or of NaCl 137 mms but without

anti-IgE give the maximum number of basophilic. They are the witnesses of reference.

- the corresponding wells with basophilic put in the presence of NaCl 137 mms and of anti-IgE without Apis mellifica give the minimum number of basophilic (maximum achromasy).

- the corresponding wells with basophilic put in the presence of Apis mellifica and of anti-IgE give a number of basophilic on which the optional modulator effect of the product is evaluated. The more this number will approach the maximum number of basophilic, the more the inhibiting effect will be large. Conversely, more this number will approach the minimum number of basophilic, more the inhibiting effect will be low or null. If it becomes low with this minimum number, the effect is activator.

The graphic representation
For each amount the antiones tested, dilutions of Apis mellifica and dilutions controls of NaCl 137 mms are spans in abscissae while the number of basophilic is carried in ordinates.

Each graphic comprises
1) a curve which represents the variations of the number of basophilic in the presence of anti-IgE according to dilutions of Apis mellifica and of NaCl 137 mms;
2) a curve which represents the variations of the number of basophilic in the presence of Tyrode-Caw plug without anti-IgE according to dilutions of Apis mellifica and of NaCl 137 mms.

c) Statistical analysis of the results
The modulator effect of dilutions of APis mellifica is studied statistically by a test of rank of Whitney-Wilcoxon at the end of a series of about fifteen independent experiments. One will compare, for each dilution of APis mellifica, the number of basophilic put in the presence of an amount of anti-IgE with the number of basophilic preincubated with NaCl 137 mms and put in the presence of the same amount of anti-IgE.

These studies are carried out independently by the responsible persons of the control of the experiments and the random interpretation of the results.

WO9114181
PROCESS FOR MONITORING THE DILUTION AND CONTAMINATION OF HIGHLY DILUTE SOLUTIONS

WO8702981
PHARMACEUTICAL COMPOSITION CONTAINING HISPIDULINE OR A DERIVATIVE THEREOF AND UTILIZATION OF SUCH COMPOUNDS IN THE PREPARATION OF ANTIASTHMATIC COMPOSITIONS

IT1063845
PROCEDE ET COMPOSITION METACHROMATIQUE POUR LA NUMERATION DES LEUCOCYTES, PLUS PARTICULIEREMENT DES BASOPHILES

NL7607012
WERKWIJZE VOOR HET ZICHTBAAR MAKEN VAN BASOFIE- LEN, IN HET BIJZONDER VOOR HET TELLEN DAARVAN, ALSMEDE VOOR DE DIAGNOSE VAN ANAFYLACTISCHE GE- VOELIGHEID, EN REAGENS OM HET TELLEN VAN BASOFIE- LEN MOGELIJK TE MAKEN ALSMEDE FARMACEUTISCH SYS- TEEM VAN HET "KIT"-TYPE