Chapter 15. “The explanation is very simple”

In contrast with other famous scientific controversies of the history of the sciences, the “Benveniste affair”, as we have already pointed out, did not succeed in going beyond the stage of a polemic. It was indeed not a controversy because, as J. Maddox insisted, there were no results! The director of *Nature* expressed this idea very explicitly in the last paragraph of conclusion of the text, which intended to close the “debate” in the columns of his journal:

> “So what is the truth about INSERM 200’s claim on behalf of high-dilution anti-IgE? One correspondent chided us with having impeded the discovery of the true explanation. My own conviction is that it remains to be shown that there is a phenomenon to be explained.” [1]

Therefore, there was no reason to look for an artifact since there was no fact for the simple motive that *it could not exist*. We have seen that the investigation report of *Nature* tried to demonstrate the non-existence of an effect of high dilution and results were assimilated to simple statistical fluctuations of the background noise. It is however pleasant to notice that after this report, *Nature* published the letters of readers for numerous weeks explaining what was the artefact responsible for the observed effect!

Thus let us examine the various suggestions of artefacts which were then proposed. Most suggestions came from the considerable correspondence which was sent to the journal during ten weeks after the publication of the investigation report.[2] The reader will notice that some of the proposed alternative explanations were often more unlikely and more fanciful than the hypothesis of a “memory of water”. These proposals had another feature: they were always expressed in a supposing manner “if we suppose that… then it is possible that in fact…” However each author of these proposals did not go farther than this “thought experiment” and never performed – except one – an experiment to try to confirm the hypothesis. .

The cork of molecules

This hypothesis was proposed by J. Ninio, researcher to the CNRS, who during summer 1988 tried to popularize it with the editorial staffs of various Parisian newspapers. According to this researcher, the molecules of anti-IgE, from a certain dilution, stayed at the surface of water and were thus transferred from one tube to the next one. The consequence was that there was no real dilution,

but a transfer of anti-IgE from tube to tube. To express his thought, he used the following analogy:

> "Uncork an old wine bottle. Pour a little, by a funnel, into another bottle. Complete by a liter of water. You will have actually diluted the wine… But not the dust of cork which was on the surface, and thus almost entirely transferred from a bottle to the other one." [3]

An analogy remains however an analogy and by which experiments was this scientist able to demonstrate that the molecules of anti-IgE really behaved like this? None. He proposed nevertheless a test to assess his hypothesis:

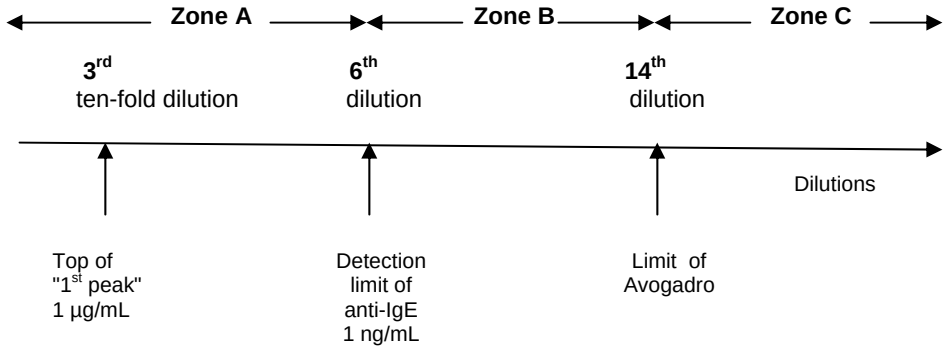
> "Let's take again the example of the wine bottle and pour two cups: the fragments of cork are found in the first one, which thus has not the same content as the second one. In the experiments of Mr Benveniste, the dilutions are made by manual pipetting and I suppose that only the first pipetting is used. Nowhere it is mentioned what occurs if the first pipetting is systematically discarded and that the following ones are used." [4]

To put it more simply, the idea of J. Ninio is that anti-IgE is transported from one tube to the next one and that the supposed concentration decrease is not respected (in particular when the concentration of anti-IgE is low). The consequence is the contamination of all tubes of the series of dilutions. The tubes which are supposed to contain only "organized water" would be in fact contaminated unknown to the experimenter by molecules of plain anti-IgE.

If we follow this reasoning, how can one explain that the heights of the peaks of the degranulating activity were similar on the entire series of dilutions? Indeed, one would expect that anti-IgE ran out gradually from tube to tube, which was not the case. Moreover, the analogy with the wine bottle seemed to suggest that the molecules of anti-IgE were transported all together (the "dust of cork" passes from a tube to the other one). In this case, all molecules of anti-IgE shall be found in the last dilution. Yet an activity was also present in the previous tubes.

But, one could object that it is possible the traces of anti-IgE were actually transported throughout the series of dilution and that these traces were sufficient to cause degranulation. We can indeed never exclude a tiny contamination.

This is quite certain, but detecting traces of anti-IgE is not enough and anti-IgE must be at a *sufficient concentration* in the presence of the cells. Here is a figure where various marks have been placed on a series of anti-IgE dilutions:



The marks on this scale allow defining 3 zones:

- Zone A is the "classic" zone (up to the 6th dilution). It is the zone that corresponds to the "first peak". Whatever the method of dilution (with or without shaking), the biological effect is the same and the dilutions of the antibody are in keeping with the successive ten-fold decreases.

- Zone B is an intermediate zone (from the 6th to the 14th dilution) where there are still anti-IgE molecules, but where they cannot be detected and where anti-IgE does not have any activity anymore (except when the dilutions are shaken).

- Zone C is the zone where there are no anti-IgE molecules.

The examination of this scale allows answering the argument about possible traces of "active" anti-IgE. Indeed, if we observe a peak of degranulation of approximately 30-40%, then – if it is indeed anti-IgE which is responsible for this activity – the presence of anti-IgE molecules should be detected after dosage. The threshold of detection of this last one is indeed of the order of 1 ng/mL. At this concentration (corresponding to the 6th ten-fold dilution of the initial antiserum), there is typically no degranulating activity anymore.

Consequently, we can reply that it is possible that the explanation of the problem of the claimed "high dilutions" is in the zone B. In this zone, the molecules of anti-IgE are present, but in undetectable small quantities. The fact of shaking them would make them much more effective for a reason which remains to discover. Therefore, we can conclude – according to this logic – that it is quite possible that traces of antibody due to a tiny contamination,

undetectable by classic methods, would be nevertheless active! One does not need to hypothesize any "memory" to explain these results.

Well, it would be indeed the end of the "memory of water" with the advent of a very big discovery! It would mean that one can transform traces of antibody into "superantibody" having the same properties as monoclonal antibodies that the pharmaceutical industry produces at high cost. It would be indeed sufficient to dilute antibodies up to traces and to shake them violently between each dilution step. And if this hypothesis applied to other molecules, pharmaceutical industry would be destabilized!

Let's dream and imagine that this explanation is the correct one. In that case, *exit* the "memory of water". We should recognize nevertheless that the initial observations of J. Benveniste deserved to be brought to the attention of the scientific community. Thanks to this debate, an important discovery would have been made. Leaving Europe to draw a new road to India and discovering America is frequent in the history of sciences and does not deserve discredit. On the contrary. But this process is possible only if "error is decriminalized" and if one does not ostracize the one who observed a fact but was not able to interpret it correctly.

To my knowledge, no patent was filed and no industrial application was developed based on this idea, rich in applications if it was true. There were however arguments – quoted in the article of *Nature* of June 1988 – that were against the hypothesis of the "efficient undetectable traces": heating at 70°C, action of ultrasounds and freezing-defrosting destroyed the effects of the high dilutions; in contrast, the active high dilutions were not modified after passage through a molecular filter that blocked plain anti-IgE but not water molecules. Taken together, these results thus suggested that the observed effects did not possess the properties that molecules – even as trace contaminants – should have possessed.

The molecules which stick on the tube (and unstick…)

At the end of 1991, Pierre-Gilles de Gennes had just just received Nobel Prize in Physics. J. Benveniste – whose a family member was in the professional circle of the physicist – asked advice through a letter. In a very brief answer, P.-G. de Gennes suggested a possible artefact for high dilutions in these terms:

> "I nevertheless wonder if the adsorption of proteins at the wall water/glass does not upset the nominal concentrations (note also that this adsorption is often reversible at high dilutions)." [5]

P.-G. of Gennes decided however to interrupt these brief exchanges in spite of several relaunchings of J. Benveniste who would like to benefit from

knowledge of the Nobel prize laureate on "soft matter", the specialty of this scientist.

The idea that molecules of anti-IgE could adhere to the walls of the tube – and consequently to falsify the ratios of dilutions – was also reported by physicists questioned by the journalist M. de Pracontal. The latter suggested the possibility for the anti-IgE molecules to adhere to the walls of the tube: "[…] from the fifth or sixth dilution, an important fraction of molecules can remain adsorbed on the walls of the tube or on the surface of the liquid." [6]

This "explanation" of the possible artefact by an adsorption on the wall of tubes does not seem very logical. Indeed if molecules stick on walls, then the diminution of the concentrations should be more rapid than expected. Consequently we should achieve the limit of Avogadro more quickly.

Paradoxically, this explanation brings rather arguments in favour of an absence of molecules in high dilutions because the test tubes which serve to make the dilutions would contribute to eliminate the contaminant plain anti-IgE. Let us remind indeed that the test tube (with the possible anti-IgE antibodies stuck on its intern walls) were not in contact with cells. The tube is simply discarded after a fraction of its contents has been taken with a pipette.

Memory of heparin

Was it a hoax? Even if the author of this correspondence to *Nature* did not express his proposal of artefact under the form of a "memory of heparin", the reading of his explanations gave a bizarre feeling. Indeed, J. Leslie Glick of the *Bionix Corporation* company in the USA noted that the physiological medium used in the article of *Nature* contains heparin. He explained that heparin molecules stick on numerous molecular structures and form aggregates that are stabilized by water and ionic environment:

> "I propose that anti-IgE antibody (or any of the other immunological stimuli noted in the paper, that were responsible for basophil dégranulation) might have acted as a template for heparin, thereby inducing a specific conformation of the heparin molecule. […] Upon dilution with heparin-containing Tyrode's solution, the stabilized heparin conformation, although lacking biological activity, would itself serve as a template, effecting a new heparin conformation which would mimic the three-dimensional structure of the antigen-binding site of anti-IgE antibody (or other immunological stimulus". [7]

According to this hypothesis, heparin would be a kind of "photocopy machine" for biological molecules. Once again the pharmaceutical industry

might be afraid for its future. Nevertheless no patent or publication tried to exploit this admirable "discovery". Did its author really believe in it?

If one makes nevertheless the bet that this proposal of artefact was seriously given out, one could answer that the experiments with high dilutions had been performed with other physiological mediums that did not contain heparin without changing the results.

The masked agent

For M.J. Escribano of the CNRS, a "very simple explanation" could exist for the reported phenomenon [8]. One could simply suppose a molecule with degranulating properties that would be fixed to one of the components of the physiological medium, for example to albumin. Agitation would release this molecule and degranulating activity would be thus observed and wrongly attributed to high dilutions of antibody.

The answer to this argument is much simpler than the "very simple explanation": there is no effect noticed with the control solution that was shaken in the same conditions.

The masked agent (bis)

This explanation was a more sophisticated version of the previous one with a "masked agent" that, in this case, would be present in the wall of the tube. Indeed, here is what A. Danchin of the Institute Pasteur proposed:

> "Since it is well known that antibodies strongly (and often specifically) interact with surfaces, it is possible that they extract some ion (or contaminant molecule), which in turn acts as a trigger for further extraction (in the absence of antibody). This would account for the requirement of strong agitation." [9]

We cannot reproach the supporter of this possible artifact not to be generous with *ad hoc* hypotheses. First, it is necessary to suppose, on one hand, that anti-IgE antibody is capable of extracting "something" from the wall of the tube, but, on the other hand, that anti-IgG is not capable of doing the same (what *per se* would be particularly interesting), that this "something" would have degranulating properties (direct or indirect) and that it would be capable of auto-extracting of the wall. One must also add – and it is the last condition – that it is necessary that agitation alone could not extract this "something" if the latter (or anti-IgE) was not already present in the solution.

The explanation being thus completely reinforced on all sides, the only possible answer is that experiments with high dilutions were performed with various types of materials (tubes in polypropylene, polyethylene, glass) and with

various molecules (anti-IgE antiserums, antigens, deganulating peptides, ionophores, histamine, phospholipase A2, etc.) We could certainly imagine a specific hypothesis for each of these various biological substances and materials by copying the above reasoning. But is it still science?

The contaminating sprays

Here also a contamination is proposed by I. Lasters and M. Bardiaux [10] of the company *Plant Gentic Systems* in Brussels. But the contamination would take place not at the time of the realization of the dilutions but when the high dilutions are put in contact with the cells. This contamination would occur step by step, from a well to the other one.

The best answer is to put emphasis on the blind experiments where "active" and "inactive" wells were both present on the same plate of cell culture.

The fragments of antibody

For R.M. Schilling, the results of the experiments with high dilutions could "be easily explained" [11]. Shaking would be responsible for the formation of fragments of antibody. To summarize the thought of this reader, one believes to use molecules of anti-IgE and in fact there are fragments – some keeping degranulating properties – which are transported through the dilution process.

However, even for fragments, the limit of Avogadro applies and the serial dilutions finally exhaust the supposed stock of fragments.

The free radicals

K.S. Suslick of the University of Illinois suggested that shaking of liquid locally creates bubbles of cavitation and high temperatures that induce chemical reactions with the following consequences:

> "We suggest that the degranulation observed by Benveniste and coworkers is an artefact of cell damage caused by reactions with small amounts of OH°, H°, H_2O_2, HO_2, etc., produced by their use of vortex turbulence." [12]

The simplest answer once gain is that controls prepared in the same manner do not induce degranulation.

Autoantibodies anti-IgE

The English scientist F. Shakib [13] pointed out that a source of anti-IgE antibody is not taken into account: anti-IgE autoantibodies, which are present in variable quantities according to the individuals. These antibodies fixed to the IgE on the basophils could be responsible for a "spontaneous" degranulation.

Here again, if this hypothesis would be correct, one should observe this phenomenon also with controls.

The oxidation of toluidine blue

The only hypothesis for which the author made the effort not only to perform an experiment, but also to publish his hypothesis of artefact was due to Jean Jacques, chemist, scientist at CNRS. We will talk in detail on this article published in 1990 in Chapter 19, because this scientist by writing this article helped J. Benveniste in a very involuntary manner. One will see how was refused to J. Benveniste on this occasion something which could have been considered as the start of a constructive controversy.

Notes of end of chapter

---

[1] J. Maddox. Waves caused by extreme dilution. *Nature*, October 27th, 1998, p. 760.

[2] *Nature* of July 28th, August 4th, 18th and 25th, September 8th, 15th, 22nd and 29th septembre, October 13th and 20th, 1988.

[3] M. de Pracontal. Les mystères de la mémoire de l'eau, p. 93.

[4] *Ibid.*, p. 97.

[5] Letter of P.G. de Gennes to J. Benveniste of October 31th, 1991.

[6] M. de Pracontal. Les mystères de la mémoire de l'eau, p. 96.

[7] J. Leslie Glick. *Nature*, August 4th, 1988, p. 376.

[8] M.J. Escribano *Nature*, August 4th, 1988, p. 376.

[9] A. Danchin. *Nature*, July 28th, 1988, p. 286.

[10] I. Lasters et M. Bardiaux. *Nature*, July 28th, 1988, p. 285.

[11] R.M. Schilling. *Nature*, October 13th, 1988, p. 584.

[12] K.S. Suslick. *Nature*, August 4th, 1988, p. 375.

[13] F. Shakib. *Nature*, October 20th, p. 664.