Chapter 6. Reproduction at Clamart… of the Israeli experiments

The bailiff, the dean and the basophils

In spite of the success of the Israeli experiments – which, we must remenber, had not been initially scheduled – the question of the “contamination” could cause damage to the credibility of the whole research program on high dilutions. Consequently, in order to escape to the trap of useless controversies, J. Benveniste took again the initiative by organizing what he proposed to M. Shinitzky, namely the repetion of an identical experiment intended to remove the doubt on the electrophoresis. This situation was rather paradoxical. The Israeli experiments were intended to reproduce those of Clamart; now, it was necessary to reproduce them in Clamart!

J. Benveniste was all the more determined not to stay on what could be interpreted as a failure that he had just received a letter of *Nature*. It was the answer concerning the manuscript, which had been sent on March 9th with documents describing the results obtained in Israel:

> “The Editor and I […] are not persuaded in favour of publication. We have decided to seek more external advice before making any decision.”[1]

J. Benveniste answered then to *Nature* that new experiments were going to consolidate the article:

> “I must say that I understand your reservation in accepting the results presented in our manuscript. However, I am afraid that more external advice will not solve this problem, since it is more a matter of personal belief and there is in fact no way for a reviewer to check the reality of the phenomenon. You have seen that these experiments were perfectly reproduced in Israel. However, they failed to properly control the lack of any contaminating compound in the diluted solutions.”[2]

It may be noted in passing how J. Benveniste diplomatically “manages” the issue of the “contamination”: the experiments were not designed to control a possible contamination.[3] Then he could move on to the description of the protocol intended to verify – now in blind conditions – that the solutions with high dilutions were not possibly contaminated with anti-IgE immunoglobulins.

He ended: "We do not expect these experiments before a month or so. Therefore, we are not in such a hurry to get a final decision". Thus, he skillfully returned the situation and he now imposed his timetable to *Nature* for the final decision.

The detailed protocols of the experiments were sent at the same moment to Z. Bentwich, M. Shinitzky, B. Robinzon, M. Oberbaum and J. Amara. Tubes would be coded at Clamart by Professor J. Dormont, dean of the Medicine Faculty and by Maître Simart, bailiff in Clamart.

The samples of the experiment were coded on April 22nd, 1987. The experiment consisted in testing blind the contents of 12 tubes: 4 control tubes, 2 tubes containing low dilutions of anti-IgE (1/100 and 1/1000) and 6 tubes containing high dilutions of anti-IgE (from $1/10^{32}$ to $1/10^{37}$). The results after unblinding (June 11th) are described in Figure 6.1.

But – the same causes leading generally to the same effects – the presence of albumin made difficult the interpretation of the electrophoresis![4] This was therefore the same situation when the experiment was performed in Israel. The photograph of the electrophoresis could not be published because it was not "clean" and thus did not achieve the objective of the experiment: to show that there was no anti-IgE in the tubes where nevertheless a degranulating "activity" was present. Nevertheless the dosage of anti-IgE performed in a laboratory at Marseilles was convincing. Anti-IgE was detected in the dilutions 1/100 and 1/1000 but not in the high dilutions.

A new blind experiment was thus performed on May 12th, but now without adding albumin in cell medium. The experiment included less experimental data because it was especially intended "to make an image". Only Maître Simart and J. Benveniste performed the double blinding. Electrophoresis was not overloaded by albumin and its results fitted the measure of anti-IgE antibodies. The results for basophil degranulation are presented in Table 6.1.

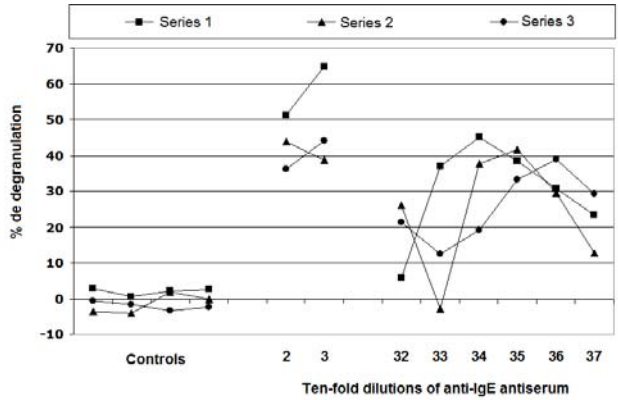

Figure 6.1. Blind experiment of April 22nd, 1987. Twelve tubes were tested in 3 sessions (series 1, 2 and 3) on basophil degranulation after a double blinding. Every series is assessed on the cells of different blood donors.
Experimental protocol: after dilution of anti-IgE up to 10^{-37} by E. Davenas, 8 tubes containing dilutions of anti-IgE (1/100, 1/1000, from $1/10^{32}$ to $1/10^{37}$) were coded together with 4 control tubes containing the medium of dilution alone. The first code was given by J. Benveniste and Maître Simart, bailiff, and the second code by Maître Simart and J. Dormont. Having put aside a part of the content for the test of basophil degranulation, the rest of each of 12 tubes was divided into 4 parts and then freeze-dried. Maître Simart sent a series of 12 samples chosen at random and sent them to two laboratories in Israel (M. Shinitzky and B. Robinzon) to perform an electrophoresis and a laboratory in Marseilles specialized in the production and the marketing of antibody to measure directly the presence of anti-IgE antibody. The scientist who performed this dosage preferred that no mention was given ("neither written, nor oral") of the name of his laboratory.
These results were published in the article of *Nature* of June 30th, 1988, which is reproduced Chapter 8 Figure 8.3.

	Donneur 1		*Donneur 2*	
Open-label	Number of basophils	% of degranulation	Number of basophils	% of degranulation
Control	54 ; 49	-	96 ; 102	-
Anti-IgE 1/100	9 ; 6	85%	53 ; 51	47%
Code	Number of basophils	% of degranulation	Number of basophils	% of degranulation
a	55 ; 51	– 3%	97 ; 99	1%
c	49 ; 53	1%	105 ; 98	1%
e	33 ; 30	39%	64 ; 65	35%
h	8 ; 12	81%	55 ; 50	47%
m	12 ; 15	74%	46 ; 48	53%
p	134 ; 135	33%	70 ; 68	30%

Table 6.1. Blind experiment of May 12th, 1987.

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This experiment is a repetition of the experiment of April 22nd because the presence of albumin overloaded electrophoresis and made them difficult to analyze. Samples received codes by a bailiff and then by J. Benveniste. They were then tested with cells from two blood donors.
Two samples were inactive (*a* and *c*), two were active (*e* and *p*) and two were very active (*h* and *m*).
This experiment was also a success after decoding, because *a* and *c* were controls, *e* and *p* were anti-IgE at high dilutions $1/10^{36}$ and $1/10^{35}$ whereas *a* and *m* were anti-IgE at "classic" dilutions, respectively at 1/100 and at 1/1000.
These results were published in Table 3 of the article of *Nature* of June 30th, 1988; this table is reproduced Chapter 8 Figure 8.3 and the corresponding electrophoresis made at Clamart is reproduced Chapter 8 Figure 8.4.

Before unblinding, the results seemed clear cut again. If results should be predicted, one would say that *a* and *c* were controls, *h* and *m* were "classical" dilutions of anti-IgE and *e* and *p* were high dilutions of anti-IgE. Before even the unblinding of the experiments, J. Benveniste wrote to P. Newmark:

> "You will receive within a week a detailed report on the blind experiments that were conducted in cooperation with the Israel laboratories. The code is not yet broken but they appear quite successful." [5]

By doing so, J. Benveniste took an important risk: to be contradicted after unblinding; but transparency was a guarantee for credibility.

"I can understand the reservation of such a prestigious journal as Nature to publish these findings"

The unblinding of the experiments (those of April 22nd and May 12th) by the bailiff on June 11th in the presence of J. Dormont and J. Benveniste were again a total success.[6] The tubes *e* and *p* were anti-IgE at $1/10^{36}$ and $1/10^{35}$, respectively. The three tests (electrophoresis, dosage of anti-IgE, test of degranulation) were positive when anti-IgE at "classical" dilution was present. In contrast, only the test of basophil degranulation detected anti-IgE at high dilutions. The effect observed with high dilutions was thus not simply due to a contamination by anti-IgE.

On the same day, J. Benveniste wrote a long letter to P. Newmark with tables of results and copy of the electrophoresis reporting the experiments of April 22nd and May 12th. Large extracts of this letter deserve to be reproduced because they enlighten the future developments of the story. At first, J. Benveniste reconstituted the history of the facts that led to these experiments:

> "You must remember the letter from Israel that the involved scientists cosigned attesting the good results of the experiments. The only missing information was to eliminate the possibility of the antibody present at normal concentration in the active highly diluted tubes. About a month after the experiment, a report came from Dr. Shinitsky's laboratory that several bands were identified in the latter that could be immunoglobulins. However, a second group of scientists in Israël (Dr. Boaz Robinzon) affirmed that these could by no means be immunoglobulins. It was clear that we were bothered by the polymerised HAS [= *albumine*] present in all solutions. Moreover, by contrast with the rest of the experiment, the electrophoresis were not performed blind and no attempt was done to measure anti-IgE activity. We therefore decided to launch to blind experiments." [7]

This passage of the letter to P. Newmark was then followed by the description of the experimental protocol of the experiments of April 22nd and May 12th. Then J. Benveniste got at the root of the affair:

> "We feel that the main requirement expressed in your letter of 24 November 1986 that these results be confirmed in another laboratory has been adequately fulfilled. […] I must say that being myself bewildered by these findings, I can understand the reservation of such as prestigious journal as Nature to publish them. […] Thus, I would like to propose you to print the article preceeded by a word of warning, or an editorial, where you express all the reservations that the editorial board can have towards such a heretic result. It could also be stated that I and the associated scientists have done all possible efforts to detect an error in methodology or interpretation, going well beyond what is usually done in similar experiments but that nevertheless we are fully ready to accept the challenge of any colleague that could detect some hidden flaw in them.
>
> Thank you for your quick reply concerning your position on this difficult but fascinating problem."

The response of P. Newmark brought along a lot of information. He reported the written answer of an expert, the oral answer of another expert and he made a proposal:

> "Thank you for your letter of 12 June, the content of which I have not fully absorbed yet. In the meantime, I think you should see the enclosed comment from a new referee of your paper;
>
> These arrived before your latest letter but I had not sent them on to you because I had been hoping to receive written comments from another referee who, by telephone, had expressed concern at some "large unexplained differences between the data obtained in France and in Israel".
>
> The comments that are enclosed reinforced our own view at the time that you had not provided us with evidence of a truely independent confirmation of your data.
>
> We will need to consider carefully the new information in your 12 June letter and the suggestion that we publish your paper with an editorial. Could you, meanwhile, let us know both your reaction to the enclosed comments whether you could, in theory, incorporate any of the new information into your manuscript." [8]

In his comment of about twenty lines, the (American) expert – who did not know the results of the last experiments, but only the experiments performed by E. Davenas at Rehovot – insisted on the necessity of reproducing the experiments because, as he wrote:

> " […] The "independent" verification in Israel doesn't count. The material dated 3/6/87 is literally unintelligible as presented. The numbers are undefined, the protocol is presented in only a fragmentary manner and the experiment was analysed by one of the original team. That's not what I would call an independent verification."

And if the experiments were to be reproduced, he considered that it would anyway be necessary to perform a large number of experiments before publishing:

> "If the findings could really be reproduced, then there are a very large number of controls that need doing. To give a couple as an example: If the anti-IgE is effective at doses of less than 1 molecule per specimen, then its action would hardly be expected to exhibit species specificity; indeed plain old goat IgG should work just as well. "

This was an interesting point raised here. The expert basically said that if one admitted that the effect was real and was due to high dilutions of anti-IgE, he did not admit that this effect could possibly be specific. In other words, according to him this is not a key that opened only one lock which was

generated during the dilution process, but rather a master key. We know now that this expert who examined the article of J. Benveniste was H. Metzger, eminent member of the NIH (*National Institute of Health*). We will see him appear on several occasions in this text.

Notes of end of chapter

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[1] Letter of P. Newmark to J. Benveniste of April 6th, 1987.

[2] Letter of J. Benveniste to P. Newmark of April 17th, 1987.

[3] The 5 experiment made in Israel by E. Davenas were nevertheless described in the article of *Nature* of June 30th, 1988 (Table 1 of the article for the 4 first ones and in the text for the 5th; see Chapter 8).

[4] It is surprising *a posteriori* that the experiment did not exclude albumin straightaway, as if the lesson of the experiment of March 2nd made in Rehovot had not been learned. An explanation could be that the idea that there was indeed a contamination was admitted by J. Benveniste and E. Davenas.

[5] Letter of J. Benveniste to P. Newmark of June 3rd, 1987.

[6] Certified report of the bailiff Me Simart of June 11th, 1987.

[7] Letter of J. Benveniste to P. Newmark of June 12th, 1987.

[8] Letter of P. Newmark to J. Benveniste (no date) as an answer to the letter of the latter of June12th, 1987.