

Chapter 23. “Benveniste-like experiments without Benveniste”?

J. Benveniste liked to say that “nine pregnancies of one month do not make a baby”. The publication of the results of the study impulsed by the laboratories Boiron and coordinated by M. Roberfroid was – whatever the reasons – long to deliver!

The announcement in 1994

One finds a first allusion to this study by one of the participants, F. Wiegant. The latter, in August 1994, sent a letter to *Nature* concerning the article of Hirst *et al* evoked previously. This letter is interesting because the sequence of the ideas explicitly unveiled the strategy to strictly keep away from J. Benveniste and his results.

First step: F. Wiegant announced that he agreed with the conclusion of Hirst *et al* and he indicated that his group of research published the same negative results two years ago.¹ This team had then noticed differences in the counts of basophils of two experimenters. He added that this fact could explain the absence of positive results with high dilutions.

Second step: F. Wiegant then specified that one of the signatories of the article of *Nature* of 1988, J. Sainte-Laudy, modified the initial method of basophil degranulation and used at present alcian blue “which allows rapid and clear-cut basophil counts without time-consuming training.”

Third step: F. Wiegant indicated that with this modified method, J. Sainte-Laudy reproduced the results published before, namely the inhibitory effect of histamine at high dilutions on basophil degranulation.

Fourth step: F. Wiegant announced that blind experiments using this model were in progress in five laboratories in United States, Ireland, Italy, France and the Netherlands. These experiments were coordinated by Marcel Roberfroid of the University of Leuven in Belgium.

He ended then: “the last word has not yet been spoken”. Let us remind that one was then in 1994. But, it will be necessary to wait until 2004 to see these results published in the journal *Inflammation Research!*²

The results unveiled in 2004

The signatories of the article³ of 2004 were from four laboratories (the US laboratory had disappeared). There were first of all the former two signatories of the article of *Nature* of 1988, P. Belon, scientific director of Boiron, and

J. Sainte-Laudy (CERBA, France) as well as Fred Wiegant (University of Utrecht, Netherlands) and two other researchers who had not previously been involved in this research: Madeleine Ennis (Queen University of Belfast, United Kingdom) and Pier Francesco Mannaioni (University of Florence, Italy). The study was coordinated by M. Roberfroid, professor of biochemistry who also blinded the dilutions to be tested. The results were analyzed by Jean Cumps, statistician (Catholic University of Leuven). The laboratories having performed the degranulation tests were French (laboratory 1), Dutch (laboratory 2), English (laboratory 3) and Italian (laboratory 4).

High dilutions of histamine were tested at 10^{-30} , 10^{-32} , 10^{-34} , 10^{-36} , 10^{-38} mol/L (there are of course "theoretical" concentrations). The effect was assessed on three concentrations of anti-IgE corresponding to the first peak (1; 0.2 and 0.04 $\mu\text{g}/\text{mL}$). Overall – and it was a remarkable result – the statistical analysis showed that the percentages of degranulation were smaller for the samples that contained histamine at high dilutions. Figure 23.1 is a summary of all results. One notices that, taken as a whole, the percentages of inhibition were observed more frequently than by chance with positive values. The statistical tests indicated a very high degree of significance ($p < 0.0001$).

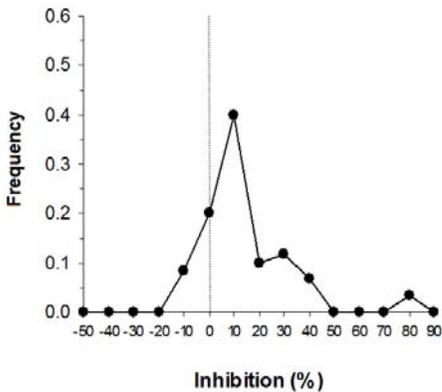
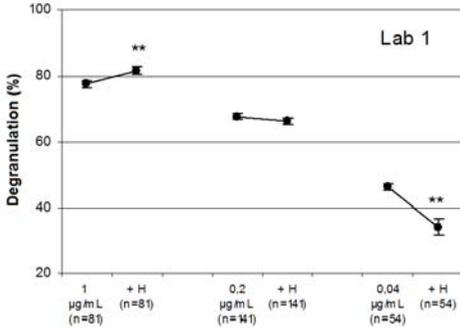


Figure 23.1. This figure shows the overall results of inhibition by histamine at high dilutions in the European study. The figure has been performed using the means reported in the article for each experimental condition (an experimental condition being, for example, inhibition by histamine at 10^{-30} mol/L with antiserum anti-IgE at 0.2 $\mu\text{g}/\text{mL}$ for the laboratory 1). One notices that the percentages of degranulation are "moved" towards the right of the point 0% of the x -axis. If there was no inhibition (null hypothesis), the distribution curve should be centered on 0%. (Each point of the x -axis corresponds to the upper limit of the interval).

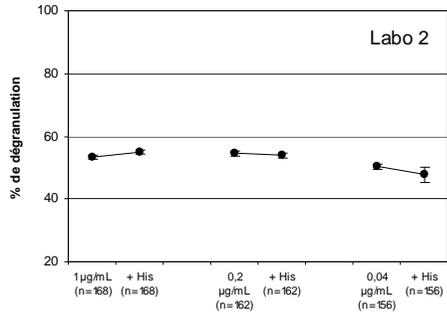
The results obtained in each laboratory are represented on Figure 23.2. One notices that the results are considerably different according to laboratories. First of all, there was no significant effect of high dilutions of histamine for the laboratory 2. This was the laboratory of F. Wiegant whose group of research had already published on the same subject and did not observe any effect of high dilutions on basophils (in the article of Ovelgonne *et al* of 1992; cf. note 2). Nevertheless, it was this laboratory that supplied the higher number of experiments. Only laboratories 1 and 4 observed an effect with the highest dose

of anti-IgE (1 µg/mL). But strangely degranulation was increased in the presence of high dilutions for laboratory 1 whereas it decreased for laboratory 4. Finally, laboratory 4 distinguished itself compared to the three others for its “efficiency”: an inhibitory effect with high dilutions of histamine was observed with all concentrations of anti-IgE.

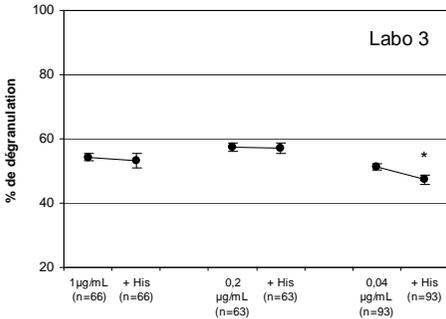
Laboratory 1 (Jean Sainte-Laudy, France)



Laboratory 2 (F. Wiegant, The Netherlands)



Laboratory 3 (M. Ennis, United Kingdom)



Laboratory 4 (P. Mannaioni, Italy)

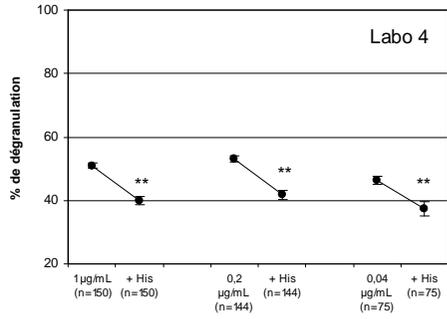


Figure 23.2. The analysis of the European study with four laboratories concluded that overall high dilutions of histamine significantly inhibited degranulation ($p < 0.0001$).

The results shown here were obtained from the data of the article published in 2004 in *Inflammation Research*, for each laboratory. The effect of high dilutions of histamine (+H) on degranulation induced by anti-IgE at various concentrations (0.04; 0.2 and 1 µg/mL) is compared with the effect of anti-IgE alone at the same concentrations (+His). It must be noted that this representation does not allow highlighting – and risks even to mask – the classic “waves”. We observe nevertheless significant differences (* $p < 0.005$. ** $p < 0.0001$) for some results, more frequently for the lowest concentration of anti-IgE (0.04 µg/mL). That is why we summarized in Figure 23.3 the results with this concentration of anti-IgE in the presence of all dilutions (from 10^{-30} to $1/10^{-38}$ mol/L).

The results are given as mean \pm standard error of the mean.

Three laboratories among the four obtained significant effects with the lowest dose of anti-IgE (0.04 µg/mL). Since the inhibitory effect was the most marked with this concentration of anti-IgE, it is interesting to study the results according to each dilution of histamine as shown in Figure 23.3.

When one looks at Figure 23.3, one can notice that the results of the European study – in "inhibition" on the first peak and with the alcian blue method – were poorly reproducible between the four laboratories. Furthermore, the differences between "active" samples and controls were small and the peaks of activity had faded. We remember that P. Belon had depreciated the results of the article in *Nature* of 1988, in particular because "the peaks of activity were not stable". The reproducibility was also not reliable according to him. The method with alcian blue and the experiments "in inhibition" were supposed to produce better results. Even if globally a significant effect persisted for this study, one notices here again that the blind procedure abolished the differences between the various high dilutions (i.e. there was no "dose-response").

Even J. Sainte-Laudy did not reproduce, in these blind experiments, the spectacular results that he previously reported. Furthermore, in contradiction with the results of the other laboratories and with his own previous results, with the highest concentration of anti-IgE (1 µg/mL), he observed an *increase* of basophil degranulation.

Yet, the new technique with alcian blue was supposed to allow counting basophils with a better reproducibility and the biological system "in inhibition" was considered as fully tried and tested. Great expectancies were therefore put on this protocol. On arrival, it was – as for the article of the *Comptes Rendus* of J. Benveniste and A. Spira – the same perplexity: one undoubtedly obtained an overall significant effect on the statistical plan, but the "message" was blurred if one considered the results in details, for each dilution. Here again, the "measurement instruments" had very different performances.

Questioned in 2001 about the results of this study which had been reported at a congress, J. Benveniste – who abandoned at that time basophils several years before to dedicate himself to what he named "digital biology" (cf. second part) – declared: "They've arrived at precisely where we started 12 years ago!"⁴

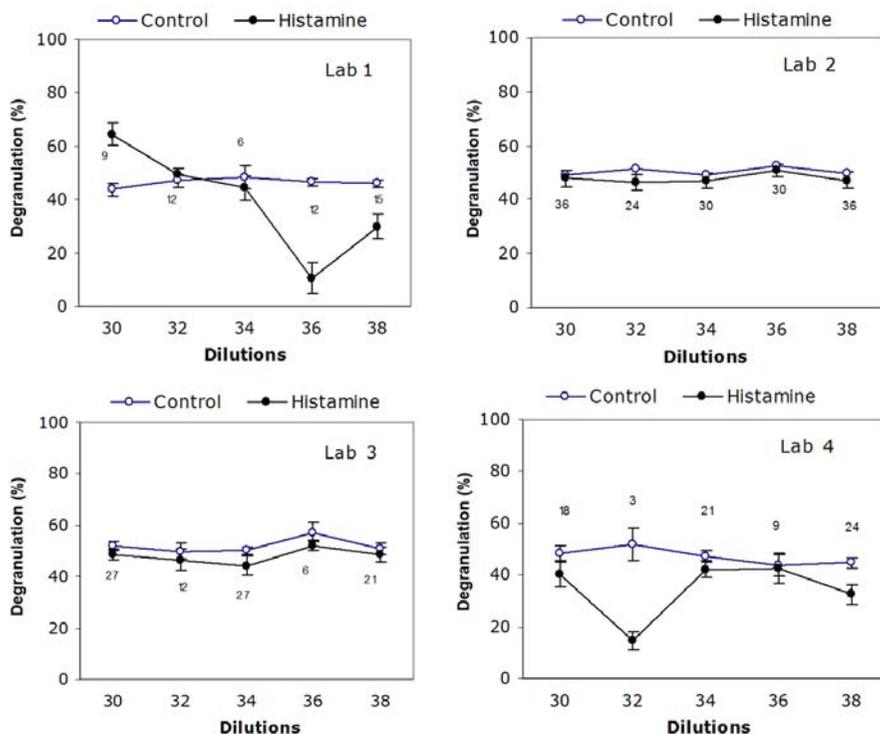


Figure 23.3. . This figure corresponds to the results of Figure 23.2 for anti-IgE 0.04 µg/mL. Indeed it was at this concentration of anti-IgE that most significant results were observed in the European study. Degranulation induced by anti-IgE 0.04 µg/mL is presented in the presence and in the absence (control) of histamine (from 10⁻³⁰ to 10⁻³⁸ mol/L). The results are thus very different according to laboratories and even if a significant global effect is found, one does not observe “waves” of inhibition. Even within each laboratory, one could not conclude if a given dilution of histamine was an “active” one. This contrasts with the previous results reported by J. Sainte-Laudy and P. Belon.

The numbers under each of the symbols are the number of experimental points (for every point, there are as many points of histamine at high dilutions as samples of corresponding controls).

On x-axis, dilution “30” corresponds to 10⁻³⁰ mol/L.

“The scourge of homeopathy”

This experiment with several European laboratories nevertheless allowed converting a “non-believer”, namely M. Ennis of Belfast who managed one of the four participating laboratories. Here is how Mr. Ennis was described in *New Scientist*:

“Madeleine Ennis, a pharmacologist at Queen's University, Belfast, was the scourge of homeopathy. She railed against its claims that a chemical remedy could be diluted to the point where a sample was unlikely to contain a single molecule of anything but water, and yet still have a healing effect. Until, that is, she set out to prove once and for all that homeopathy was bunkum. In her most recent paper, Ennis describes how her team looked at the effects of ultra-dilute solutions of histamine on human white blood cells involved in inflammation. [...] The study, replicated in four different labs, found that homeopathic solutions – so dilute that they probably didn't contain a single histamine molecule – worked just like histamine. Ennis might not be happy with the homeopaths' claims, but she admits that an effect cannot be ruled out.”⁵

One knows that recent converts are often proselytes! They do not hesitate to tell the conditions of their “conversion” in terms such as “I did not want to believe it but the results were there”. M. Ennis reported her evolution in the following manner:

“I was incredibly surprised and really had great feelings of disbelief, but I know how the experiments were performed and I couldn't see an error in what we had done.”⁶

At another occasion, she declared:

“Despite my reservations against the science of homoeopathy [...] the results compel me to suspend my disbelief and to start searching for a rational explanation for our findings.”⁷

In order to explore these results in more detail, M. Ennis set up in her laboratory a method which avoided counting basophils manually. To put it simply, this method was based on the measurement of a molecule from basophil granules which is “transported” on cell surface during the degranulation process. Specific fluorescent antibodies recognize this molecule and therefore degranulation could be quantified. This method had already been used by J. Sainte-Laudy⁸ and M. Ennis applied his experimental protocol. In 2001, M. Ennis published, in the form of a communication at a congress, preliminary results using this method; the results were in favour of an effect of high dilutions of histamine.⁹

The results of M. Ennis generated some publicity in United Kingdom because during a television program of the BBC2, an attempt of replication of these – preliminary – results was presented. The initiative did not come from

M. Ennis, but from the producer of the scientific series *Horizons*. The purpose was to win the million dollar prize offered by the foundation chaired by J. Randi (still him). This prize was intended for anyone who can prove the reality of a “paranormal” effect (high dilutions being apparently filed under this denomination...) A scientific team was constituted (unrelated to M. Ennis and her laboratory) and the emission was broadcasted on November 26th, 2002. The result was considered as a failure and the million dollars stayed in the bank account of J. Randi...

Not long after a debate developed because the protocol which had been followed by the scientists in charge of the study was apparently not the one that M. Ennis used.¹⁰ Moreover, none of the scientists “recruited” for the occasion had a particular skill concerning this research area.

We will not comment beyond these “studies” for which we only have indirect information because the results of the experiment and the protocol were of course not published. Once again we can notice here a recurrent and now familiar situation: auto-appointed experts, atmosphere of circus where science is done on stage with the media as witnesses. Finally, it is the confusion of the ideas that prevails and the truth – if there is a truth – cannot find its way.

Notes of end of chapter

¹ This article was the following: Ovelgonne JH, Bol AW, Hop WC, van Wijk R. Mechanical agitation of very dilute antiserum against IgE has no effect on basophil staining properties. *Experientia* 1992; 48:504–8 (Department of Molecular Cell Biology, State University of Utrecht, The Netherlands).

In fact, in this article, Ovelgonne *et al* compared two series of anti-IgE at high dilutions (from $1/10^{21}$ to $1/10^{30}$) in 24 experiments. One of the series of anti-IgE was shaken and the other one was obtained “by pipetting very gently and tilting the test tubes 10 times to mix the contents after diluting”. Unfortunately, the authors did not perform a series of controls (without anti-IgE). The method of “soft” dilution was thus not controlled and it was consequently difficult to know to which extent a certain “activity” of anti-IgE was not present in these high dilutions. We saw in the article of Hirst *et al* that the dilutions performed without shaking had an activity which was intermediate between that of “true” controls (i.e. shaken control samples) and “true” high dilutions of anti-IgE (i.e. shaken dilutions of anti-IgE).

² There was a congress abstract in 1991 in *Inflammation Research* 48 (Suppl 1): S17-8. The article of 2004 was submitted to *Inflammation Research* in December 2002 and accepted in November 2003.

³ Belon P, Cumps J, Ennis M, Mannaioni PF, Roberfroid M, Sainte-Laudy J, Wiegant FA. Histamine dilutions modulate basophil activation. *Inflammation Research* 2004; 53:181–8.

⁴ L. Milgrom. Thanks to the memory. *Guardian*, 15 mars 2001.

⁵ Michael Brooks. 13 things that do not make sense. *New Scientist* n°2491, March 19th, 2005.

⁶ Interview of M. Ennis during the TV program *Horizons* of BBC2 of November 26th, 2002.

⁷ L. Milgrom. Thanks to the memory. *Guardian*, 15 mars 2001.

⁸ Sainte-Laudy J, Belon P. Analysis of immunosuppressive activity of serial dilutions of histamine on human basophil activation by flow cytometry. *Inflammation Research* 1996; 45 Suppl 1:S33–4.

⁹ Brown V, Ennis M. Flow-cytometric analysis of basophil activation: inhibition by histamine at conventional and homeopathic concentrations. *Inflammation Research* 2001; 50 Suppl 2:S47–8.

¹⁰ Robert Matthews. TV homeopathy trial was 'flawed'. *New Scientist*, 7 décembre 2002.