Chapter 17. “She only lacks a bit of makeup”

Light years away from the investigation of Nature

The protocol drafted in common by Benveniste’s and Spira’s teams planned to reproduce first of all the experiments reported in *Nature* with high dilutions of anti-IgE antiserum (ten-fold dilutions from $1/10^{21}$ in $1/10^{30}$). As a control, anti-IgG antiserum, ineffective on basophil degranulation, was diluted in the same conditions.

Another series was performed including experiments with inhibition of the first degranulation peak by high dilutions of *Apis mellifica*. One remembers that experiments with this homeopathic product were initially included in the manuscript intended for *Nature* and then had been finally published in another journal (see Chapter 4). The inhibitory experiments with *Apis mellifica* performed during the collaboration with A. Spira contained 6 dilutions (ten-fold dilutions from $1/10^{30}$ in $1/10^{40}$) of the initial solution of *Apis mellifica* the effect of which was compared with the solvent of this solution diluted in the same conditions.

The experiments were performed by E. Davenas (ED) and S. Gonnord (SG) from October to December 1989. As already said, the protocol planned to select for analysis only the experiments which met a series of quality controls concerning minimal number of basophils in control wells, significant percentage of degranulation of the first peak and absence of spontaneous degranulation of basophils. Among 45 experiments performed in the first series, 18 were included in the analysis according to the predefined selection criteria and among the 38 experiments performed for the second series with inhibition, 19 were included. The readers can make their own analysis with the counts of basophils obtained during this study that are given in the appendix of the first part.

The effect of high dilutions was confirmed

Overall, the statistical analysis highlighted that high dilutions of anti-IgE were associated with counts of basophils lower than high dilutions of anti-IgG. In other words, it was as if high dilutions of anti-IgE had a degranulating effect on basophils. Therefore, the main result of the article of *Nature* was reproduced. It was thus an essential result. As J. Benveniste said, degranulation with high dilutions was present, “beautiful and faithful”.

Indeed, the statistical analysis performed by the team of A. Spira indicated that the observed differences were not simply due to statistical fluctuations (for the familiar reader of the statistical tests, a value of $p < 0.01$ was achieved). To make these results more concrete, we will build several figures.

The figure below represents all counts in terms of percentage of degranulation with anti-IgE at high dilutions. The percentage of degranulation associated with each of the 10 high dilutions of anti-IgE was calculated in comparison with the mean of the 10 anti-IgG (controls) [1]. Let us remind that each of these points was counted blind. Consequently, if only chance was at work, one should obtain a cloud of points centered on the horizontal line (corresponding to degranulation equal to 0%). We notice that ED's cloud is moved upward, indicating that chance was "biased" towards the positive values. In other words, it was as if high dilutions of anti-IgE (compared with high dilutions of anti-IgG) had a degranulating effect on basophils. The statistical analysis confirmed this observation. It was a very important result. In contrast, for the experimenter SG, the cloud remained centered on the line 0% of degranulation.

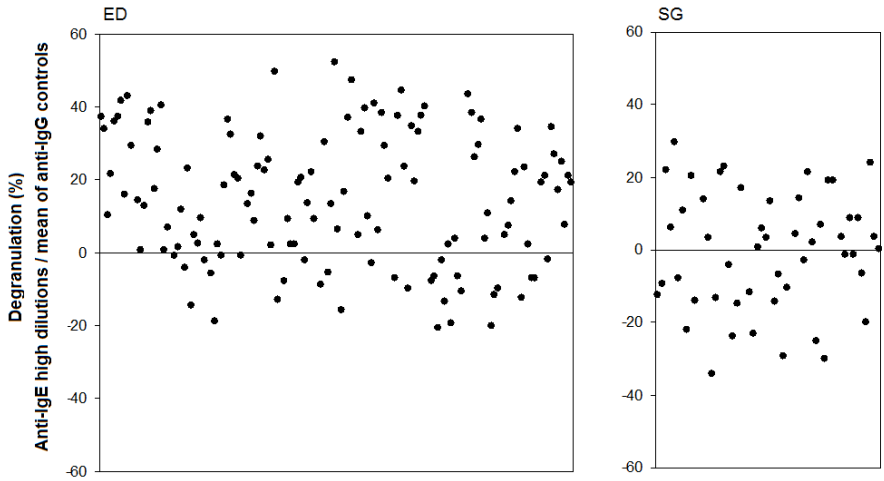

Figure 17.1. Each point is the percentage of basophil degranulation in the presence of high dilutions of anti-IgE assessed by experimentaters ED and SG. The calculation of the percentage of degranulation corresponding to a well X is made in the following way:
(mean of the 10 anti-IgG counts – count of the well X) / mean of the 10 anti-IgG counts
The values of the counts of basophils are given in Appendix 4.

We can also represent these clouds of points in a more concise manner by calculating the distributions of the percentages of degranulation with high dilutions of anti-IgG and with high dilutions of anti-IgE (Figure 17.2). This type of represention clearly highlights that the "behavior" of the basophils was not the same if they were in the presence of high dilutions of anti-IgE or the

inactive controls, namely "high dilutions of anti-IgG". We can also separate ED's and SG's results (Figure 17.3).

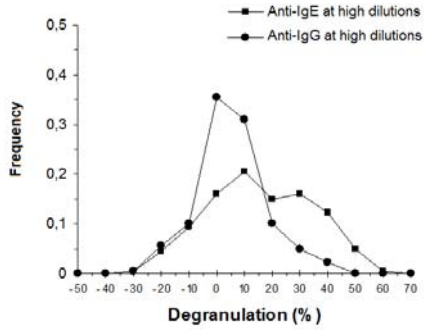

Figure 17.2. This figure presents the results as the distribution of the percentages of degranulation obtained with anti-IgG and anti-IgE. The percentages of degranulation of anti-IgE at high dilutions are calculated as indicated in Figure 17.1 by taking as controls the mean of the 10 anti-IgG controls. For high dilutions of anti-IgG, the mean is equal to 0 by definition. We observe that basophils that were incubated with high dilutions of anti-IgE were more frequently "degranulated".
(NB. On this figure and the next ones, each value of the *x*-axis corresponds to the upper limit on the interval).

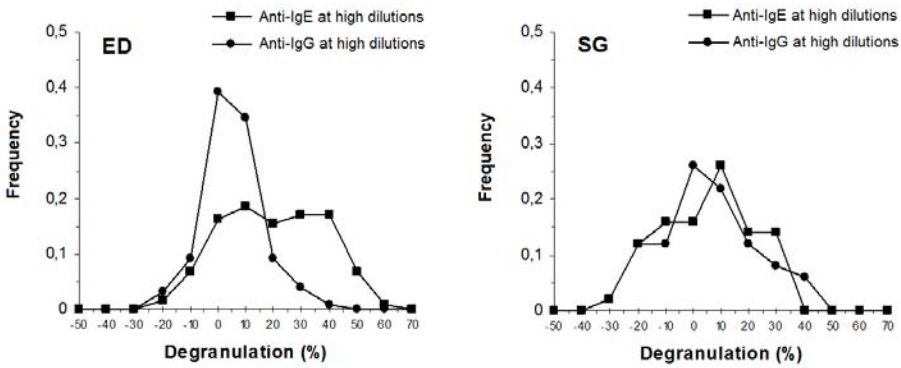

Figure 17.3. These two figures are built exactly as for Figure 17.2. Simply the results of both expérimenters (ED and SG) are separated. We notice that both "measurement instruments" did not achieve comparable "performances". In particular the important dispersion of anti-IgG at high dilutions (control) on the right figure suggests that getting a "signal" with SG would be more difficult.

Both experimentaters thus obtained very different results. What was striking with SG was the wide dispersion of the percentages with anti-IgG controls. Obviously there were two "measurement instruments" with very different "performances". It was possible that the detection of the loss of affinity of basophils for the staining agent required a good or specific view of colors. The manual dexterity or even the sensory acuteness of the experimenter could be crucial. These are only hypotheses. However if we were to speak about physics instruments, this would seem obvious. These results illustrate the difficulties to achieve a good reproducibility for some experiments in biology, even, as depicted here, within the same laboratory.

J. Benveniste attributed these differences to the different duration of practice for basophil counting of both experimenters.[2]

But where are the "sinusoids" of yesteryear?

The above presentation of the results does not take into account however the rank of the dilution, only the characteristics "high dilution of anti-IgE" or "high dilution of anti-IgG" was considered. One can also show the percentages of degranulation of the dilutions of anti-IgE by taking into account the rank of the dilution from $1/10^{21}$ to $1/10^{30}$. The 18 experiments are shown in Figure 17.4.

What is striking is the chaotic aspect of the results. One is very far from the regular curves that had been previously reported and had very much intrigued. As J. Benveniste noticed: "she lacks just a little makeup". Nevertheless, as we have seen before, points are more often above the line 0% (for ED) than allowed by chance only.

The inhibitory experiments with Apis mellifica

Let us examine now the results performed with the homeopathic product *Apis mellifica*. We represented the effect of this product as percentages of degranulation inhibition (Figure 17.5). Here again, if only chance were at work one should have an equal distribution around the line 0% inhibition. We notice here again that better performances were obtained with ED compared to SG. The latter obtained nevertheless an inhibitory effect of the homeopathic product, but less marked than her colleague.

The distribution of these points is also shown in Figure 17.6 (results of ED and SG are not separated). One notices as expected that, overall, the high dilutions of *Apis Mellifica* had an inhibitive effect.

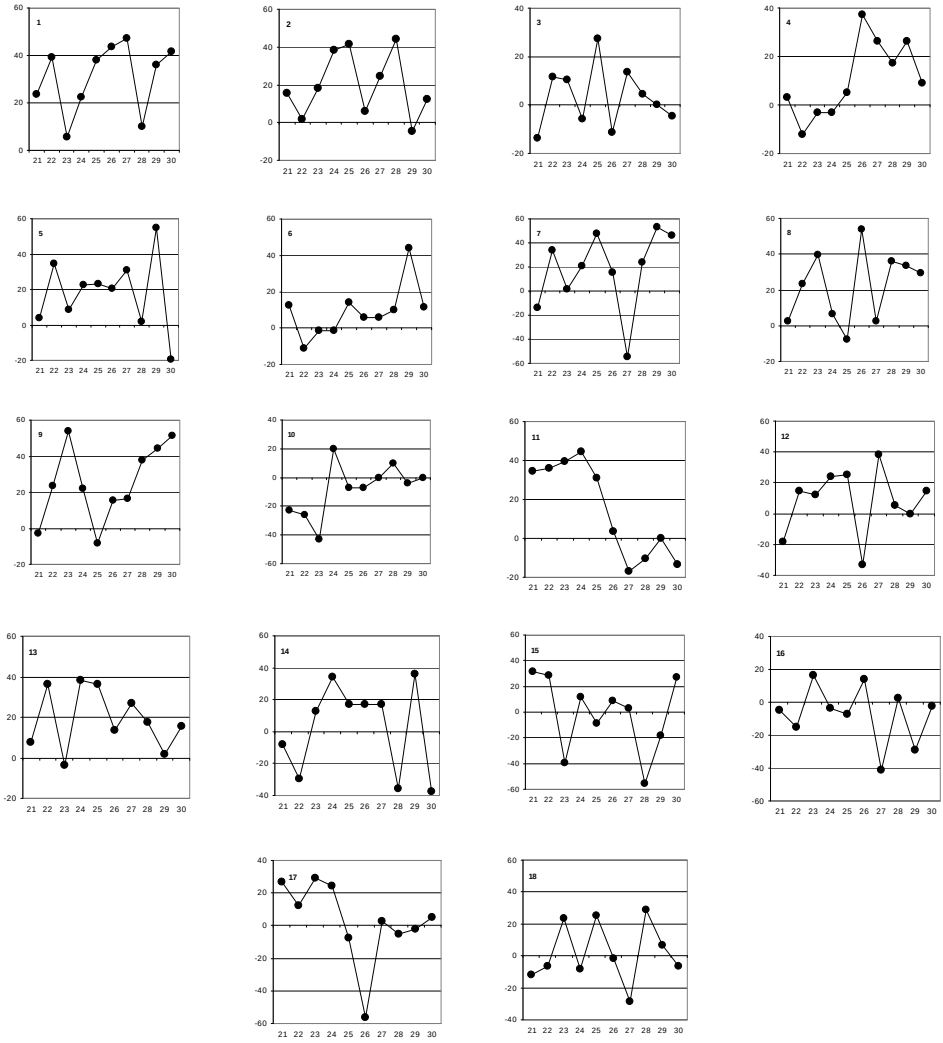

Figure 17.4. The 18 experiments are shown separately on this figure (n°1 to 13 for ED and n°14 to 18 for SG). Overall, there is an impression of chaos that prevails and one observes only rarely "sinusoids" or regular "waves". Nevertheless, overall, there is a statistically significant effect. In other words, points are more often above the line 0% of degranulation (in approximately two-third of cases) than allowed by chance (if only chance was at work we should have comparable numbers of points on each side of this line).

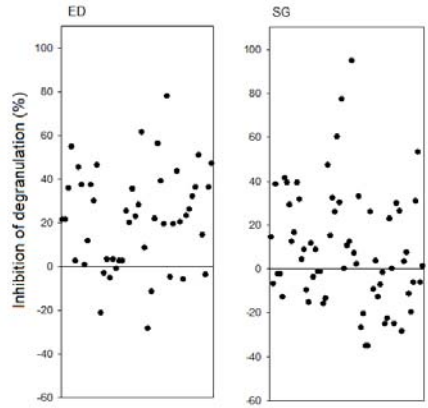

Figure 17.5. Each point is the percentage of inhibition of basophil degranulation by *Apis mellifica* assessed by the two experimenters ED and SG.

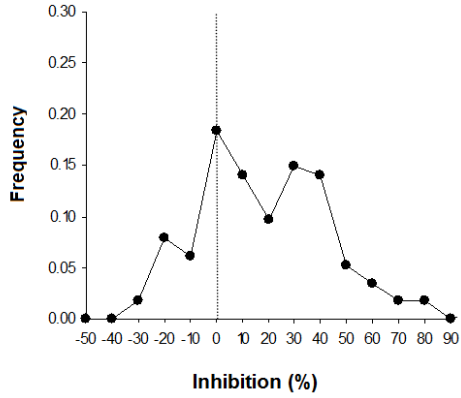

Figure 17.6. This figure summarizes the results of inhibition of degranulation by *Apis mellifica* as the distribution of all experimental values. We observe that the percentages of degranulation are more frequently on the right of the *x*-axis 0% thus indicating an overall inhibitory effect. If there was no overall inhibition (null hypothesis), then the distribution should be centered on 0% of inhibition.
The counts of basophils are given in Appendix 4.
(Each value of the *x*-axis corresponds to the upper limit on the interval).

A. Spira and his collaborators analyzed the results, dilution by dilution, and found a statistically significant effect (from $p < 0.05$ to $p < 0.01$) for the dilutions $1/10^{30}$, $1/10^{32}$, $1/10^{34}$ and $1/10^{40}$. We can draw the profile of

inhibition according to the dilutions of *Apis Mellifica* in the following way (Figure 17.7).

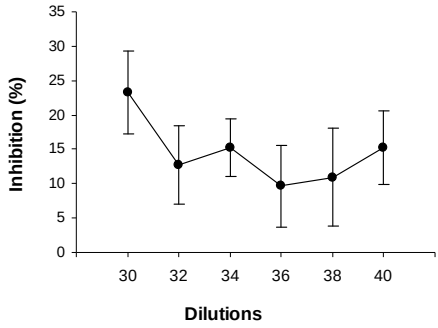

Figure 17.7. This figure is another representation of the results with *Apis mellifica*; the inhibitory effect is represented for each dilution of this product. Each point is the mean ± standard error of the mean of 19 experiments of inhibition of the degranulation with *Apis mellifica*. If the results obtained were only due to random fluctuations, we should find points on both sides of the horizontal line corresponding to 0% of inhibition.

As above, the results of both experimenters can be shown separately. If we consider results of ED who overall found a higher inhibition, 6 dilutions gave results which were statistically not different between them (Figure 17.8).

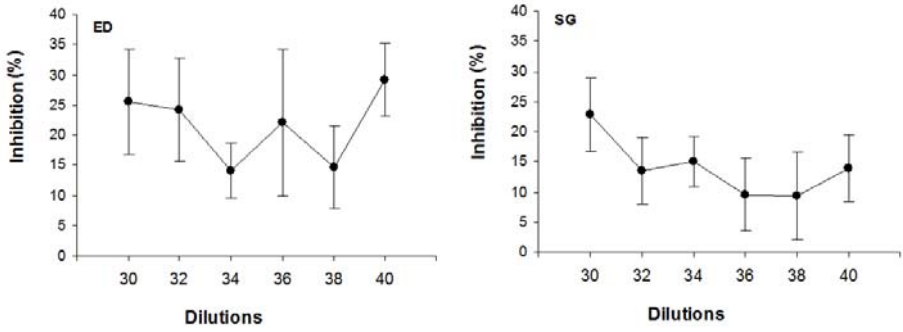

Figure 17.8. These figures show the same results as those of Figure 17.7. Simply, the results of ED and SG are shown separately. It is difficult to conclude that a given dilution has higher efficacy, even if overall the inhibition is statistically significant.

What about the law of small numbers?

As in Chapter 12, we can study the distribution of the ratio variance/mean for the 10 anti-IgG controls in each of the 18 experiments of the first series of experiments (Figure 17.9).

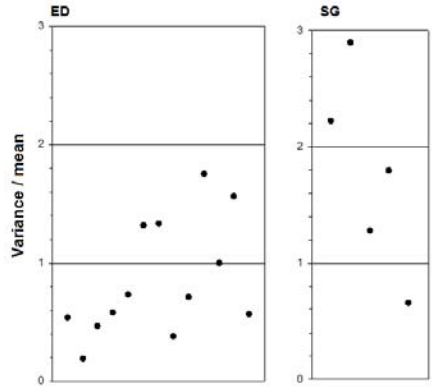

Figure 17.9. The distribution of the ratio variance/mean of the controls (anti-IgG at high dilutions) of the experiments with "direct" activation by anti-IgE is represented on this figure. Each point is the ratio of the variance of 10 anti-IgG controls divided by their mean. For the experimenter ED, we notice that the variance is lower more frequently than the mean (variance/mean < 1). See Chapter 12 for explanations concerning the interest of this representation.

One thus notices that in the 13 experiments performed by ED, 9 had a variance which was lower than the mean. In the 5 experiments of SG, 4 had a variance which was higher than the mean and two of them had a variance which was at least twice the mean. Therefore, with the "successful" ED experimenter, one finds again this notion of weak variability (here for 69% of the counts). Let us remind however that with a sampling of n=10 we should expect that 56% of the variances would be lower than the mean (cf. Chapter 12). These results are thus not incompatible with the law of small counts (if one does not take into account a possible added statistical noise).

In the conclusion of the article, the authors mentioned this issue of the conformity of the results with the law of small numbers:

> "Indeed, the variability for each of the 18 experiments of the number of basophils counted for the dilutions 21 till 30 of anti-IgG, showed that in 15 cases the test was compatible with the law

> of small numbers; in 2 cases the variance was higher to a variance conform to the law and in 1 case was lower."

This precision, which insisted on the compatibility with the law of small numbers, differed a little bit from the initial statement of A. Spira quoted at the end of the previous chapter ("It is surprising, unusual") when the first analysis were just done.

A consequence of the collaboration with A. Spira

At the end of December 1989, P. Lazar announced that he maintained J. Benveniste in his functions of director of Inserm U200 until June 30th, 1992.

According to the journal *Le Monde*:

> "This decision, taken in the context of the affair of the "memory of water", puts thus an end to the kind of testing imposed last July to Doctor Benveniste by Mr Lazar (*Le Monde* of July 8th and 12th, 1989). The latter had then recommended to Doctor Benveniste to adopt a "code of good practice" supposing in particular that he gives up for a while expressing himself on the effects of high dilutions except in high-level scientific journals in order to reconstitute a reliable capital "largely dissipated" in the eyes of his colleagues, said Mr Lazar.
> As well as Doctor Benveniste actually refrained since last July from making statements in the media, the decision of the director of INSERM could be also motivated by the fact that the results of the "second opinion" expertise led jointly for several months by doctors Benveniste and Alfred Spira (director of the unit 292 of INSERM) confirm for the moment the data published in June 30th, 1988 in the journal *Nature* on the molecular effects without molecule." [3]

There was however an important obstacle necessary to overcome: informing the scientific community on these results.

Notes of end of chapter

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[1] One could also calculate for each high dilution of anti-IgE the degranulation with regard to anti-IgG at the same corresponding dilution. Close results are obtained that change nothing to the demonstration.

[2] In the first versions of the manuscript, the initials of the two experimenters were reported. In the version published in the *Comptes Rendus de l'Académie des Sciences* (cf. Chapter 19), only overall results were presented. The difference of results between SG and ED was explained in these terms in the early version of the manuscript of March 6th, 1990: "These differences of performance can be probably attributed to the longest experience of manipulation and counting of basophils of E.D. (five years) compared with that of S.G., a newcomer in this area (6 part-time months)."

[3] F. Nouchi. Une décision du directeur général de l'INSERM. Le docteur Benveniste est maintenu dans ses fonctions jusqu'en 1992. *Le Monde*, January 3rd, 1990.