Chapter 3. An uncharted continent

Before continuing, the reader who is not biologist or not familiar with this area of research can refer to Appendix n°1. Information about the experimental model and interpretation of the results are given.

An unexplored peak is observed

It is tempring to believe that the god of the scientists watched then over J. Benveniste. Indeed, a modification of the experimental conditions perfomed at the end of 1985 timely arrived. It is the spark which sets fire to powder and propeled this research subject towards unhoped and unexpected summits.

On November 5th, 1985, there was a meeting in the Unit 200 of Inserm in Clamart with some people – including the author of this text – who worked in the laboratory on high dilutions. J. Sainte-Laudy who has been mentioned in the previous chapter, also participated in the meeting.

J Sainte-Laudy explained us that he often observed a second peak of basophil degranulation when he diluted an allergen more than usual. Our interest was raised, but we pointed out to him that this type of phenomenon was more or less already described and could be explained because allergens are complex molecules (they have several epitopes the immunologists say). J. Sainte-Laudy agreed, but he immediately added that he had observed this effect not only with allergens but also with anti-IgE antibodies, what was more difficult to explain. More importantly, he said that he noticed that high dilutions of histamine “flattened” this second peak. He specified that this inhibitory effect exceeded by far the effect on the first peak of degranulation that both laboratories used until now to assess the effect of homeopathic substances.

We were torn between doubt and desire to believe him. On one side, we knew well J. Sainte-Laudy; we appreciated him for his imagination and his creativity. But getting details on the precise experimental conditions, how many experiments had been performed and what was the reproducibility was often a challenge.

On the other hand, if this story of second peak was true, maybe it was the perfect model to assess high dilutions with obvious effects, in white or black.

We dreamed about such an experimental model, because we could then advance further in the understanding of the physico-chemical characteristics of high dilutions.

In any event, checking the reality of this "second peak" was very simple and the experiment was immediately performed. In contrast with J. Sainte-Laudy, we had only rarely at our disposal samples of blood from allergic subjects and we thus used blood cells from not allergic donors that were stimulated with anti-IgE antiserum. Indeed, anti-IgE antiserum plays the role of a "universal allergen" (see Appendix n°1).

After counting the stained basophils under a microscope, we noticed, with a mixture of surprise and excitement, a rise of the curve of degranulation with the low concentrations of anti-IgE antiserum (Figure 3.1).

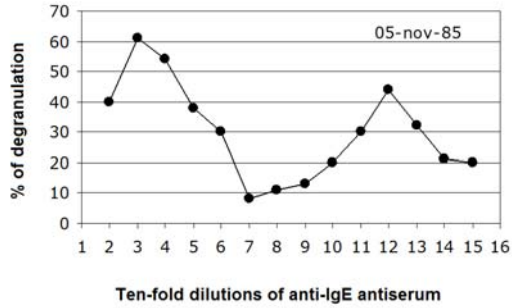

Figure 3.1. First attempt at Inserm Unit 200 to get basophil degranulation with low concencentrations of anti-IgE. Samples were obtained after serial ten-fold dilutions. Between each dilution, the tube was shaken for 10 secondes using rotating shaker. The left "peak" is the classical degranulation curve; the right peak is the "second" peak the observation of which was unexpected. Note that the "left" peak could be also obtained without shaking between each dilution.

In the weeks that followed November 5th, 1985, we explored this new avenue which unexpectedly appeared under our feet. We had the feeling that an important lock had been broken. We were in the state of mind of somebody who would discover a hidden door in his own house for new rooms that he would explore gradually. From November 6th, we repeated the experiment and the second peak (which we improperly called the "second curve") was still there (Figure 3.2)

From November 5th, 1985 to April 11th, 1986, 39 double peaks of degranulation in various experimental conditions were obtained as depicted in Figure 3.3.

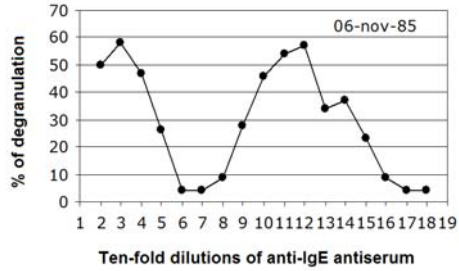

Figure 3.2. Repetition of the experiment of November 5th, 1985.

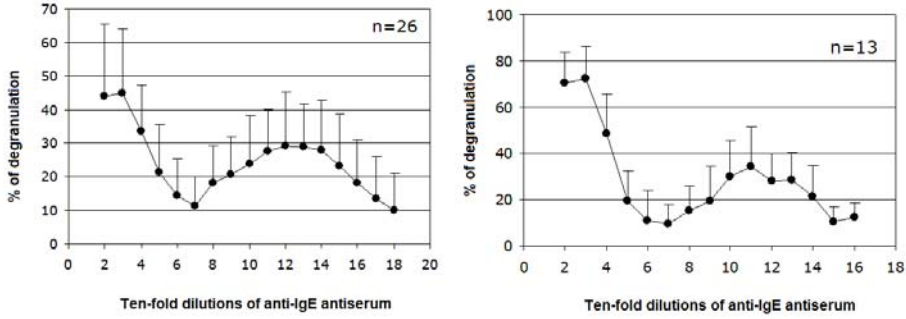

Figure 3.3. Summary of 39 experiments confirming the existence of a second peak of basophil degranulation. Results are presented with mean ± standard error of the mean. The experiments were performed with two different saline buffered-solutions: 26 experiments for the first one and 13 for the second one.

The second peak keeps its promises

But, for the moment, we were impatient to assess the effect of high dilutions of histamine on the second peak. Histamine indeed is not only released by basophils, but it can also inhibit its own release. This phenomenon was known for histamine at "classic" concentrations and in previous experiments on the "first peak", the same inhibitory phenomenon was observed with histamine at high dilutions.

We thus chose the dilution "18 C" of histamine with which we had already observed inhibitory effects on the first peak. Traditionally, homeopathic dilutions are performed by factor 100 and are named "C" (it is this "C" that we can read on the tubes of homeopathic pills; cf. Appendix 1). Therefore, the 18th

1/100-dilution – that is a dilution $1/10^{36}$ – "theoretically" contains 10^{-36} mol/L of histamine. We also decided to test this "18 C" dilution on all dilutions of anti-IgE to kill two birds with one stone: to accumulate results with the second peak and to begin exploring the possible inhibitory effect of histamine at high dilutions. We decided to perform these experiments in blind conditions to convince ourselves of the reality of the results.

During November 1985, four blind experiments were performed that compared the effect of 18 C dilution of histamine and a control dilution obtained exactly in the same conditions, but without histamine in the first tube. Control samples and samples of histamine at high dilution were given to the experimenter under a code label. We then noted with pleasure that a moderate inhibition of the first peak was obtained – thus reinforcing our previous results – and most importantly that a very large inhibition of the second peak was obtained (Figure 3.4 A).

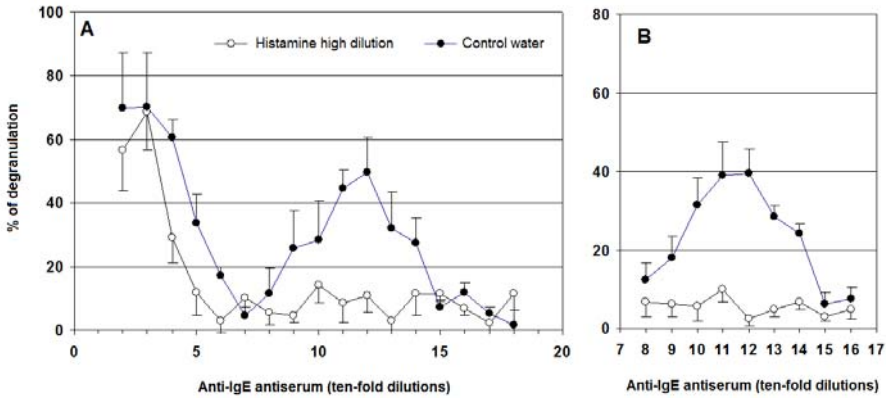

Figure 3.4. This figure shows the effect of high dilution of histamine on the first and second peaks (figure A) and on the second peak alone (figure B). High dilutions of histamine were obtained by performing 18 serial 1/100-fold dilutions with 10 secondes-shaking between each dilution (dilution $1/10^{36}$). The "theoretical concentration" of histamine was about 10^{-38} mol/L. "Water control" was obtained with the same process without histamine at the onset. Results are presented as mean ± standard error of the mean of 4 experiments for A and 8 experiments for B. All these experiments were performed blind: the tube containing high dilution of histamine and the tube containing its control were given under a code.

Then, in order to save time, we focused on the second curve and 8 new blind experiments were performed with similar results (Figure 3.4 B). We then assessed the effect of a series of dilutions of histamine on the "top" of the

second peak. The results of three experiments confirmed the results that we had previously obtained on experiments restricted to the first curve with two zones of inhibition the first one around 5–6 C and the second around 17–18 C.

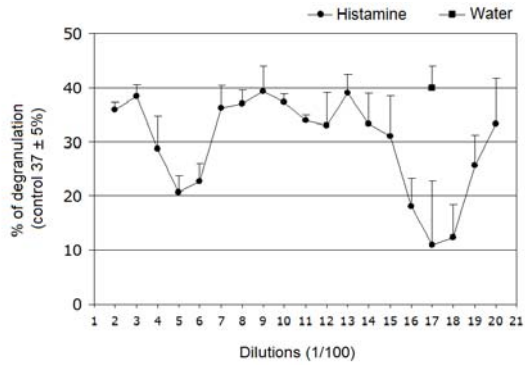

Figure 3.5. For these experiments, a solution of histamine was 1/100-fold serially diluted with 10-second shaking between each dilution (mean ± standard error of the mean of 3 experiments). The effect of these solutions was assessed on the second peak of degranulation. A control "water in water" diluted in the same conditions had no effect (square).

Simultaneously, experiments were undertaken to obtain an exit of histamine from cells ("release of histamine") in the presence of high dilutions of anti-IgE (see Appendix 1). Various experimental conditions known to favor the release of histamine were tested, but were unsuccessful. Finally, other experiments were performed to assess the effect of homeopathic products *Apis mellifica* and *Lung histamine* on the second peak.

A third peak appears and also the next ones

Only six months after the first observation of the second peak, an experiment was performed to answer the following question: what was beyond the second peak? An infinite plain? A mountain range? On May 13th, 1986, cells of a patient allergic to dust mites were incubated with allergen dilutions and waves of degranulation were observed beyond the second peak.

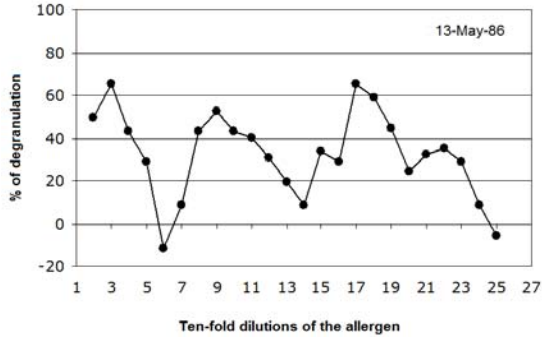

Figure 3.6. First evidence of degranulating effect after the second peak.

On June 11-12th, 1986, an attempt to activate basophils until the dilution $1/10^{60}$ of anti-IgE was performed, giving the result shown below.

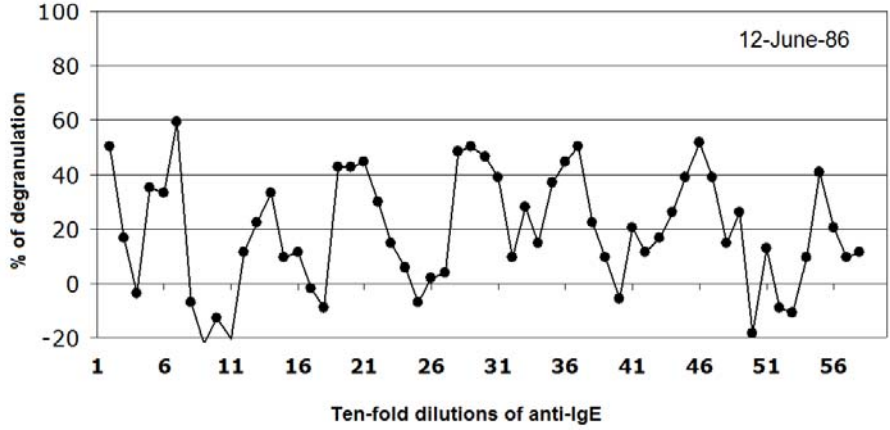

Figure 3.7. This figure shows the first attempt up to dilution $1/10^{60}$. As soon as the phenomenon began, it seemed to self-reproduce at the infinite with successive waves of biological activity.

The dilutions of anti-IgE were continued until $1/10^{120}$ (Figure 3.8). In front of these oscillations, which seemed to continue endlessly, we were bewildered. The vertigo which seized us with the early experiments when we performed these (very) high dilutions was now a little blunted because it seemed that the same phenomenon was self-sustaining throughout the whole dilution process.

Naturally, we were also concerned about the "specificity" of this phenomenon. We observed that an anti-IgG antiserum, which did not produce a first peak, had also no effect at high dilutions.[1] This result was excessively

intriguing. Indeed, an anti-IgE immunoglobulin and an anti-IgG immunoglobulin have very similar structures. Only a small portion of the protein – the one that "recognizes" IgE or IgG – is different. Nevertheless, an immunoglobulin is a voluminous molecule and if water keeps the "memory" of this molecule, it was as if water also keeps in memory all the fine details of this big protein structure (Figures 3.8 and 3.9).

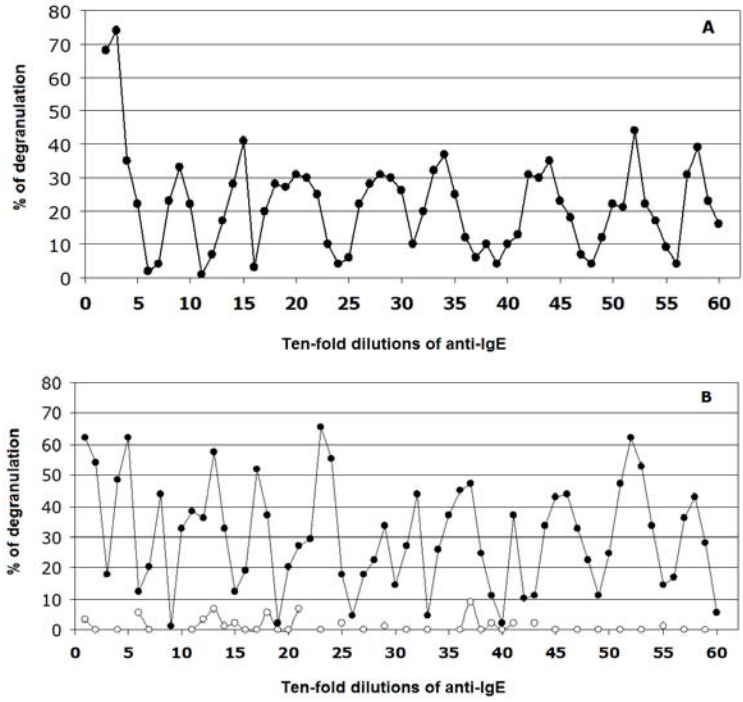

Figure 3.8. These experiments that repeat those of Figure 3.7 were published in *Nature* in 1988. They were reproduced a dozen times with anti-IgE antiserum dilutions (including four experiments with anti-IgG controls: open circles). Ten-fold dilutions were performed in experiment A and hundred-fold dilutions in experiment B. The last dilutions (60) in B is thus a $1/10^{120}$ dilution.

The apparent specificity of the high dilutions was at least as problematic as the absence of molecules. Indeed, even if one could accept that the properties of water could be modified during the successive dilutions in the absence of the starting molecules, the maintenance of the specificity was much more difficult to conceive.

We also used other basophil degranulating agents at high dilutions (calcium ionophore, phospholipase A2, etc.) and the waves of degranulation were always

there. Thus, rabbits were immunized with an antigen named peroxydase. A degranulation of their basophils was also observed in the presence of high dilutions of this antigen. But here once again, ther was no release of histamine (Figure 3.10).

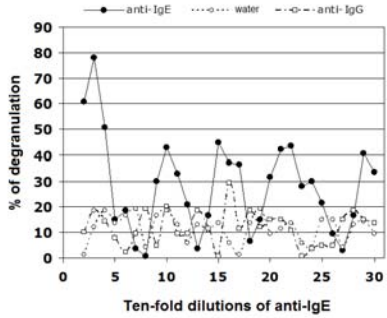

Figure 3.9. In this experiment, anti-IgE dilutions were prepared until $1/10^{30}$ before coming into contact with basophils. The controls (antiserum anti-IgG or water) were prepared in the same conditions. The "water" control showed that the shaking-dilution process alone was not sufficient to trigger the phenomenon; the "anti-IgG" control showed that diluting a protein was not sufficient – an immunoglobulin in this case – to obtain the same results as with anti-IgE.

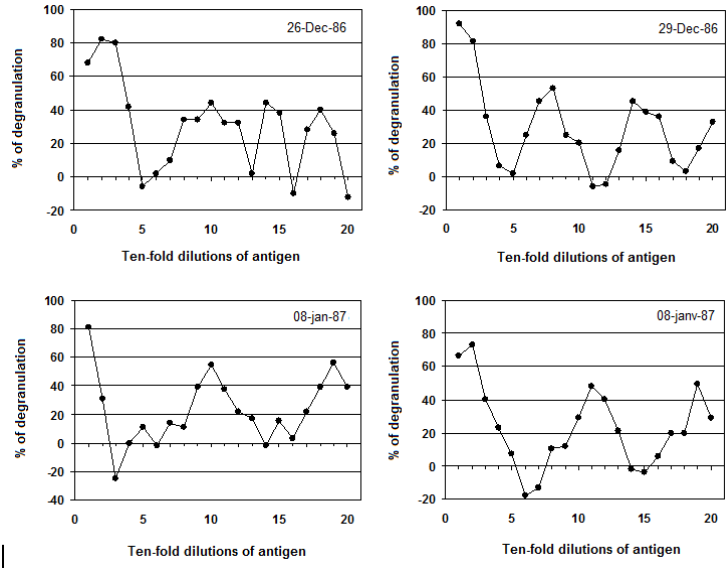

Figure 3.10. Rabbits were immunized against an allergen (peroxydase). In the presence of a series of dilutions with this allergen, rabbit basophils also degranulated with "waves" according to the strength of the dilution. Other agents that degranulated basophils at "classic" concentrations were also able to induce rabbit basophil degranulation at high dilutions.

Ghosts and their footprints, predators and their preys

Such oscillations of the biological effect of a substance according to its dilution are unusual in cell biology and in pharmacology. At the most, it is sometimes reported in some biologic systems "bell-shaped" responses according to the concentration. Some opponents to high dilutions sometimes took these oscillations as an argument to state that these results were "impossible" [2]. In fact, the oscillations observed with high dilutions reminded models described in population biology describing evolution over time of two animal populations, one being a predator and the other one a prey. The variations of the numbers of preys and predators can be modeled by the classic equation of Lotka-Volterra, which was developed in the 1920s. [3] This model rests on the idea that the number of preys decreases according to the number of predators and that the number of predators increases when the number of preys increases. We can easily transpose this model in the field of the high dilutions.

Indeed, to explain these oscillations, we can suppose that water has the property to keep a kind of imprint in counter-relief by "molding" a dissolved molecule. This molding would then generate a "ghost" – a kind of copy of the initial molecule – which in turn would leave an imprint. The successive generation of these imprints (biologically inactive because in "counter-relief") and "ghosts" of the molecule (biologically active because in "relief") could in this manner explain the succession of the peaks of biological activity. It is therefore necessary that "real" molecules are present in sufficient amounts at the start of the reaction, but the process could then self-generate when the initial molecules would have disappeared during the serial dilution process.

According to the model of Lotka-Volterra, we define X_t as the number of preys and Y_t the number of predators at time t. We then obtain:

$X_{t+1} – X_t = rX – aXY$ et $Y_{t+1} – Y_t = bXY – mY$ with:
r = rate of reproduction of preys in the absence of predators
a = rate of mortality of preys due to predators
b = rate of reproduction of predators according to eaten preys
m = rate of mortality of predators in the absence of preys.

We can conceive from this model a simple mechanism to explain the oscillations observed with high dilutions (Figure 3.12). The graphic representation of the equation of Lotka-Volterra with parameters specifically chosen gives the curves depicted in Figure 3.13.

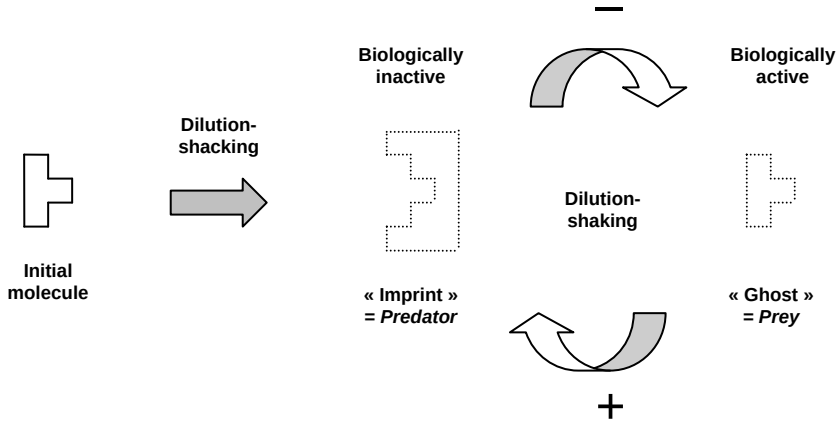

Figure 3.12. The model of Lotka-Volterra applied to high dilutions.

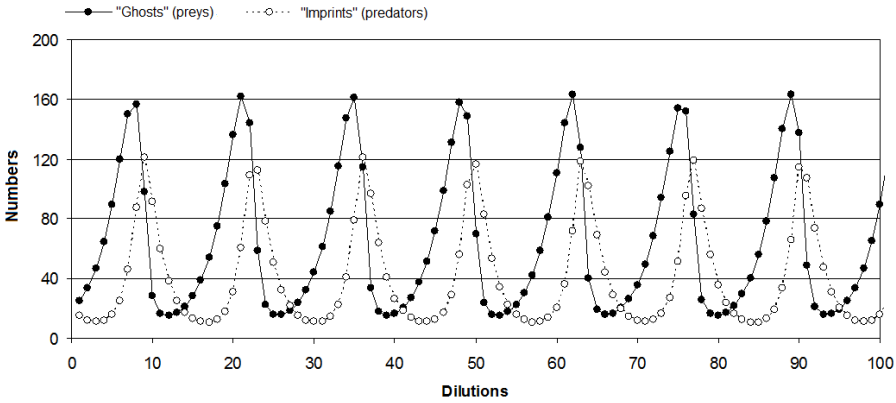

Figure 3.13. The model of Lotka-Volterra, which is usually applied in ecology to model dynamics of animal populations, can be be used to model the experimental oscillations observed with high dilutions. One can indeed make the simple hypothesis of the existence of two "entities" that, as in the initial model, interact on each other as one prey and its predator do. They are "ghosts" and "imprints", respectively. Only "ghosts" have biological activity (closed circles); "imprints" (open circles) are present in the solution but are biologically inactive. During each step of the dilution-shaking process, "ghosts" produce "imprints" that in turn destroy a certain amount of "ghosts". By correctly choosing the parameters of the model, oscillations of the number of "ghosts" – biologically active because they mimick the molecular structures of the starting molecule – appear. Each step of the dilution-shaking process plays the same role as time in the model of Lotka-Volterra.

Hunted by Avogadro's ghost

Of course, this modeling is hypothetical. In spite of our difficulties in naming the underlying processes of the effects that we observed, it was however such images ("molecules ghosts", "imprints", "copies", "moldings", "virtual molecules", "structuring of water") that we had then in mind and that allowed us to dilute beyond what was *a priori* reasonable and without feeling ourselves ridiculous. It was indeed psychologically difficult – because of the nonsense of this process for someone who knows a minimum of physical chemistry – to dilute a biological substance beyond a dozen ten-fold dilutions. Indeed, every biologist knows that it is very rare to observe biological effects at concentrations below 10^{-14} mol/L. Diluting beyond the limit fixed by the Avogadro number [4] needs either an unshakable faith or a total ignorance of an elementary scientific principle. We should not bury our heads in the sand, after this limit, we diluted water in water! One might as well add zeros to zeros hoping that a non-null number would appear.

But thanks to such theoretical speculations, we could imagine when we made this strange manipulation that, in spite of the disappearance of the initial molecules, it was not impossible that the process of dilution-agitation would generate "entities" that in turn would convey biological activity. In fact, these speculations remained mechanistic and very close to a molecular description of biology. It was also by means of "structures" that cell activity would be modified. These "structures" would be as "true" biological molecules able of interacting with cell receptors. We were far from a "new state of the matter" prophesied by some people and far from the "questioning of two centuries of scientific discoveries". The law of mass action was not indeed modified in this conception. At the most it would be necessary to take into account additional interactions in some circumstances. These conceptions were hardly formalized within the framework of the experiments at Clamart, but they allowed not to be stopped by the argument of the unsurpassable limit fixed by the Avogadro number.

However, the highlighting of physical modifications of the solvent related to its possible structuring appeared as a distant objective when the experiments which we have described above were performed. In the meantime, it was necessary to convince the other scientists – including those of Inserm U200 who worked on more "classic" subjects – and especially to convince ourselves that these biological effects were very real. It was then dozens of experiments that were performed.

Michel Schiff analyzed in 1992 all the laboratory notebooks concerning the period that followed the observation of the "second peak". Scientist at the

CNRS (*National Center for Scientific Research*), initially in physics and subsequently in sociology of sciences, M. Schiff participated in 1992-1993 to the life of the laboratory of Clamart to understand the causes of the controversial debate. We will talk more about M. Schiff in the second part of this text. Firstly sceptic about the results of J. Benveniste on high dilutions and "transmission of biological signal", he ended up being convinced about the reality of the results by investigating himself and by participating in the experiments. When he began to observe the life of the laboratory and to participate in the experiments, basophils had been replaced by another biological model than we will describe in the second part. Here are some extracts of his observations:

> "What I want to underline here is the caution with which the researchers of the Unit 200 moved forward in the study of the high dilutions. Partially depending on the the most qualified person for the counting of basophils (Elisabeth Davenas), they wanted to take precautions to make sure against the risks of bias in the sequence of operations. In the reports of the blind experiments of the first six months *[after the "discovery" of the second peak]*, I did not find less than twelve different names among the people involved in coding!" [5]

M. Schiff estimated that about 350 experiments had been performed before the investigation of *Nature*. And in another extract, he expressed the impoverishment of the research on high dilutions when a logic of proof had been substituted to it:

> "While an original work had begun on the physical properties of high dilutions and on the phenomenon of "waves", this work was stopped. Among 200 experiments performed after the investigation of *Nature*, less than 5% were new. So, during two years, the researchers of U200 dedicated the major part of their efforts to repeat indefatigably the two same experments to convince their colleagues." [6]

The events that led to the publication in *Nature* in 1988 are described next chapter onwards.

Notes of end of chapter

[1] Some anti-IgG antisera can also induce basophil degranulation. The one that was chosen had no effect at usual concentrations in order to be a proper control.

[2] For example, here is what F. Jacob (French Nobel prize laureate in 1965) answered in 1996 to the journalist E. Fottorino: " […] "The curve that Benveniste showed me indicated an incredible character". François Jacob sketched in front of us the figure that Benveniste should have shown if he had really discovered an effect at high dilution. A simple straight line parallel to the x-axis and not a series of domes as depicted by Benveniste." (E. Fottorino. La mémoire de l'eau. Une vérité hautement diluée. *Le Monde*, January 23rd, 1997.)

[3] Lotka AJ. 1925. Elements of physical biology. Baltimore: *Williams & Wilkins Co.* ; Volterra V. 1926. Variazioni e fluttuazioni del numero d'individui in specie animali conviventi. *Mem R. Accad Naz dei Lincei*. Ser. VI, vol. 2.

[4] The number of Avogadro is the number of elementary entities (atoms, ions, molecules, etc) contained in a mole of matter. It is usual to consider this number equal to 6.023×10^{23} even if is 6.022×10^{23} seems to be a better approximation. This number is a relation between the amount of matter of a mass m with the molecular mass of the elementary entity. Let us take the example of a molecule of anti-IgE. It is an immunoglobulin of molecular mass 150 000 (that is 150 000 g for a mole). Consequently, a solution of 1 mg/mL (or 1 g/L) of anti-IgE contains 1/150 000 moles of anti-IgE by liter (that is 6.67×10^{-6} moles/L or 4.0×10^{18} molecules). In the basophil degranulation test, the volume of anti-IgE solution added to the assay is 10 µL (that is 4.0×10^{13} molecules for the initial solution); we calculate easily that the 14th ten-fold dilution contains less than 1 molecule.

[5] Michel Schiff. Un cas de censure dans la science. L'affaire de la mémoire de l'eau, p. 47.

[6] *ibid.* p. 52.

Crossed portait #3

By Eric Fottorino

"The memory of water was his joker"

"Son of a general praticionner, completing "baccalauréate" at fifteen years, hospital resident, brilliant, bragging, a little bit of a show-off, Jacques Benveniste took a different direction for research in 1969, the year of his departure for San Diego (California). During three years, he works in the laboratory that will isolate the famous PAF-Acether. For this breakthrough he wins the silver medal of the CNRS.

Committed to the left-wings politics, he was also the "Mr. Medicine" of Jean-Pierre Chevènement, between 1981 and 1983, when this one was Minister of Research. He is finally a scientific member of Inserm council. What he says has clout. The retort will be accordingly. Behind the knowledge hides the issue of power.

In 1982, an American team received the Nobel Prize for work nearby that of Doctor Benveniste. His close relationships assert that he felt bitterness, that the "memory of water" was his joker to pick up the supreme reward. He denies, a little irritated. At the age of twenty, Jacques Benveniste saw himself as a racing driver. He competed for races at Montlhéry (Essonne). He was offered to become a rally pilot. He chose another way, as risky."

(*Le Monde,* January 21st, 1997)

"To meet Jacques Benveniste was to be immediately exposed to this mark of the meeting. The mark of the intelligence in the raw, fast, in perpetual movement. An embodied intelligence, capable of speeding and skids, but very generous, opener of horizons, of unknown worlds and infinite hopes."

(*Le Monde*, October 6th, 2004)