# Appendices of the first part

## Appendix 1. The world of basophils and allergy

*Basophils, mast cells and allergy*

Blood while cells contain less than 1% of basophil polymorphonuclear cells (we will call them basophils in short). The cells contain granules (a kind of small bags) that can release their content outside the cell. This phenomenon is called degranulation. Histamine which is thus released is responsible for some of clinical symptoms of allergy: redness (due to the increase of blood flow in capillary vessels dilated by histamine), local swelling (due to liquid leakage from blood to tissues) and tingling and itching (due to stimulation of nerve ends). Other cells – such as mast cells – share the same characteritics, but contrary to basophils they are located in tissues.

In the case of a individual who suffers from hay fever (or allergic rhinitis), the symptoms are due to the following sequence of events: pollen irritates the nasal mucosa, mastocytes are "stimulated" and they release histamine and other compounds that participate to the allergic reaction; basophils are attracted by some chemical mediators on the site of inflammation and they also release the content of their granules thus participating to the inflammatory reaction. In the case of hay fever, the consequence of inflammation is nasal discharge, tingling and sneezing.

But, outside allergic phenomena, what is the role of these cells since anybody – allergic or not – possesses mast cells and basophils? These cells play a role in the control of the diameter of small vessels (and thus control blood flow) and in the early phase of immune response in tissues. Paradoxicallly, the physiology of these cells is better known in allergic individuals than in healthy ones.

These two cell types, basophils and mast cells, are thus studied to better understand and hopefully control allergic reactions. Basophils have the advantage that they can be obtained by taking a simple blood sample. However their purification is difficult and only small numbers are obtained.

*Another actor of the allergic reaction: IgE.*

Why some individuals are allergic but others are not? Allergic people have high amounts of antibodies called IgE (= Type-E immunoglobulins). These antibodies synthesized by the immune system when the body encounters some molecules contained, for example, in pollens, animal hair, food, etc. Non-allergic people synthesize also these antibodies, but at considerably lower levels. IgE antibodies have an important property: their "foot" binds at the surface of

basophils and mast cells, whereas their "head" remains free, in order to "catch" foreign molecule for which they have been specifically synthesized. When an allergenic molecule is close to specific IgE molecules, they bind all together due to the complementarity of their surfaces (lock and key model). Thus, IgE molecules immobilize allergens as Velcro strips does. An important diffference with Velcro is however specificity. Thus, IgE molecules that "recognize" only cow milk antigens are not able to bind to pollen molecules. When an IgE molecule binds to a molecule, other IgE molecules – due to their mobility on cell membrane – come in close contact with the allergen molecule and progressively an aggregate of antibodies is forming around the allergen molecule. This immobilization of IgE molecules is responsible for the triggering of a series of enzymatic reactions that lead finally to the onset of "degranulation".

*Tests for allergy diagnosis*

This reaction can locally be induced in the skin of the allergic subject. Indeed, eventhough the initial contact between allergen (pollen) and the body is located in nasal mucosa (in the case of allergic rhinitis), IgE that are synthesized by the immune system spread out the whole body and bind on basophils and mast cells whatever their localization. If the sensitizing allergen is introced into the skin of an allergic individual, it is "recognized" by specific IgE molecules and maintained at the surface of mast cells present in dermis. As described above, histamine and other pharmacologically-active compounds are released by mastocytes and induce a small inflammatory reaction where allergen has been introduced with redness (the diameter of blood capillaries increases), formation of a small bubble that lifts up the epidemidi (edema due to fluid leak outside capillaries) and itching (nerve ends are stimulated). This method is the basis of a diagnosis test used by allergy specialists (skin tests).

Scientists like to reproduce *in vitro* the *in vivo* biological mechanisms in order to manipulate them easily. Thus, let us put into a tube maintained at 37°C: 1) blood from an individual allergic to pollen; 2) the specific allergenic. After about 15 min, histamine can be measured at the outside the cells. Of course, if we do the same experiment without adding allergenic extract we have no histamine outside the cells. This last experimental condition is called a "control". It allows checking that allergen is indeed responsible of the release of histamine. This notion of control could seem obvious. This is the case indeed in this simple exemple. This notion is however very important in any experimental procedure. It is a constant obsession in experimental sciences to wonder whether the observations are "real" and have not been created by the

experimental conditions themselves. It is important to know what a control is to understand some of the arguments durig the controversy on "high dilutions".

*Staining of basophils and their degranulation*

Basophil granules have the property to bind some stains (called basic stains). Toluidine blue is one of them. It is precisely because basophils "like" basic stains that they receive this name. Due to electrostatic charges, toluidine blue binds on structures that have many electronegative charges, such as basophil granules. When these stained structures are observed under a microscope, they appear as dark red. This shift from blue to red is called metachromasia. Basophils that appear red after staining with toluidine blue can be counted under a microscope.

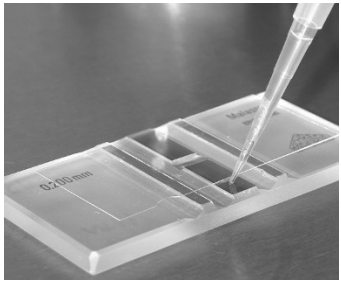
What is the consequence of the "degranulation" of the basophil? Not only histamine is released, but degranulated basophils cannot be stained. It is the basis of the basophil degranulation test that can be used for diagnosis purposes or for experimental reserach. In particular, there is a kind of "universal allergen" that allows inducing basophil degranulation as an allergen does. It is an anti-IgE antiserum that contains antibodies able to bind the IgE at the surface of basophil membrane. This binding of antibodies to IgE "mimicks" the effect of an allergen.

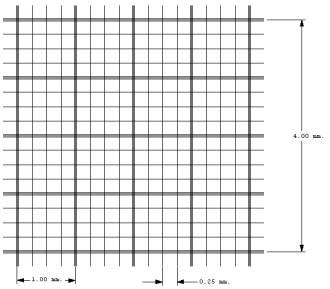
*How to assess degranulation?*

How to induce basophil degranulation with allergen of anti-IgE? In a first step, some simple handlings (blood sedimentation, recovery of supernatants, centrifugation) – that we will not detail – allow obtainig a concentrate of white blood cells suspended in physiological medium. Remember that this cell concentrate contains about one basophil for hundred white blood cells. Small volumes of this concentrate are put in tubes or more often in wells of plates designed for cell culture and widely used in biology labs ("96-well plates"). Then the allergen at different concentrations is placed in each well. The plate is then warmed at 37°C for half an hour.

After the time is up, a fixating and staining solution is added in each well. This solution allows also to get rid of contaminating red cells that disturb counting. Basophils are then counted under a microscope. Because one tries to assess the percentage of basophils that have degranulated in comparison with a control (which is a well prepared in the same conditions except that no allergenic extract was added), it is very important to count basophils in comparable volumes. Therefore basophils are counted by using a hemocytometer that allows the biologist to count cells in an accurate volume. A

small volume of the content of a well is placed unded the coverslip of the hemocytometer and cells are counted on the grid pattern engraved in glass. On the left image below, we can see what a hemocytometer looks like. We see the two surfaces that are recovered with a coverslip before placing fixed blood cells in each of the two chambers. On the right, there is the grid pattern seen under a microscope (here at low magnification). Basophils are counted by scaning systematicallly the whole surface row by row.





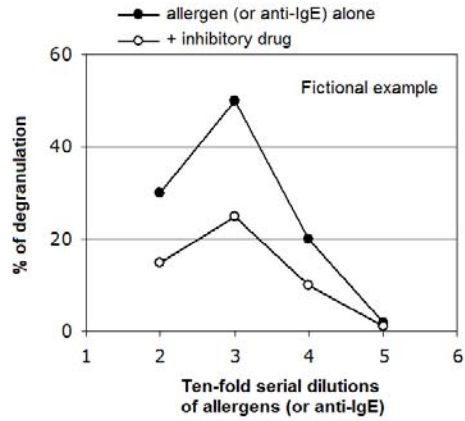

Suppose that the following results have been obtained (of course by counting the same surface for each result). For simplification, we suppose that each experimental point was performed only once.

| | |
|---|---|
| Well 1: control | 60 basophils |
| Well 2: allergen diluted 1/100 | 42 basophils |
| Well 3: allergen diluted 1/1000 | 30 basophils |
| Well 4: allergen diluted 1/10.000 | 48 basophils |
| Well 5: allergen diluted 1/100.000 | 59 basophils |

We observe that, in comparison with control, the number of basophils decreases according to the dilution of anti-IgE antiserum. We can show these results as above, but it is often more demonstrative to express the results as percentages of degranulation, namely the percentage of basophis that are no more visible. For this purpose, one calculates the difference of basophils in control well and in well to be calculated and one divides by the number of basophils in the control well. For example, for the well 1/100, we obtain: (60 – 42)/60 = 30%. A graph is usefull to show these results. The percentages have been calculated below for the same wells. In *y*-axis, we put the percentages of degranulation of the different dilutions.

| | |
|---|---|
| Well 2: antiserum anti-IgE diluted 1/100 | 30% of degranulation |
| Well 3: antiserum anti-IgE diluted 1/1000 | 50% of degranulation |
| Well 4: antiserum anti-IgE diluted 1/10.000 | 20% of degranulation |
| Well 5: antiserum anti-IgE diluted 1/100.000 | 2% of degranulation |

One can also slightly modify this biological model for the study of substances or drugs that inhibit the allergic reaction. The drug to be tested is added in wells containing the allergen (or anti-IgE) and results with and without the study drug are compared.




*Release of histamine and degraulation*

Of course, reality is rarely as simple as in texbooks. Moreover, what is not yet in texbooks is a possible definition of research. In particular, we showed at Inserm U200 in some experimental situations (not detailed here) that it was possible to get basophil "degranulation" evidenced by toluidine blue staining without simultaneous release of histamine. In other words, the basophil granules remained in place with their histamine content, but the granules nevertheless lost their capacity to retain toluidine blue. We thus hypothesized that the decrease of the ability to retain the dye was the consequence of ion movements. We all know for example that depolarization of neve cells is – schematically – the consequence of an entry of sodium ions in nerve cells. This entry of sodium is an example of ion movement. There are many cell models where the first step of "cell activation" is an entry of ions into the cell though "ion channels". In the case of a "degranulation" withour histamine release, our hypothesis was that an entry of ions was evidenced (no fixation of the dye to electronegative charges), but that the activation of the cell stopped. One possibility was that obtaining

release of histamine required "stronger" stimuli. The demonstration of this hypothesis was not completely achieved. Nevertheless, we published several articles, more particularly on the inhibitory role of extracellular sodium on the release of histamine and on ion channels present on basophil membrane. [1]

As described in this text, we observed that degranulation associated with high dilutions was not accompanied with a relase of histamine. If histamine had been released, the reproduction of the high dilutions experiments by other laboratories would have been more confortable. We thus proposed that high dilutions were "weak stimuli" that induced physicochemical changes of granules evidenced by the loss of affinity for the dye but were not able too induce the release of histamine. This is generally the case with people allergic to drugs, for example. This does not prevent allergy specialists to use this test for the diagnosis of drug allergy.

The use of the word "degranulation" has been criticized when the article has been published in *Nature*. It has been argued that a "true" degranulation must be accompanied, by definition, with a release of histamine. Afterwards, we coined the term "achromasia", which was purely descriptive and did not prejudge the release of histamine.

*How to make "high dilutions"*

Homeopathic dilutions are traditionally given as CH (hahnemannian centesimal dilutions) which are serial 1/100 dilutions. One could express concentrations as moles per liter, but rapidly the units have no sense (eventhough it is frequently used) because molar concentration refers to a number of molecules. One calculates indeed – it is the main polemical point on high dilutions – that there is less than one molecule after un number of dilutions. Let us take for example the case of anti-IgE at high dilutions which was the subject of the article of *Nature* in 1988. Starting from an antiserum that contains 1 mg/mL of anti-IgE, one calculates that after the dilution $1/10^{14}$, there is less than one molecule in the assay.

It is for this reason that one prefers talking about what is obtained (i.e. the dilution) thus referring to an experimental process that does not prejudge the presence or not of molecules. The adjective "hahnemannian" corresponds to a particular method of preparation: between each dilution, the solutions are vigourously shaken. In practice, we used a vortex mixer that allows a rapid mixing of solutions. This device is widely used in biology laboratories. For homeopaths, this shaking is very important because it is supposed to be a necessary condition to get active homeopathic dilutions.

*Notes of end of chapter*

[1] F. Beauvais *et al. J Allergy Clin Immunol* 1991; 87: 1020 ; *J Allergy Clin Immunol* 1992; 90: 52; *Fundam Clin Pharmacol* 1992; 6: 153; *J Immunol* 1992; 148: 149; *Fundam Clin Pharmacol* 1994; 8 :246 ; *Clin Exp Immunol* 1994; 95: 191; *Immunol Lett* 1995; 46: 81.

## Appendix 2. Israel results (February-March 1987)

| | **23 Feb.** | **1** | **2** | **3** | **26 Feb.** | | **1** | **2** | **3** | **27 Feb.** | | **1** | **2** | **3** | **1st Mar.** | | **1** | **2** | **3** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Open-label** | | | | | | | | | | | | | | | | | | | |
| Contrôle | | 79 | 82 | 83 | | | 85 | 95 | 87 | | | 86 | 79 | 80 | | | 110 | 106 | 104 |
| Anti-IgE 1/100 | | - | - | - | | | 33 | 45 | 49 | | | - | - | - | | | 57 | 63 | 55 |
| Anti-IgE1/1000 | | 33 | 38 | - | | | - | - | - | | | 27 | 29 | 27 | | | 37 | 42 | 41 |
| **Blind** | **Code R** | | | | **Code S** | **Code R** | | | | **Code S** | **Code R** | | | | **Code S** | **Code R** | | | |
| Control | B | 82 | 79 | 84 | 3 | G | 88 | 85 | 90 | 5 | 5 | 85 | 82 | 82 | 4 | A | 107 | 103 | 105 |
| Controle | E | 83 | 79 | 78 | 8 | H | 92 | 88 | 84 | 6 | G | 79 | 85 | 81 | 8 | G | 104 | 107 | 106 |
| Anti-IgE 1/100 | | - | - | - | | | - | - | - | | | - | - | - | 3 | H | 32 | 40 | 39 |
| Anti-IgE $1/10^{32}$ | F | 78 | 76 | 79 | 4 | A | 85 | 88 | 89 | 7 | 7 | 64 | 64 | 71 | 9 | I | 96 | 95 | 90 |
| Anti-IgE $1/10^{33}$ | G | 78 | 76 | 74 | 7 | B | 86 | 92 | 88 | 8 | A | 75 | 77 | 81 | 7 | B | 69 | 78 | 77 |
| Anti-IgE $1/10^{34}$ | D | 54 | 51 | 56 | 1 | C | 55 | 50 | 53 | 3 | D | 39 | 37 | 38 | 6 | D | 45 | 47 | 53 |
| Anti-IgE $1/10^{35}$ | C | 44 | 46 | 45 | 2 | E | 34 | 34 | 37 | 1 | 1 | 38 | 42 | 44 | 1 | F | 51 | 50 | 47 |
| Anti-IgE $1/10^{36}$ | A | 46 | 49 | 52 | 5 | E | 51 | 49 | 51 | 4 | B | 52 | 59 | 54 | 2 | C | 70 | 78 | 75 |
| Anti-IgE $1/10^{37}$ | H | 75 | 79 | 83 | 6 | F | 84 | 86 | 86 | 2 | F | 70 | 75 | 75 | 5 | E | 104 | 106 | 106 |

These results are the first 4 experiments performed by E. Davenas in Israel during her stay in February-March 1987. See Chapter 4 for detail on experiments and Chapter 11 and 12 for additional comments. The figures corresponding to these results are shown in Chapter 5.

Three wells of cells to be counted (numbered 1, 2 and 3) were associated with each dilution (or control). A part of each experiment was "open-label" in order to check that the experimental conditions were correct and the other part was performed "blind". Tubes were blinded with a simple code for the experiment of February 23rd and with a double code with two successive coders for the other ones (Code R = code of B. Robinzon ; code S = code of M. Shinitzky). In the case of a double code, no participant to the experiment was able to identify the tubes. These results were reported in Table 1 of *Nature*'s article 1988, which is also reproduced Chapter 8 (Figure 8.2). (*NB.* there are small differences for rounding of decimal numbers with *Nature*'s article).

## Appendix 3. Results obtained during the survey of *Nature* (July 1988)

| | ED | | FB | |
|---|---|---|---|---|
| | *1* | *2* | *1* | *2* |
| Control 1 | 45 | 56 | 36 | 24 |
| Control 2 | 44 | 56 | 36 | 31 |
| Control 3 | 35 | 49 | 39 | 32 |
| Control 4 | 32 | 28 | 37 | 20 |
| Control 5 | 31 | 31 | 25 | 47 |
| Anti-IgE $1/10^2$ | 11 | 30 | 13 | 14 |
| $1/10^3$ | 30 | 37 | 32 | 22 |
| $1/10^4$ | 28 | 34 | 39 | 31 |
| $1/10^5$ | 41 | 45 | 39 | 36 |
| $1/10^6$ | 58 | 59 | 43 | 33 |
| $1/10^7$ | 48 | 60 | 20 | 42 |
| $1/10^8$ | 31 | 45 | 36 | 29 |
| $1/10^9$ | ?* | 56 | 28 | 31 |
| $1/10^{10}$ | 59 | 61 | 30 | 40 |
| $1/10^{11}$ | 43 | 52 | 37 | 19 |
| $1/10^{12}$ | 35 | 56 | 40 | 37 |
| $1/10^{13}$ | 44 | 42 | 35 | 35 |
| $1/10^{14}$ | 58 | 49 | 29 | 33 |

| | ED | | FB | |
|---|---|---|---|---|
| | *1* | *2* | *1* | *2* |
| $1/10^{14}$ | 58 | 49 | 29 | 33 |
| $1/10^{15}$ | 32 | 54 | 26 | 17 |
| $1/10^{16}$ | 36 | 56 | 43 | 34 |
| $1/10^{17}$ | 52 | 30 | 28 | 41 |
| $1/10^{18}$ | 54 | 36 | 35 | 31 |
| $1/10^{19}$ | 47 | 42 | 40 | 38 |
| $1/10^{20}$ | 49 | 54 | 19 | 40 |
| $1/10^{21}$ | 26 | 33 | 37 | 37 |
| $1/10^{22}$ | 63 | 45 | 39 | 42 |
| $1/10^{23}$ | 59 | 44 | 36 | 34 |
| $1/10^{24}$ | 40 | 32 | 30 | 39 |
| $1/10^{25}$ | 56 | 36 | 34 | 44 |
| $1/10^{26}$ | 41 | 40 | 27 | 29 |
| $1/10^{27}$ | 39 | 60 | 32 | 34 |
| $1/10^{28}$ | 26 | 54 | 38 | 41 |
| $1/10^{29}$ | 44 | 65 | 41 | 38 |
| $1/10^{30}$ | 13 | 65 | 34 | 36 |

* Only one value (error of W. Stewart).

These results correspond to the experiment counted by two experimenters (ED and FB) on Thursday July 7th, 1988 (two counts for each well). These results (67 pairs) were used to calculate the distribution of the difference of the counts of basophils performed in duplicate. For more details, see Figures 9.5 of Chapter 9, Figures 11.3 and 11.4 of Chapter 11 and also Chapters 9 to 12.

## Appendix 4 (First part). Results of the article of *Comptes Rendus de l'Académie des Sciences* of 1991

### ED

| | *Exp 1* | | *Exp 2* | | *Exp 3* | | *Exp 4* | | *Exp 5* | | *Exp 6* | | *Exp 7* | | *Exp 8* | | *Exp 9* | | *Exp 10* | | *Exp 11* | | *Exp 12* | | *Exp 13* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Dil.** | **E** | **G** | **E** | **G** | **E** | **G** | **E** | **G** | **E** | **G** | **E** | **G** | **E** | **G** | **E** | **G** | **E** | **G** | **E** | **G** | **E** | **G** | **E** | **G** | **E** | **G** |
| $1/10^{21}$ | 56 | 77 | 56 | 66 | 88 | 76 | 105 | 108 | 93 | 97 | 76 | 85 | 64 | 56 | 74 | 76 | 77 | 75 | 75 | 59 | 33 | 53 | 72 | 58 | 42 | 46 |
| $1/10^{22}$ | 59 | 94 | 65 | 66 | 86 | 96 | 118 | 106 | 90 | 127 | 64 | 56 | 41 | 61 | 52 | 70 | 45 | 62 | 74 | 56 | 36 | 57 | 70 | 81 | 41 | 60 |
| $1/10^{23}$ | 80 | 85 | 57 | 69 | 77 | 86 | 97 | 94 | 98 | 107 | 69 | 68 | 62 | 63 | 47 | 78 | 40 | 79 | 84 | 54 | 43 | 66 | 65 | 74 | 53 | 51 |
| $1/10^{24}$ | 70 | 90 | 42 | 67 | 91 | 86 | 100 | 97 | 82 | 106 | 69 | 68 | 51 | 63 | 70 | 75 | 55 | 71 | 71 | 85 | 41 | 67 | 59 | 77 | 34 | 54 |
| $1/10^{25}$ | 57 | 91 | 40 | 67 | 67 | 91 | 81 | 86 | 73 | 98 | 57 | 67 | 28 | 56 | 80 | 74 | 79 | 73 | 79 | 74 | 37 | 55 | 50 | 69 | 38 | 57 |
| $1/10^{26}$ | 56 | 95 | 54 | 58 | 100 | 90 | 63 | 100 | 83 | 105 | 56 | 60 | 55 | 64 | 46 | 88 | 47 | 58 | 68 | 63 | 56 | 58 | 85 | 60 | 43 | 50 |
| $1/10^{27}$ | 52 | 94 | 47 | 63 | 83 | 95 | 67 | 93 | 80 | 113 | 72 | 76 | 68 | 36 | 73 | 75 | 58 | 70 | 83 | 83 | 52 | 42 | 58 | 87 | 39 | 53 |
| $1/10^{28}$ | 75 | 84 | 39 | 68 | 85 | 89 | 78 | 95 | 105 | 107 | 61 | 68 | 49 | 63 | 48 | 76 | 48 | 75 | 67 | 74 | 70 | 64 | 74 | 78 | 48 | 57 |
| $1/10^{29}$ | 51 | 83 | 65 | 62 | 79 | 79 | 79 | 105 | 54 | 113 | 55 | 86 | 37 | 68 | 55 | 81 | 45 | 77 | 74 | 71 | 65 | 65 | 81 | 81 | 41 | 42 |
| $1/10^{30}$ | 63 | 100 | 61 | 69 | 89 | 85 | 100 | 109 | 121 | 100 | 64 | 72 | 31 | 58 | 62 | 85 | 43 | 80 | 77 | 77 | 64 | 56 | 81 | 92 | 42 | 50 |

These results are for the 18 experiments with "activation" (i.e. experiments aimed for evidenving an effect of anti-IgE at high dilutions).
These results are presented as follows: columns E and G correspond to the counts of basophils in the wells containing anti-IgE and anti-IgG (controls), respectively, from $1/10^{21}$ to $1/10^{30}$.
The results from the two experimenters ED (13 experiments) and SG (5 experiments) are presented separately.
For more explanations see Chapters 16 to 19; see also the original article: J. Benveniste, E. Davenas, B. Ducot, B. Cornillet, B. Poitevin, A. Spira. L'agitation de solutions hautement diluées n'induit pas d'activité biologique spécifique [*Agitating highly diluted solutions does not induce specific biological activity*] ]. *C R Acad Sci* tome 312 série II n°5, February 28th, 1991 p.461–466.

*(Continued on next page)*

## Appendix 4 (Second part). Results of the article of *Comptes Rendus de l'Académie des Sciences* of 1991

*(Continued from previous page)*

**SG**

| | ***Exp 14*** | | ***Exp 15*** | | ***Exp 16*** | | ***Exp 17*** | | ***Exp 18*** | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Dil.** | **E** | **G** | **E** | **G** | **E** | **G** | **E** | **G** | **E** | **G** |
| $1/10^{21}$ | 57 | 78 | 72 | 67 | 89 | 85 | 39 | 50 | 57 | 50 |
| $1/10^{22}$ | 64 | 83 | 70 | 51 | 98 | 86 | 35 | 40 | 60 | 56 |
| $1/10^{23}$ | 89 | 63 | 50 | 58 | 79 | 92 | 42 | 54 | 54 | 68 |
| $1/10^{24}$ | 75 | 83 | 60 | 82 | 75 | 72 | 32 | 42 | 60 | 55 |
| $1/10^{25}$ | 52 | 46 | 45 | 56 | 77 | 71 | 40 | 37 | 54 | 69 |
| $1/10^{26}$ | 51 | 57 | 69 | 80 | 69 | 80 | 51 | 28 | 63 | 62 |
| $1/10^{27}$ | 69 | 71 | 57 | 68 | 91 | 58 | 38 | 39 | 71 | 54 |
| $1/10^{28}$ | 82 | 45 | 78 | 55 | 85 | 87 | 53 | 51 | 45 | 62 |
| $1/10^{29}$ | 76 | 64 | 51 | 74 | 103 | 80 | 33 | 32 | 57 | 61 |
| $1/10^{30}$ | 55 | 73 | 73 | 49 | 88 | 86 | 33 | 35 | 59 | 55 |

**Appendix 4 (Third part). Results of the article of *Comptes Rendus de l'Académie des Sciences* of 1991**

| | ***ED*** | | | | | | | | ***SG*** | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ***n°1*** | ***n°2*** | ***n°3*** | ***n°4*** | ***n°5*** | ***n°6*** | ***n°7*** | ***n°8*** | ***n°9*** | ***n°10*** | ***n°11*** | ***n°12*** | ***n°13*** | ***n°14*** | ***n°15*** | ***n°16*** | ***n°17*** | ***n°18*** | ***n°19*** |
| Control | 76 | 96.5 | 84.5 | 70.5 | 66.5 | 72 | 64.5 | 57.5 | 130 | 108.5 | 197.5 | 84 | 94.5 | 127 | 101.5 | 98 | 90 | 112 | 73 |
| Anti-IgE 1/100 + contrôle diluted and agitated ($1/10^{40}$) | 34 | 41.5 | 35.5 | 31 | 36.5 | 31 | 30 | 30 | 63.5 | 60 | 90.5 | 43.5 | 48 | 70 | 53 | 44 | 46 | 64.5 | 32.5 |
| Anti-IgE 1/100 + *A. Mel.* $1/10^{30}$ | 43 | 62 | 25 | 32 | 45 | 54 | 45 | 40 | 73 | 80 | 124 | 42 | 70 | 114 | 54 | 58 | 35 | 77 | 30 |
| Anti-IgE 1/100 + *A. Mel.* $1/10^{32}$ | 43 | 42 | 34 | 32 | 55 | 47 | 37 | 44 | 59 | 79 | 95 | 47 | 55 | 70 | 69 | 39 | 36 | 51 | 45 |
| Anti-IgE 1/100 + *A. Mel.* $1/10^{34}$ | 49 | 48 | 37 | 41 | 39 | 39 | 28 | 34 | 89 | 74 | 100 | 43 | 63 | 76 | 40 | 46 | 56 | 66 | 54 |
| Anti-IgE 1/100 + *A. Mel.* $1/10^{36}$ | 57 | 62 | 33 | 39 | 28 | 63 | 38 | 29 | 62 | 66 | 80 | 43 | 60 | 77 | 43 | 37 | 46 | 68 | 30 |
| Anti-IgE 1/100 + *A. Mel.* $1/10^{38}$ | 35 | 58 | 37 | 45 | 33 | 29 | 39 | 40 | 62 | 68 | 74 | 37 | 76 | 124 | 36 | 40 | 35 | 59 | 33 |
| Anti-IgE 1/100 + *A. Mel.* $1/10^{40}$ | 53 | 67 | 35 | 40 | 43 | 39 | 41 | 43 | 55 | 79 | 103 | 38 | 62 | 74 | 36 | 43 | 59 | 55 | 31 |

These results are for the 19 experiments with "inhibition" (i.e. experiments aimed for evidencing an effect of the homeopathic medicine *Apis mellifica* at high dilutions).

These results are presented as follows: the first line corresponds to the counts for control (maximal number of basophils at resting state), the second line is the counts of basophils in the presence of anti-IgE and control (minimal number of basophils after degranulation induced by anti-IgE) and next lines are the counts of basophils in the presence of *Apis mellifica* and anti-IgE.

Each number of the first ("control") and second line ("Anti-IgE 1/100 + control diluted and agitated ($1/10^{40}$)" is the mean of two counts.

The results from the two experimenters ED (8 experiments) and SG (11 experiments) are presented separately.

For more explanations see Chapters 16 to 19; see also the original article: J. Benveniste, E. Davenas, B. Ducot, B. Cornillet, B. Poitevin, A. Spira. L'agitation de solutions hautement diluées n'induit pas d'activité biologique spécifique [*Agitating highly diluted solutions does not induce specific biological activity*] ]. *C R Acad Sci* tome 312 série II n°5, February 28th, 1991 p.461–466.

## Appendix 5. Results of the article of Hirst *et al* (*Nature*, 1993)

Note that results are given as degranulation percentages in this table.
For more explantions, see Chapters 20 and 21; see also the original article (Hirst SJ, Hayes NA, Burridge J, Pearce FL, Foreman JC. Human basophil degranulation is not triggered by very dilute antiserum against human IgE. *Nature* 1993;366:525-7).

**Table 1 out of 3**

| | **Diluted and agitated anti-IgE** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **$1/10^{12}$** | **$1/10^{14}$** | **$1/10^{16}$** | **$10^{18}$** | **$10^{20}$** | **$10^{22}$** | **$10^{24}$** | **$10^{26}$** |
| 5 sessions with 8 high dilutions at $1/10^{12}$, $1/10^{14}$ … $1/10^{26}$ | ***1*** | -12 | -18 | -25 | -17 | -3 | -10 | -28 | -3 |
| | ***2*** | -10 | -3 | -8 | -15 | 1 | 3 | -12 | -2 |
| | ***3*** | -1 | 4 | -2 | -4 | 8 | 11 | 2 | 9 |
| | ***4*** | 6 | 7 | 7 | 7 | 10 | 12 | 14 | 10 |
| | ***5*** | 10 | 11 | 17 | 25 | 17 | 16 | 25 | 14 |
| | | **$1/10^{30}$** | **$1/10^{32}$** | **$1/10^{34}$** | **$1/10^{36}$** | **$1/10^{38}$** | **$1/10^{40}$** | **$1/10^{42}$** | **$1/10^{44}$** |
| 5 sessions with 8 high dilutions at $1/10^{30}$, $1/10^{32}$ … $1/10^{44}$ | ***1*** | -16 | -14 | -12 | -4 | -22 | -13 | -13 | -30 |
| | ***2*** | -14 | -13 | -8 | -3 | -2 | -6 | -12 | -16 |
| | ***3*** | -2 | -11 | -7 | 2 | 4 | -1 | -6 | 1 |
| | ***4*** | 2 | -4 | 12 | 9 | 13 | 10 | -1 | 14 |
| | ***5*** | 28 | 34 | 13 | 14 | 25 | 28 | 33 | 22 |
| | | **$1/10^{46}$** | **$1/10^{48}$** | **$1/10^{50}$** | **$1/10^{52}$** | **$1/10^{54}$** | **$1/10^{56}$** | **$1/10^{58}$** | **$1/10^{60}$** |
| 5 sessions with 8 high dilutions at $1/10^{46}$, $1/10^{48}$ … $1/10^{60}$ | ***1*** | -8 | -4 | -19 | -10 | -9 | -1 | 7 | -2 |
| | ***2*** | 4 | 6 | -10 | 20 | 1 | 1 | 9 | -1 |
| | ***3*** | 5 | 9 | 8 | 21 | 11 | 2 | 17 | 7 |
| | ***4*** | 8 | 13 | 10 | 22 | 26 | 7 | 28 | 32 |
| | ***5*** | 14 | 24 | 16 | 41 | 30 | 29 | 30 | 35 |

*(Suite)*

**Table 2 out of 3**

| | **Diluted anti-IgE without agitation** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **$1/10^{12}$** | **$1/10^{14}$** | **$1/10^{16}$** | **$10^{18}$** | **$10^{20}$** | **$10^{22}$** | **$10^{24}$** | **$10^{26}$** |
| 4 sessions with 8 high dilutions at $1/10^{12}$, $1/10^{14}$ … $1/10^{26}$ | ***1*** | 0 | -7 | -22 | -15 | -9 | -12 | -10 | -21 |
| | ***2*** | 1 | 1 | -6 | -6 | -2 | -6 | -6 | -9 |
| | ***3*** | 16 | 13 | 3 | 2 | 10 | -4 | 0 | 9 |
| | ***4*** | 21 | 15 | 5 | 24 | 11 | 11 | 13 | 10 |
| | | **$1/10^{30}$** | **$1/10^{32}$** | **$1/10^{34}$** | **$1/10^{36}$** | **$1/10^{38}$** | **$1/10^{40}$** | **$1/10^{42}$** | **$1/10^{44}$** |
| 4 sessions with 8 high dilutions at $1/10^{30}$, $1/10^{32}$ … $1/10^{44}$ | ***1*** | -4 | -11 | 9 | -15 | -11 | -4 | -12 | -8 |
| | ***2*** | 4 | -4 | 2 | 2 | 1 | -2 | -6 | -6 |
| | ***3*** | 5 | -1 | 4 | 9 | 2 | 13 | 1 | 2 |
| | ***4*** | 7 | 3 | 20 | 14 | 4 | 15 | 13 | 4 |
| | | **$1/10^{46}$** | **$1/10^{48}$** | **$1/10^{50}$** | **$1/10^{52}$** | **$1/10^{54}$** | **$1/10^{56}$** | **$1/10^{58}$** | **$1/10^{60}$** |
| 4 sessions with 8 high dilutions at $1/10^{46}$, $1/10^{48}$ … $1/10^{60}$ | ***1*** | -4 | -9 | -14 | -1 | -11 | -7 | -11 | -12 |
| | ***2*** | 4 | 5 | -12 | 2 | -4 | -3 | -1 | -3 |
| | ***3*** | 6 | 7 | 5 | 5 | 0 | 4 | 3 | 3 |
| | ***4*** | 11 | 8 | 6 | 7 | 8 | 12 | 13 | 7 |

*(Suite)*

**Table 3 out of 3**

| | **Diluted control with agitation** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 3 sessions with 8 high dilutions at $1/10^{12}$, $1/10^{14}$ … $1/10^{26}$ | | **$1/10^{12}$** | **$1/10^{14}$** | **$1/10^{16}$** | **$10^{18}$** | **$10^{20}$** | **$10^{22}$** | **$10^{24}$** | **$10^{26}$** |
| | ***1*** | -10 | -11 | -7 | 1 | -8 | -9 | -5 | -8 |
| | ***2*** | -9 | -8 | -1 | 2 | -4 | 1 | 1 | -1 |
| | ***3*** | 21 | 8 | 5 | 15 | -3 | 5 | 15 | 11 |
| 3 sessions with 8 high dilutions at $1/10^{30}$, $1/10^{32}$ … $1/10^{44}$ | | **$1/10^{30}$** | **$1/10^{32}$** | **$1/10^{34}$** | **$1/10^{36}$** | **$1/10^{38}$** | **$1/10^{40}$** | **$1/10^{42}$** | **$1/10^{44}$** |
| | ***1*** | -18 | -11 | -2 | -6 | -3 | -12 | -3 | -7 |
| | ***2*** | -5 | 1 | 1 | -4 | -2 | -6 | 1 | -6 |
| | ***3*** | 4 | 2 | 4 | 14 | 1 | -4 | 4 | 3 |
| 3 sessions with 8 high dilutions at $1/10^{46}$, $1/10^{48}$ … $1/10^{60}$ | | **$1/10^{46}$** | **$1/10^{48}$** | **$1/10^{50}$** | **$1/10^{52}$** | **$1/10^{54}$** | **$1/10^{56}$** | **$1/10^{58}$** | **$1/10^{60}$** |
| | ***1*** | -7 | -13 | -8 | -5 | -1 | -10 | -12 | -5 |
| | ***2*** | -5 | -10 | 0 | -2 | 2 | -1 | -6 | 0 |
| | ***3*** | -2 | 1 | 1 | -1 | 6 | 3 | -3 | 8 |