Chapter 4. When hearts get tangled

A blind experiment for all the participants

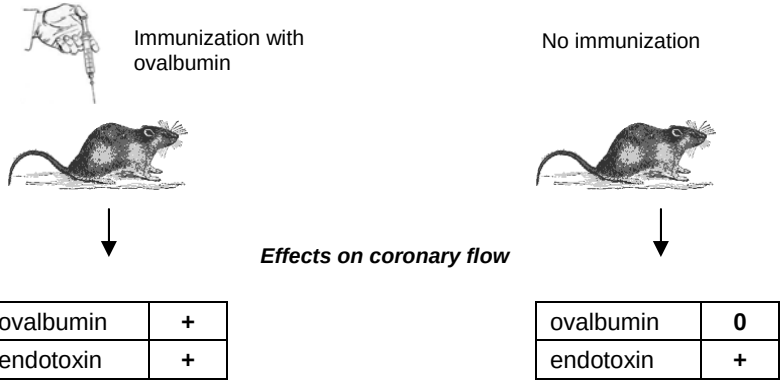
We thus resume the story after the summer 1992 when a successful transmission experiment had been performed on July 9th with visitors. One remembers that this experiment had upset the Director of Inserm. A new experiment was organized on September 28th. The purpose of J. Benveniste was to manage demonstrations with witnesses not belonging to the laboratory before drafting an article. Six new visitors attended this session.[1]

The design of this public experiment looked like the one of July 9th (see technical sheet). It was however a little more complex. Indeed, the design contained 16 tubes “to be guessed” versus only 12 in the experiment of July. Furthermore, an additional refinement was introduced: it was planned to discriminate not only the active tubes from the inactive ones, but also to determine the initial molecule from the active tubes: ovalbumin or endotoxin (LPS). The purpose was to demonstrate that during the transmission the specific activity of the initial molecule was transmitted. For that purpose, samples were tested on hearts of guinea pig immunized or not with ovalbumin. If the activity was ovalbumin-like then the heart of immunized animals shall react; if it was an endotoxin-like activity, the heart had to react whatever the state of immunization of the animal (Figure 4.1).

At first, the transmission experiment was performed. Three types of samples were prepared: samples of transmitted ovalbumin (from a solution containing 10^{-8} mol/L of ovalbumin), transmitted endotoxin (from a solution containing 10^{-8} mol/L of endotoxin) or “control” samples (from a tube of water without biological compound). The transmission experiment was described in these terms by J. Benveniste:

> “On September 28th, the transmission experiment began in the presence of Gérard Chaouat and Pierre Richard. A first vial of 10 ml-distilled water was randomly chosen by P. Richard and given to G. Chouat who distributed it in 10 tubes of 1 ml. Vials having undergone a transmission from vials of distilled water, ovalbumin 10^{-8} M and endotoxin 10^{-8} M, were also chosen at random by Pierre Richard and given to Gérard Chaouat […]. Most of the participants having then arrived, the blinding was performed in the

presence of Gérard Chaouat, Pascale Pacaud, Pierre Richard, Michel Schiff and Jean Staune."[2]



ovalbumin	+
endotoxin	+

ovalbumin	**0**
endotoxin	+

Figure 4.1. Assessment of the specificity of the "electromagnetic transmission". How to discriminate between samples "transmitted" with an ovalbumin-type or an endotoxin-type activity? Proteins such as ovalbumin have no effect on the heart of "naive" animal. If an animal has been injected with albumin (with specific experimental conditions), its immune system synthesizes allergic-type antibodies which get fixed onto organs, heart in particular. When the heart is in the presence of ovalbumin, it "reacts" (this reaction can be recorded by measuring different cardiac parameters). The heart does not react in the presence of a protein against which the animal has not been immunized. Endotoxin has an effect on the heart whatever the immunization state of the animal.
NB. For the experiment of September 28th, 1992, guinea pigs were immunized.

The random choice of samples and blinding were performed by the six participants according to a method proposed by M. Schiff. This method named "method of the envelopes" allowed blinding so that the initial label was unknown to everyone.

The method of envelopes is simple and cunning. Let us summarize it briefly. Each of the tubes for random blinding is marked with a label that identifies it. One unsticks the label which one sticks *inside* an envelope where the tube now without label is also placed. Envelopes are not sealed and are mixed. Then, for each envelope, an observer takes the tube, without looking inside the envelope, and he writes the same sign (a figure or a letter) *both on the tube and on the outside of the envelope*. He then seals the envelope. The tube can be then given to the experimenter who can test it. All the envelopes are then placed in a big envelope which is then sealed and the participants sign on its flap. For unblinding, each

inside label is placed beside the outside code. Thanks to the method of envelopes, nobody can have the information – consciously or unconsciously – because all participants are not aware of the code *including those who were directly involved in the process of blinding.*

Coherent results

During the days which followed September 28th, the contents of the tubes were tested. But the measurements were done on a slow pace. At first, the isolated hearts poorly reacted to stimuli. Thus, the measurements began late (from October 7th to 14th) and samples were tested as a precaution 10 times (on 7 hearts of immunized animals and on 3 hearts of not immunized animals). In order to inform the participant, J. Benveniste wrote:

> "We took our time to measure the experiment blinded on September 28th. Indeed, animals are currently slower to immunize, with reactions that are not as strong as before summer […] This allows us to have clear effects but not as spectacular as in the past." [3]

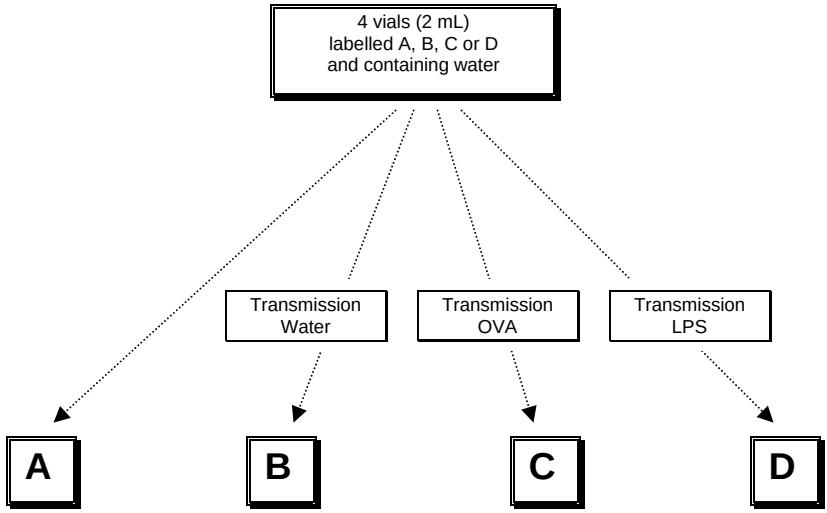
Nevertheless, the results appeared homogeneous and seemed to correspond to what was expected. J. Benveniste could thus announce:

> "Overall the results are coherent and we are particularly impressed by three experiments on hearts from animals having received alum alone, without ovalbumin *[i.e. not immunized]*, which, as expected, reveal only a single active tube and we have to hope it is the endotoxin tube."

Indeed, the open-label tubes gave expected results and, among the blind samples, five of them strongly changed the coronary flow (but had no effect in not immunized animals) and among them, as reported by J. Benveniste, only one was effective on hearts of immunized or non-immunized animals. Taking the coherence of the results into account, one is unable to help but thinking that these results were not accidentally obtained and that this experiment should be a success.

Technical sheet of the experiment of September 28th, 1992

Type of experiment: electromagnetic transmission on September 28th
Place of experiment: Clamart (for transmission and assessment of samples)
Blinding: on September 28th by 6 participants not belonging to U200; unblinding on October 22nd
Number of samples to be tested: 16 tubes tested between October 7th and 14th on 10 hearts (7 from ovalbumin-immunized animals and 3 not immunized); one part of the measurements was performed on the two Langendorff devices in parallel.
Additional in-house blinding: no



Blinding of 16 tubes* numbered from 1 to 16 (*blind tests*):

5 tubes "**A**"; 5 tubes "**B**"; 5 tubes "**C**"; 1 tube "**D**"

+

4 tubes not blinded (*open-label tests*):

1 tube "**A**"; 1 tube "**B**"; 1 tube "**C**"; 1 tube "**D**"

*Dilution at 1/1000 in physiological saline for heart infusion

"There is no crucial experiment"

On October 22nd, the experience was unblinded in the presence of an audience of about ten people.[4] M. Schiff prepared an introductory document in which he reminded some principles of "applied psycho-socio-epistemology":

> "In a chain of reasonings, the skeptic looks for the weakest link, according to the logical idea that a chain has the solidity of its weakest link.
> Thus the game of the critics consists in raising questions such as "Did he calibrate his test of degranulation? Did he perform kinetics? Did he cover his tubes with a silicone film?" etc… The trap for the experimenter consists in adopting one of the two attitudes, which are both indefensible. You begin by considering these arguments, with more or less conviction, then at one point you say "they piss off me, they are dishonest". Even if it is true that some opponents have an irrational attitude, this is not reason enough to be irrational oneself." [5]

M. Schiff then explained his own conception of an approach susceptible to be constructive during a change of scientific theory:

> "I believe that the correct attitude from both an epistemological point of view and from a point of view of balance of power in the context of any change of scientific theory consists in examining the relevance or the absence of relevance in the arguments. From this point of view, the statistical reasoning and the use of blind manipulations can bring solid arguments, without being however sufficient. I am repeating my personal conviction to you that there is no crucial experiment. The change of paradigm occurs following a convergence of presumptions going to the same direction, which eventually achieves general agreement."

Then he explained how the statistical reasoning could bring forceful arguments, particularly in the field of biology where the studied objects are never identical making the application of the experimental method more delicate:

> "Let a biological object O1 to which I apply a treatment T, and which becomes different. To prove that the change is really attributable to the treatment, I have to compare the evolution of the object O1 to that of another biological object O2. The statistical reasoning allows comparing not objects but populations

> of objects. The fact that, individually, objects inside a population are different, both intrinsically and because of errors or fluctuations in the manipulations becomes *not relevant*, or to be more precise, it acts only on the signal to noise ratio".

It is thus an experimental approach totally different from a "horse-race approach" as J. Benveniste was accustomed. M. Schiff specified:

> "In other words, when you use a statistical approach, in which you analyze two populations statistically equivalent by randomization, all the arguments about the lack of reliability of your operations turn against the skeptics: the fact that a result is statistically significant *in spite* of the inevitable fluctuations and the inevitable unknowns show that the physical or biological meaning is *bigger* than the one empirically observed, and not weaker as one often believes."

M. Schiff finally explained the best possible strategy using a statistical approach:

> "To resume the argument of the chain, the strategy consists in concentrating the argumentation and the attempt of proof on a single link, at the same time easy to display and difficult to attack from a logical point of view. The reasoning consists in saying that. Let two samples P1 and P2, which are obtained from the same original population of objects P. I applied the treatment T1 to the population P1 and I applied the treatment T2 to the population P2, with T2 identical to T1, except a part t. I observed a statistically significant difference between P1 and P2. I attribute the difference of the effects to the difference of treatments, which is symbolized by t."

And he concluded:

> "All the difficulty consists in proving, or rather in convincing oneself and then the others, that the treatment T2 actually differs from the treatment T1 only by the part t and not by a hidden part e. In the arguments of the skeptics, this hypothetical hidden difference e (e symbolizes the error) can contain the unconscious biases of the observer, the errors of manipulation such as the accidental contamination of a sample, and even, without being explicitly stated, a fraud.
>
> I think that it is impossible to individually counter each objection, and that it is better to consider the unknown effects as

> black boxes, and it is here that the procedures of randomization and blinding are involved."

Finally, the unblinding …

Then, after the theory, the practice succeeded and the experiment was unblinded. The big envelope was opened and the small envelopes were extracted. The codes and the corresponding transmitted activity were successively announced.

There was some disappointment. Among 16 tubes, 12 fitted with what was expected, but for the 4 other tubes there was some bewilderment (Table 4.1). Indeed, sample n°11 that was supposed to contain endotoxin-like activity turned out to be "naive" water which directly came from the vial and did not even undergo any transmission process.

A discussion began with two dominant attitudes among the participants:

> "After the unblinding […], two points of view expressed themselves. The first one consisted in trying to understand the imperfect character of the results, in particular with the hypothesis of a double inversion of tubes. This first point of view is argued by J. Benveniste who points out that the blind results with tubes 10 and 11 do not concern transmission, because these tubes were supposed to come from the pure water batch. The second point of view, argued by M. Guyot and M. Schiff, consisted in centering the attention on the results of the statistical analysis."[6]

One understands that J. Benveniste who tried "to guess" the "good" tubes preferred this type of explanation. He clanged consequently to the idea of an error when blinding was done. He thus pointed out that a simple inversion of two couples of tubes would allow obtaining the correct results (Figure 4.2). M. Schiff, on the contrary, faithful to the probabilistic approach that he had developed into the introduction, calculated that the odds of success were only 1 on 60 to find 4 of the 5 ovalbumin-type transmitted tubes among 15.

Tested recordings	Maximal changes of coronary flow (%)		Increasing order of biological activities (immunized animals)	Unblinding
	Immunized animals (7 measurements)	Non-immunized animals (3 mesuremenets)		
Blind tests				
n°12	3.0 ± 1.0	6.0 ± 1.7	1	**Ova tr.**
n°6	3.4 ± 1.6	2.7 ± 1.2	2	Water tr.
n°13	3.4 ± 1.9	2.7 ± 1.2	3	Water
n°8	3.4 ± 2.8	4.3 ± 2.5	4	Water tr.
n°2	3.6 ± 1.0	3.7 ± 1.5	5	**LPS tr.**
n°4	4.0 ± 1.6	3.7 ± 1.5	6	Water tr.
n°3	4.1 ± 2.0	4.3 ± 2.5	7	Water tr.
n°16	4.7 ± 2.4	3.7 ± 1.5	8	Water
n°9	4.9 ± 1.7	3.7 ± 1.5	9	Water tr.
n°14	6.4 ± 3.4	3.3 ± 1.2	10	Water
n°11	10.0 ± 2.1	13.7 ± 1.5	11	Water
n°5	15.4 ± 2.9	6.7 ± 1.5	12	**Ova tr.**
n°1	15.4 ± 4.5	2.3 ± 0.6	13	**Ova tr.**
n°10	15.9 ± 4.0	3.3 ± 2.3	14	Water
n°7	16.7 ± 3.6	3.7 ± 1.5	15	**Ova tr.**
n°15	20.0 ± 8.0	4.3 ± 0.6	16	**Ova tr.**
Open-label tests				
Water	2.6 ± 0.8	3.3 ± 2.3	-	-
Water tr.	4.4 ± 2.1	4.0 ± 2.0	-	-
Ova tr.	17.3 ± 3.1	6.3 ± 2.5	-	-
LPS tr.	12.0 ± 2.4	14.3 ± 3.5	-	-
Ova 0.1 µmol/L	24.9 ± 5.0	6.7 ± 4.0	-	-

Means ± standard deviations

Tableau 4.1. Results of the experiment of September 28th, 1992. This table describes the results obtained with the 7 hearts from ovalbumin-sensitized animals (i.e. hearts that were expected to react as well to "ovalbumin activity" as to "endotoxin activity") and with the 3 hearts from non-sensitized animals (i.e. expected to react only to "endotoxin activity".) Open-label samples also included ovalbumin at 0.1 µmol/L. This control was always the last tested sample on a given heart in order to assess the sensitivity of the physiological preparation and to check the immunization state of the animals for albumin.

Open-label samples gave expected results. With blind samples, 6 out of 16 were associated with a change of coronary flow in albumin-sensitized animals and only 1 sample in non-sensitized animals. Before unblinding, observed results were thus consistent with expected results. After unblinding (last column), there were some inconsistencies in the results.

● : transmitted ovalbumin ; ○ water (naive or transmitted) ; ■ : transmitted endotoxin

n° tube	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
code	●	■	○	○	●	○	●	○	○	○	○	●	○	○	●	○
result	●	○	○	○	●	○	●	○	○	●	■	○	○	○	●	○

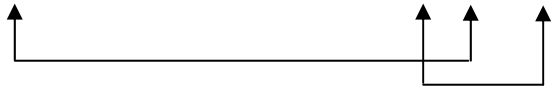


Figure 4.2. In the experiment of September 28th, 12 tubes out of 16 were correctly "guessed". To explain this imperfect result, J. Benveniste suggested that two couples of tubes (2-11 and 10-12) had been inverted by mistake.

For this reason, trying to explain the cause of this partial failure, J. Benveniste again performed during the next days the measurements by using samples which had been prepared on September 28th, but which had not been included in the blinding tests (only a part of the the tubes that have been prepared were used after random selection). He asked to Jacques Testart (the "biological father" of the first French "test-tube baby" who worked in the same building) to blind the tubes:

> "On October 23rd, J. Testart blinded 13 remaining tubes, which had not been used for the blind experiment of September 28th: 4 ovalbumin, 4 naive water, 4 transmitted water, 1 endotoxin. We measured them on October 23rd and 26th and J. Testart unblinded them on October 27th. Result: 100% of the measurements are correct. The hypothesis of the inversion of 2 tubes [7] – at which moment? – is strengthened by these experiments."

J. Benveniste suggested for next experiments that two people managed each stage and he concluded:

> "In spite of some errors and uncertainties, which we will try hard to avoid afterward, the experiment of September 28th goes in the same direction as our recent open-label observations and also this one which was performed on July 9th in blind conditions: the hypothesis of a transmission of biochemical information by a magnetic way appears to us at present as the most economic one."

An error of manipulation was indeed always possible, but the precautions and the important number of participants who mutually watched themselves implied that this hypothesis was admitted only by default. The fact that the "good results" were obtained after unblinding of the new experiments made

with the original samples was actually in favour of an error during the blinding process. However, this *a posteriori* argument could satisfy only those who were already convinced as for the reality of the phenomenon supposed to be highlighted.

Notes of end of chapter

[1] P. Richard (Scientific director, Bouygues), G. Chaouat (biologist, CNRS, Hospital Antoine Béclère, Clamart), A. Fiebig (Ecole Normale Supérieure Cachan), J. Staune (European University of Paris), P. Pacaud (SAUR), M. Schiff.

[2] J. Benveniste. Compte rendu du décodage de l'expérience du 22 octobre 1992. *[Report of the unblinding of the experiment of October 22nd, 1992]*.

[3] Letter of of J. Benveniste of October 13th, 1992 "to the participants of the treansfert experiments".

[4] Participants present at the unblinding meeting of October 22nd, in addition to J. Aïssa, J. Benveniste and M. Schiff: Gérard Chaouat (biologist, CNRS, Hospital Antoine Béclère, Clamart), Raphaël Douady (CNRS, Ecole Normale Supérieure, Paris), Alexandre Fiebig (Ecole Normale Supérieure Cachan), Jean-Yves Follézou (physician, Hospital Pitié-Salpétrière, Paris), Marcel Guyot (physicist, CNRS, Meudon-Bellevue), Geneviève Potier de Courcy (ISTNA-CNAM, Paris), Pascale Pacaud (SAUR), M. Reynier (from the laboratory of Henri Laborit, Hospital Boucicaut, Paris), Alfred Spira (epidemiologist, Inserm U 292), Jean Staune (vice-president of the European University of Paris), Jacques Testart (biologist, Inserm U335, Clamart), Yolène Thomas (CNRS, Inserm U200).

[5] M. Schiff. Note de préparation à la séance d'ouverture du code pour l'expérience de transmission du 28 septembre 1992 ; datée du 15 octobre 1992. *[Preparatory note of the unblinding meeting for the experiment of September 28th, 1992]*.

[6] J. Benveniste. Compte rendu de l'expérience du 28 septembre 1992. *[Report on the experiment of September 28th, 1992]*.

[7] In fact, according to this logic, there were two inversions of two tubes.