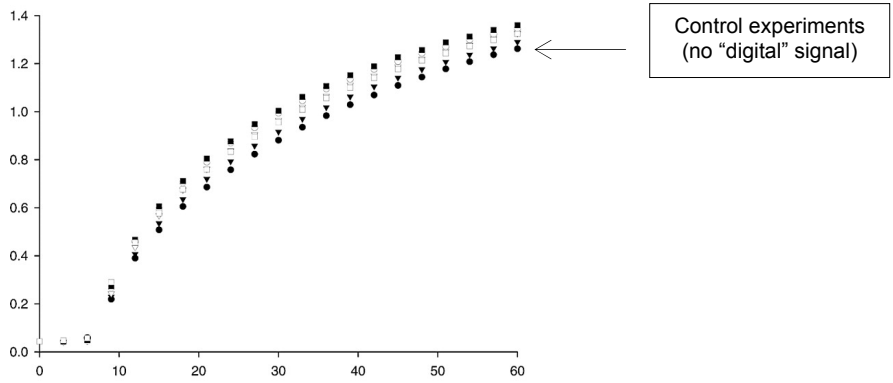
Chapter 23. "The digital signal appeared to work!"

"The results are highly significant"

The robot analyzer acquired by Digibio thanks to the credits of the DARPA was thus settled in a laboratory of the National Institute of Health in Bethesda (Maryland). J. Aïssa and D. Guillonnet came to run it from July 14th to 21th, 2001 during the phase of the expertise named pre-pilot study. The purpose of this pre-pilot study was to verify that everything worked correctly. J. Aïssa and D. Guillonnet also did some informal experiments which allowed to notice in a satisfactory manner that the inhibitory digital signal was also efficient on the other side of the Atlantic Ocean and they explained the functioning of the robot.

After the pre-pilot phase, the control experiments (without any digital signal) were performed by the American experts that indicated high degree of reproducibility. On the basis of these trials, it was calculated that four experiments would be sufficient from a statistical point of view to detect a 20% difference of the active digital signals compared with control conditions (Figure 23.1).



(Reproduced from W. Jonas et al, Faseb J 2006 ; 20 : 23)

Figure 23.1. Exemple of an experiment performed by the robot analyzer (in the absence of any "digital" signal) by the U.S. experts between the pre-pilot and pilot phases. These trials allowed evidencing the low variation from one sample to another. According to the U.S. experts, the variation was less than 1% for 10 experiments performed during 5 different days.
x-axis in min and *y*-axis in optical density units.

Before continuing, it is necessary to note that a modification was introduced by the team of J. Benveniste to make the experiments. In order to avoid being dependent on the supply of plasma, which could sometimes be difficult, it was now the effect of thrombin on fibrinogen that was measured. The purpose of the experiment did not change since fibrinogen is the soluble plasma molecule which is transformed into insoluble fibrin by thrombin and participates in clot. There was thus a purely biochemical system. This *in vitro* reaction could be also easily measured by a spectrophotometer because insoluble fibrin absorbs light.

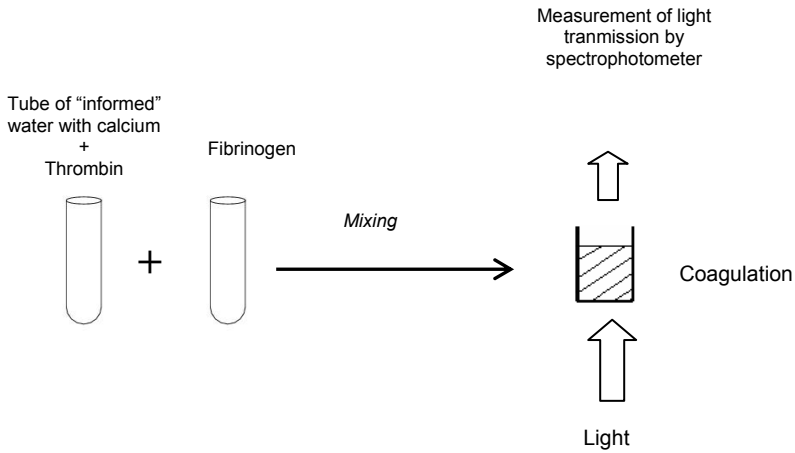


Figure 23.2. Principle of the transformation of fibrinogen (24 mg/mL) in fibrin by thrombin (0.3 µg/mL) *in vitro* with reading of the optical density by the spectrophotometer of the robot analyzer. Thrombin is an enzyme which transforms soluble fibrinogen into insoluble fibrin. The more fibrin is produced, the more light is absorbed. Water "imprinted" with "digital signal" which inhibits the effect of thrombin (active recording) could be assessed in comparison with water "imprinted" with "water signal" (inactive control).

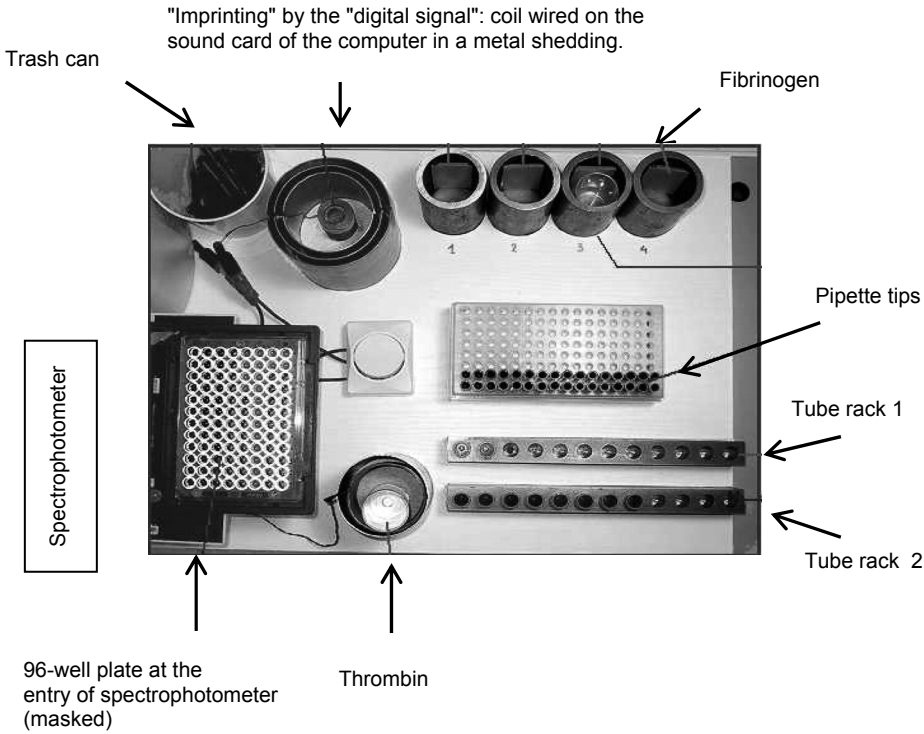
The solution of thrombin was exposed to the digitized thrombin inhibitor or to digitized water (control), mixed with fibrinogen and added to wells. Everything was automatic except the preparation of stock solutions of fibrinogen and thrombin. Indeed, the functioning of the robot was the

following one. Having placed the solution of fibrinogen and the solution of thrombin in their respective places, the software which piloted the machine was started. The arm of the robot which was equipped with a pipette distributed thrombin in tubes and placed them in the coil which broadcasted the digitized signal. The tube of water containing thrombin was "informed" during 10 minutes and replaced on the tube rack. The same tasks were performed for the other tubes. The order of the "digitized signals" was random. Fibrinogen was then added to each of the "informed" tubes the content of which was put into two wells of a "96-well plate". The optical density of the fibrinogen-thrombin mixture was automatically measured every 60 seconds during one hour (Figure 23.3).

Then, the next phase, namely the pilot study, was performed from October 30th to November 3rd, 2001 with J. Aïssa, D. Guillonnet and J. Benveniste. The aim of this phase was to verify in a formal way that the team of J. Benveniste obtained the claimed results. A protocol was defined and was accepted by all participants. The protocol consisted in "informing" samples containing thrombin according to the "information" of three different recordings: digital thrombin inhibitor (DTI), signal water and no signal, that is one active signal and two inactive signals. Every signal was transmitted to the output coild and "played" during ten minutes. The digital signals were recordings of 3 seconds played in loop during ten minutes. Every experimental point was performed in duplicate. The experimenter did not know in which order the various experimental points were performed. He knew the result of the experiment only at the end of the experiment.

J. Ives told how this pilot phase took place:

> "The next phase was the pilot phase. During this phase Benveniste's team were present and ran the experiments using the robot. They performed twenty-one (*sic*) experiments, each consisting of the three conditions in duplicate. A twenty-one to twenty-eight percent inhibition by digital thrombin [*inhibitor*] (DTI) was observed compared to the water signal (WAT) or the no-signal (NS) condition. Statistical analysis indicates that the results are highly significant ($P < 0.0001$). The digital signal appeared to work!" [1]



(Reproduce from W. Jonas et al, Faseb J 2006; 20: 23)

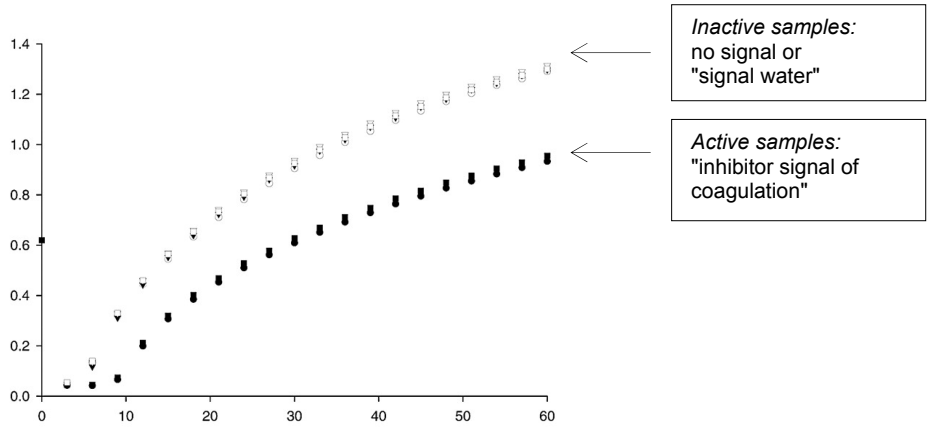
Figure 23.3. Sequence of the operations performed by the robot analyzer. In order to prepare the robot, 12 tubes were placed (manually) on the extreme left of the tube racks 1 and 2 (6 tubes per rack), containers for thrombin and fibrinogen are placed in their respective locations. Note that containers and tubes were placed in metallic shields (muffs or racks) to protect theirs contents from electromagnetic waves. Then the robot was started up.
(1) The articulated arm (not visible) of the robot which carried a pipette arms took a single-use tip and distributed equal volumes of thrombin in each of the tubes of the rack 2.
(2) The arm placed each of these tubes of the rack 1 in the coil to expose it to the electromagnetic field; the tube received one of the three possible signals: "signal water" as control, "anticoagulant signal" or "no signal". After 10 minutes of exposure, each tube was put back in place. The single-use tip was thrown in the trash can and a new tip was placed at the end of the pipette carried by the arm of the robot.
(3) After 60 minutes, fibrinogen was added to the first tube (on the left) of the rack 2.

(To be continued next page)

(continued from previous page)

(4) Thrombin of tube 1 in portoir 1 was taken and added to tube 1 in rack 2 and then mixed by repeated aspiration-expulsions.
(5) The content was put down in two adjacent wells of the 96-well plate (duplicate).
(6) The same process from (3) to (5) was repeated for the 5 other tubes.
(7) The 96-well plate was introduced in the spectrophotometer and a measurement of optical density was performed for each well every 3 minutes for 1 hour.

More exactly, the article co-authored by W. Jonas indicated that 16 experiments were performed during the pilot phase. The differences of coagulation did not exceed 1% between the experimental points of "digitized water" (control) and "no signal" (that is the controls). In 7 experiments, a decrease from 21 to 28% of coagulation was observed with samples "informed" with digitized anticoagulant compared to controls (Figure 23.4).



(Reproduced from W. Jonas et al, Faseb J 2006 ; 20 : 23)

Figure 23.4. Pilot phase (October 30th–November 3rd, 2001): example of an inhibitory effect obtained during the expertise with a "digital signal". During each experiment, three experimental conditions were compared; each of the three experimental conditions was performed in duplicate. The three experimental conditions were: no signal (open circles, open square), signal "water" (closed triangle, open triangle), "inhibitory" signal (closed circle, closed square). On the figure, the effects observed with 4 controls (2 wells "no signal" and 2 wells "signal water") and 2 "active" signals (2 wells with "inhibitory signal") are represented. One notices that the "inhibitory digital signal" actually inhibited coagulation.
Out of 16 experiments performed during the pilot phase, inhibition was evidenced for 7 of them (mean inhibition from 21 to 28%). Consequently, the results obtained during the pilot phase were in favour of "digital biology".
x-axis in min and *y*-axis in units of optical density.

Everything was thus fine and the future of "digital biology" looked bright. Overall, during the pre-pilot phase and the pilot phase, 11 experiments out of 23 gave outcomes in favor of the reality of the effects of the "digital signals" which inhibited coagulation by 24% on average; statistically speaking, these results were extremely significant.

The successful experiments having been performed in blind conditions and with an automatic device, a complete failure would be surprising after the departure of the French team. Indeed, the manipulation of the robot did not require a great manual skill or specific expertise. As already mentioned, the work of the operator was limited to set up consumables (tips of pipettes, tubes) and reagents. Then, one pushed a button and the experiment was automatically performed from the random choice of digital recordings to the printing of the results.

However, the U.S. team made the following observation:

> "A subgroup analysis of pilot phase data showed that all DTI *[digital thrombin inhibitor]* effects occurred when experiments were conducted by one member of Benveniste's team (Jamal Aissa) and that this usually occurred when using a split sample technique in which he interrupted the operation of the ABA *[automated bio-analyzer]* machine to do manual plating followed by the automated plating. Two of the 16 experiments done only by the ABA machine (no interruption) showed effects when Jamal was present. In three instances Jamal set up experiments and then left for the day. None of these showed DTI effects." [2]

The French team was hardly surprised with this possible "influence" of J. Aïssa and it revealed to the U.S. researchers that indeed it had been noticed that some individuals were "facilitators" while others were "inhibitors"; J. Aïssa was obviously a member of the first category. But the fact that this observation could question the reality of "digital biology" did not appear to be a source of concern within the team of Clamart. Indeed, the U.S. researchers noticed that when the results were not in accordance with the expectations, the French team still put the blame on material failure.

What were the results obtained during the test phase after the departure of J. Benveniste and of his collaborators? Did the absence of J. Aïssa affect these promising results?

Notes of end of chapter

[1] J. Ives. Evaluating unusual claims and devices using a team approach: A case study. *Subtle Energies & Energy Medicine* 2002; 13: 39–59.

[2] Jonas WB, Ives JA, Rollwagen F, Denman DW, Hintz K, Hammer M, Crawford C, Henry K. Can specific biological signals be digitized? *FASEB J* 2006; 20: 23–8.