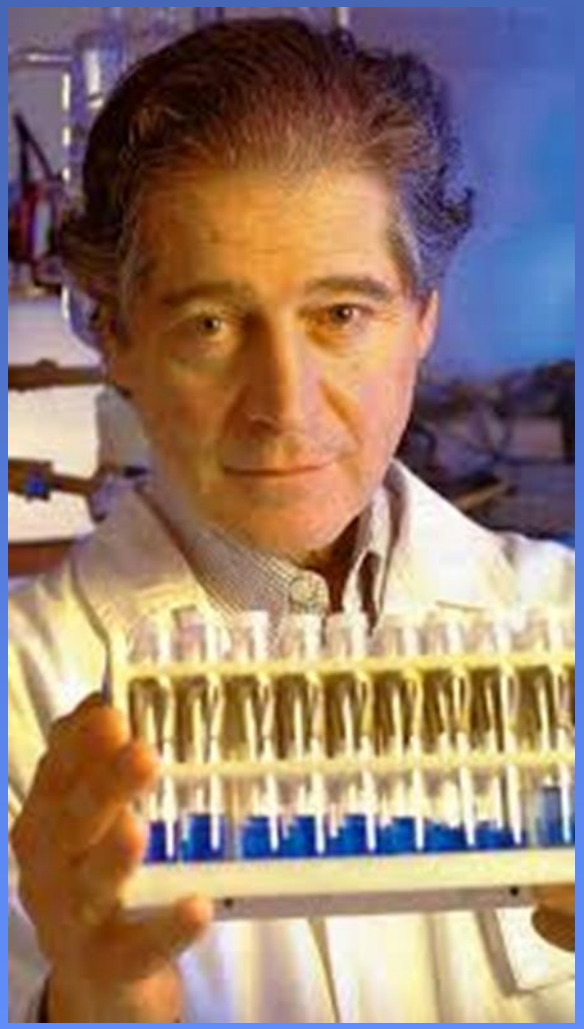
In memory

Dr Benveniste



From high dilutions to digital biology: 30 years of experiments

Jamal Aïssa, PharmD (France)

History

The high dilution story (dubbed the "Memory of Water")

1984 Basophil degranulation triggered by high dilution of serum anti-IgE.

1988 Publication in *Nature*, followed by an “inquiry”.

1991 Erasing of high dilution activities by an oscillating magnetic field. (collaboration with a CNRS team)

1992 Electronic transfer (via an amplifier) of biological information to a tube of water.

1995 Digitization: recording then replay of the biological message using a computer.

1998 Activation by agitation of a solution at very low concentration (down to 10^{-14}M)

Biological Sensitive Systems

Systems___

1984-1986	- Basophil degranulation
1990-1998	- Isolated guinea-pig heart (Langendorff)
	- Neutrophil activation
1997-1998	- Ag/Ab precipitation
	- Skin test (guinea-pig or rabbit)
1999	- Delayed (or shortened) blood coagulation

Biological Systems (1)

- 1984-1990 1) **Basophil degranulation*** by highly dilute anti-IgE antibodies *(Nature*, 1988*);* 2) Inhibition of basophil degranulation by highly dilute histamine.

  *(in fact loss of staining by alkaline dyes, without histamine release)

- 1990-1998 3) **Isolated perfused guinea-pig heart** (Langendorff). Over 30 substances tested, first at high dilution, then by direct transmission using an amplifier and finally by recording/reply of the molecular signal by means of a computer. 4) **Neutrophil activation** by Phorbol-Myristate-Acetate transmitted in real time by an amplifier. (See article in « Medical Hypotheses »)

Publication of a controversial paper

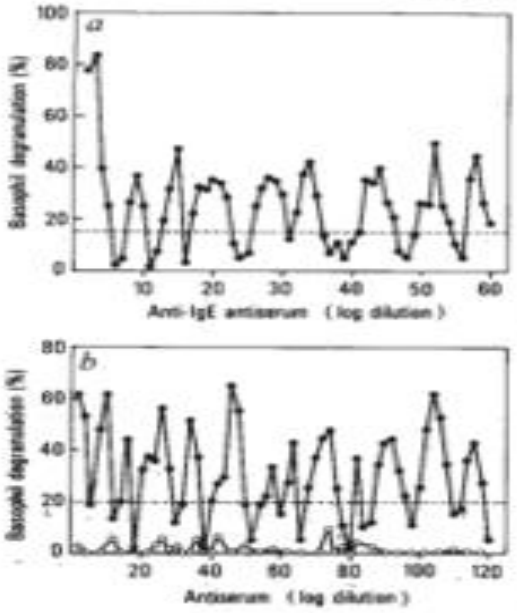


Fig. 1 Human basophil degranulation induced either by anti-IgE anti-serum (●) diluted tenfold from 1×10^{-1} down to 1×10^{-40} (*a*) or hundredfold down to 1×10^{-120} (*b*) or by anti-IgG anti-serum (○) diluted hundred-fold from 1×10^{-2} down to 1×10^{-120} (representatives of at least 10 experiments for anti-IgE and 4 experiments for anti-IgG). The significant ($P < 0.05$) percentage of degranulation was 15% (*a*) and 20% (*b*). (.....) relation to the number of counted basophils from control wells[15].

Methods Goat anti-human IgE (Fc) antiserum or as a control, goat anti-human IgG (Fc) antiserum (Nordic Immunology, The Netherlands) was serially diluted as indicated above in HEPES-buffered Tyrode's solution (in g l^{-1}: NaCl, 8; KCl, 0.195; HEPES, 2.6; EDTA-Na_4, 1.040; glucose, 1 human serum albumin (HSA), 1.0; heparin, 5000 U per l; pH 7.4). Between each dilution, the solution was thoroughly mixed for 10 s using a Vortex. Given the molecular weight of IgG molecules (150,000), the 1×10^{-40} and 1×10^{-120} dilutions correspond in the assay to 2.2×10^{-46} M (th) and 2.2×10^{-126} M (th) respectively. Venous blood (20 ml) from healthy donors was collected using heparin (1 U per ml) and a mixture of 2.5mM EDTA-Na_4/2.5 mM EDTA-Na_2 (final concentrations) as anticoagulants and allowed to sediment. The leukocyte-rich plasma was recovered, twice washed by centrifugation (400*g*, 10 min) and finally resuspended in an aliquot of HEPES-buffered Tyrode's solution. The cell suspension (10 µl) was deposited on the bottom of each well of a microtitre plate containing 10 µl $CaCl_2$ (5 mM final) and 10 µl of either of anti-IgE or anti-IgG antiserum dilutions. To a control well were added 10 µl $CaCl_2$ and 10 µl Tyrode's but no anti-IgE or anti-IgG antiserum. Plates were then incubated at 37°C for 30 min. Staining solution (90 ml: 100 mg toluidine blue and 280 µl glacial acetic acid in 100 ml 25% ethanol, pH 3.2 — 3.4) was added to each well and the suspension thoroughly mixed. Specifically redstained basophils (non-degranulated basophils) were counted under a microscope using a Fuchs-Rosenthal haemocytometer. The percentage of basophil degranulation was calculated using the following formula: Basophil no. in control − basophil no. in sample/ basophil no. in control × 100

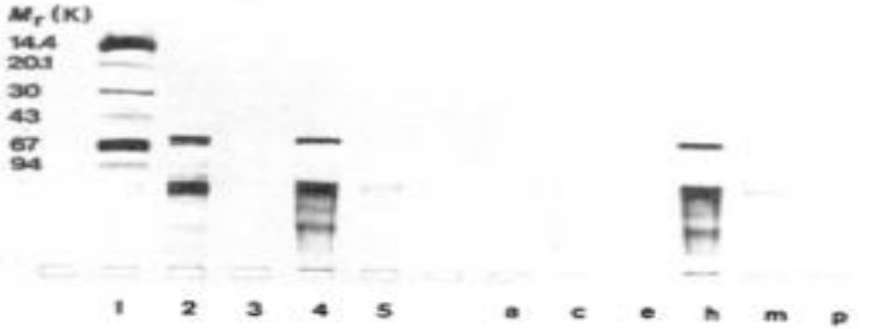


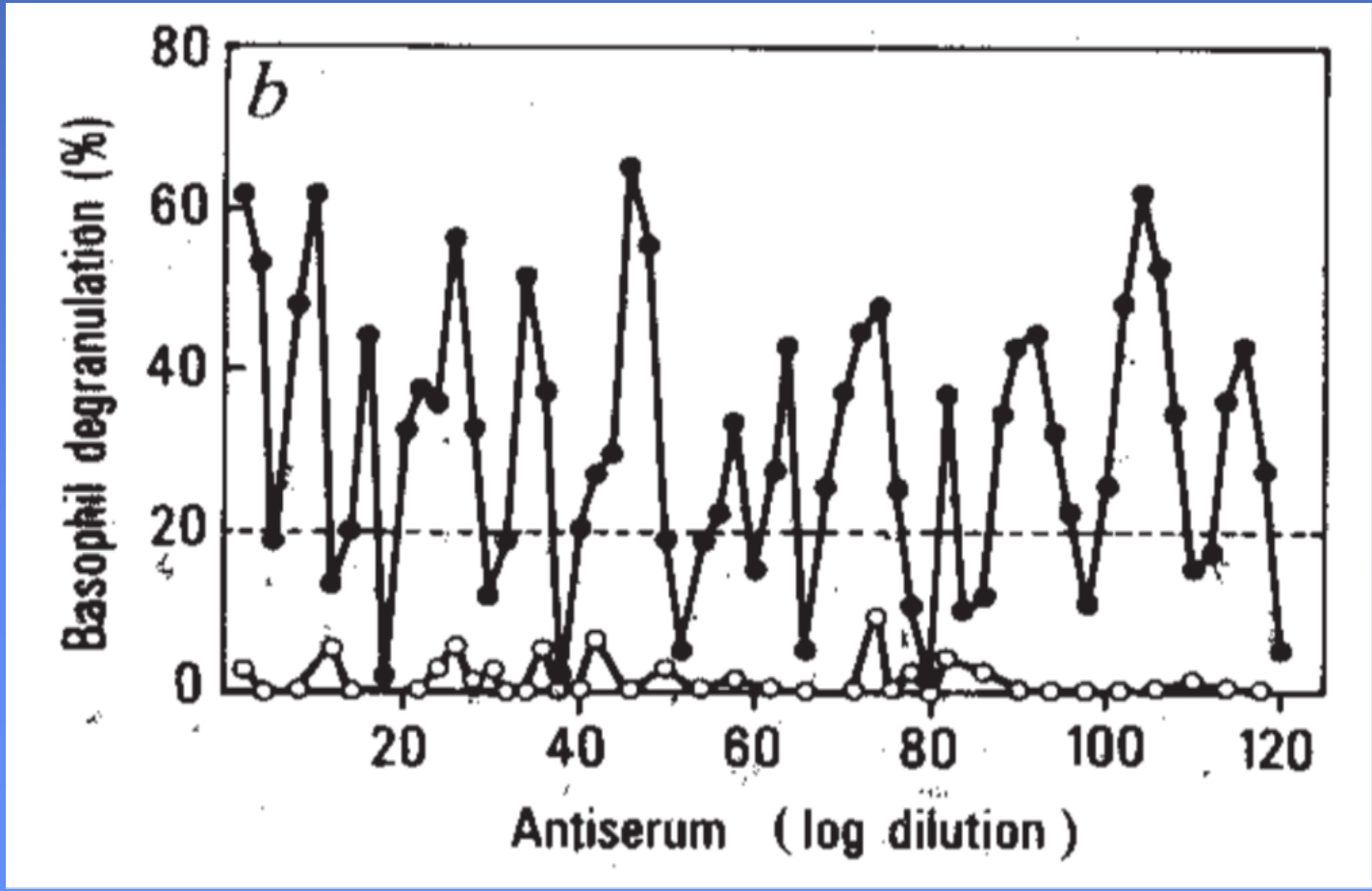
Fig. 2 Electrophoresis (polyacrylamide 7–15%, bands revealed by silver staining): samples numbered 1 to 5 are standards for the blind experiments *a*, *c*, *e*, *h*, *m*, *p*. Lane 1, Molecular weight standards for electrophoresis; lane 2, monoclonal IgG added with human serum albumin; lane 3, Tyrode's buffer without human serum albumin; lane 4, 1×10^{-2} anti-IgE dilution; lane 5, 1×10^{-3} dilution. Samples tested blind: *a* and *c*, buffer; *e*, 1×10^{-40} anti-IgE dilution; *h*, 1×10^{-2} anti-IgE dilution; *m*, 1×10^{-3} anti-IgE dilution; *p*, 1×10^{-40} anti-IgE dilution.

So we performed another almost identical experiment, using 6 tubes containing unlyophilized samples and buffer without HSA. Four tubes contained antibody at 1×10^{-2}, 1×10^{-3}, 1×10^{-40} and 1×10^{-80} dilutions, and 2 contained buffer alone. These tubes were coded and assayed according to the above protocol. The decoded results were clear-cut, high basophil degranulation being obtained with 1×10^{-2}, 10^{-3}, 10^{-40} and 10^{-80} dilutions, but no anti-IgE activity or immunoglobulins were detected either in the control tubes or in assays containing the 1×10^{-40} and 10^{-80} dilutions (Tables 2 and 3 and Fig. 2). Thus there is no doubt that there was basophil degranulation in the absence of any detectable anti-IgE molecule.

These results may be related to the recent double-blind clinical study of Reilly *et al.*[4] which showed a significant reduction of symptoms in hay-fever patients treated with a high dilution (1×10^{-40}) of grass pollen versus placebo, and to our *ex vivo* experiments in the mouse[5]. We have extended these experiments to other biological systems: using the fluorescent probe fura-2, we recently demonstrated changes in intra-

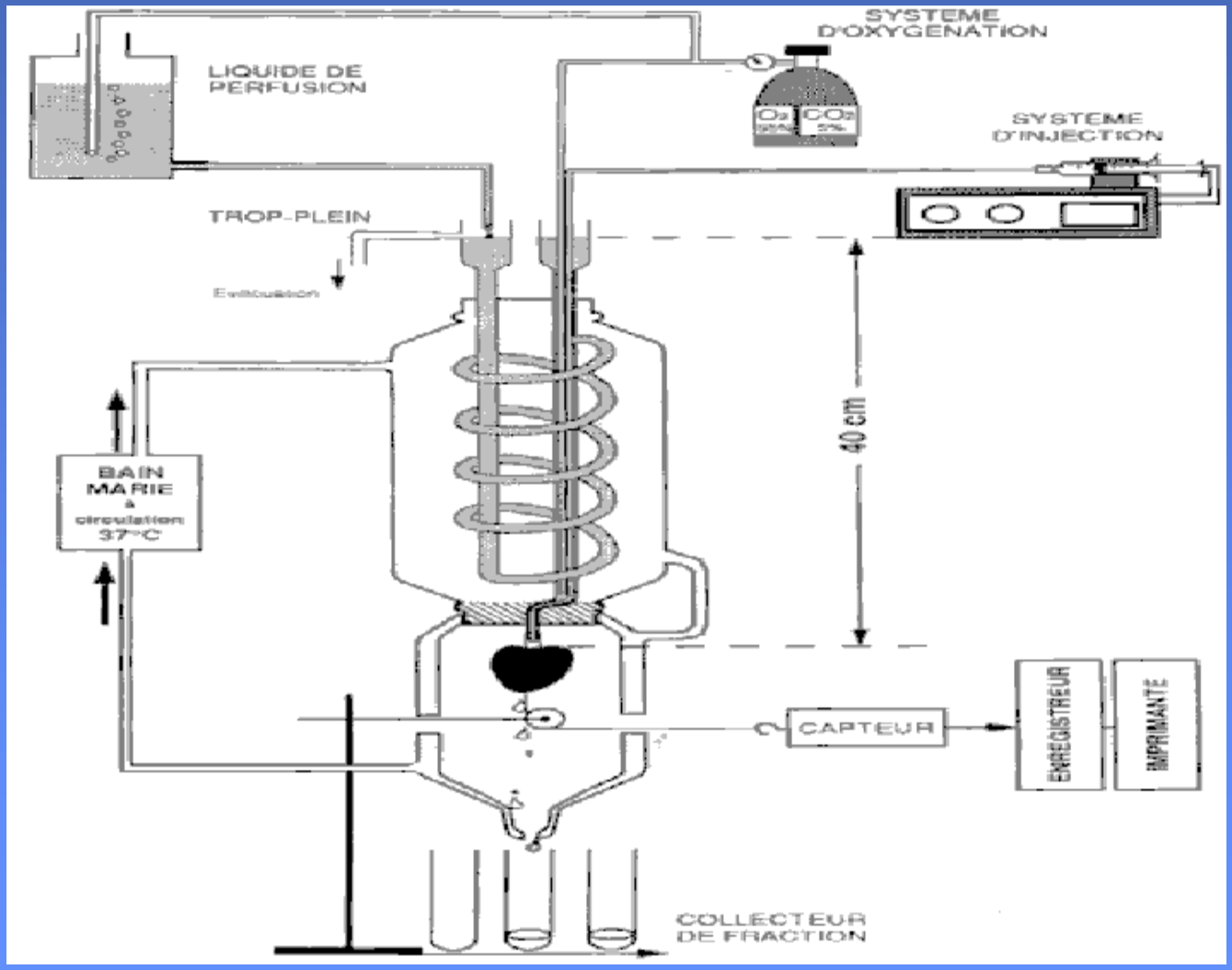
Publication of a controversial paper

Basophil degranulation triggered by very dilute antiserum against IgE " Davenas E, Beauvais F, Amara J, et al, Nature 1988;333: 816-8

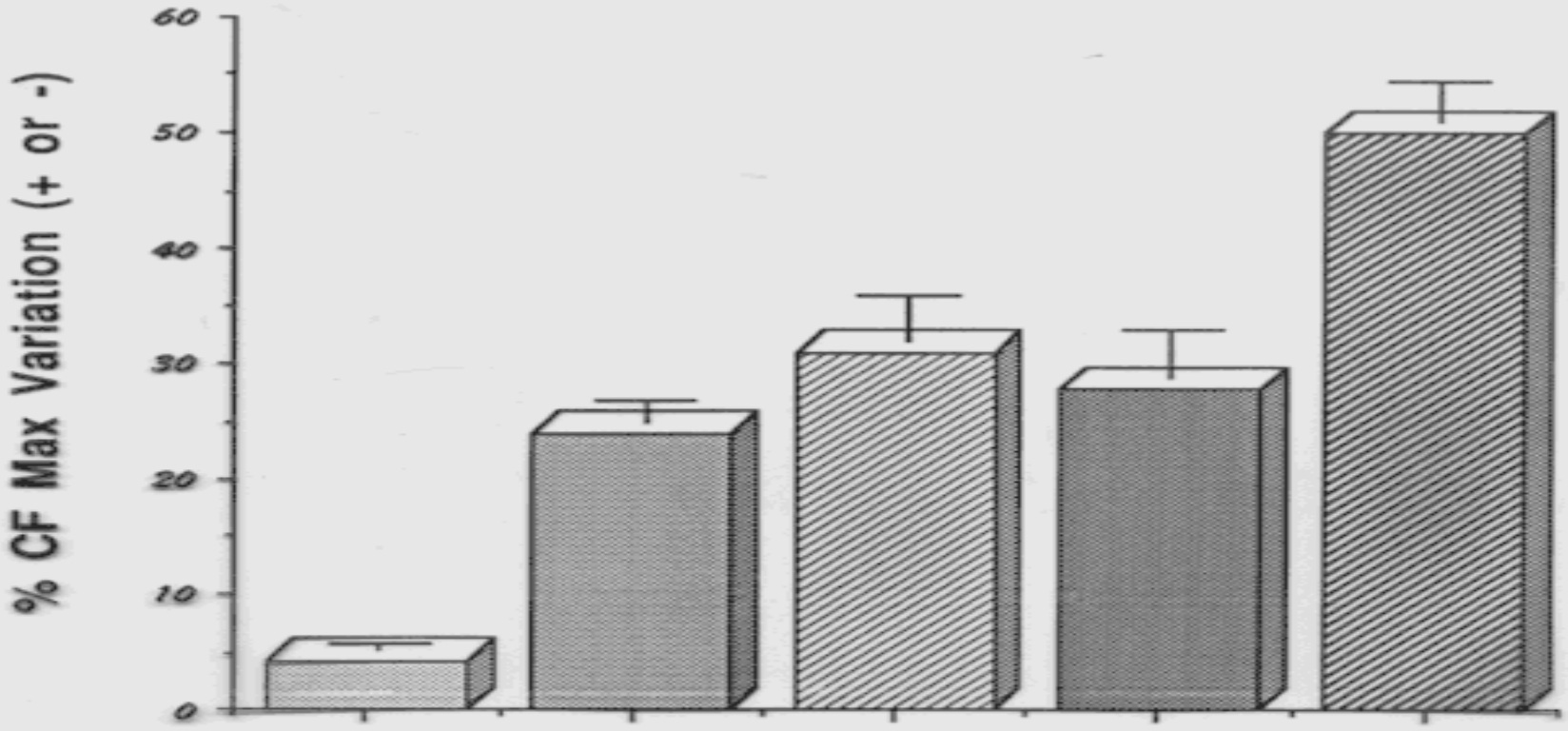


- **Anti-IgE anti-serum**
- **Anti-IgG anti-serum**

Isolated guinea-pig heart (Langendorff)



EFFECT ON CF OF VARIOUS AGONISTS
(GP immunized with Ova-Alum)



(log 31 - 41)

	TKH	Hist	Ova	H -7M	O -7M
n =	38	77	43	30	37
Comp. w.					
TKH : p=		7.7e-10	1.8e-8	2.4e-8	1.6e-16
H : p=			*	*	
O : p=					7.1e-4

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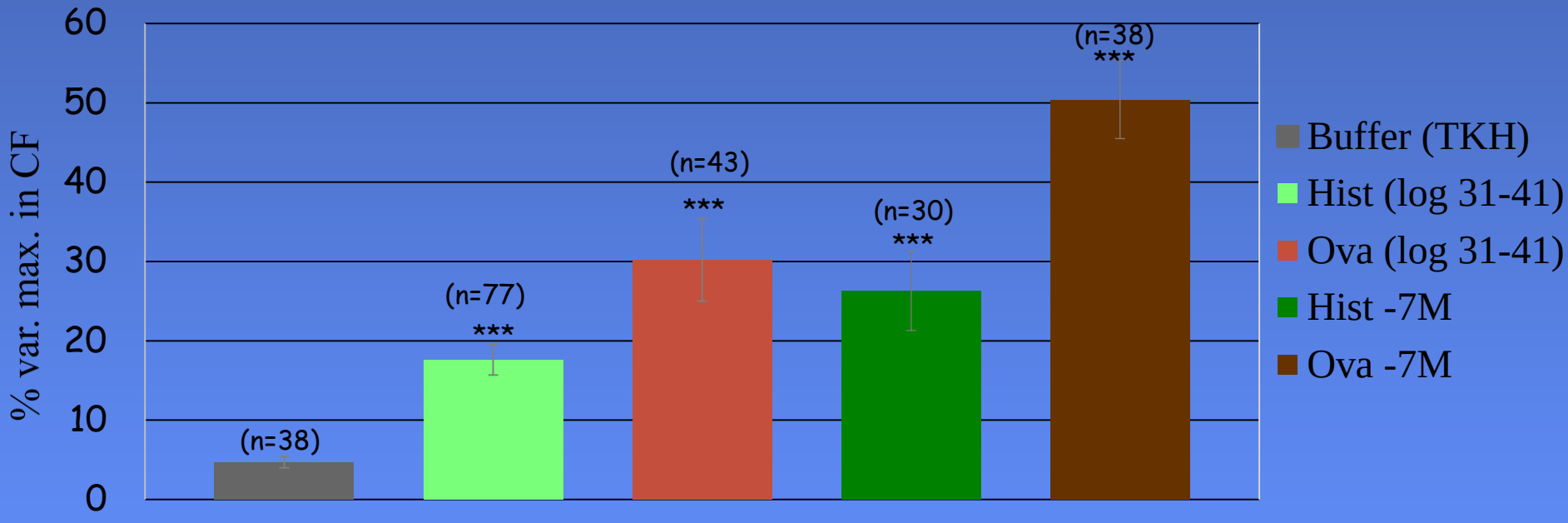
* ns at 0.05

Effect on CF of various agonists at HD

(Guinea-pig isolated heart - % var. in coronary flow)



*** p < 0,001 compared with buffer

BIOLOGICAL ACTIVITY IN HOMEOPATHIC GRANULES
(Arnica montana et Histaminum)
(Guinea-pig isolated heart - % var. in coronary flow)

% increase in coronary flow	mean + 1 SD (n)
Arnica montana 15CH granules	16,0 ± 1,2 (4)*
Arnica montana 30CH granules	13,7 ± 8,7 (3)*
Inert granules (negative control)	3,6 ± 3,0 (4)
Arnica montana MT ($1/10^6$)(positive control)	29,9 ± 10,6 (3)
Histaminum 30CH (blind)	17,2 ± 4,0 (9)*
Placebo 30CH (blind)	4,5 ± 1,24,5 (9)

* $p < 0,05$ compared with controls

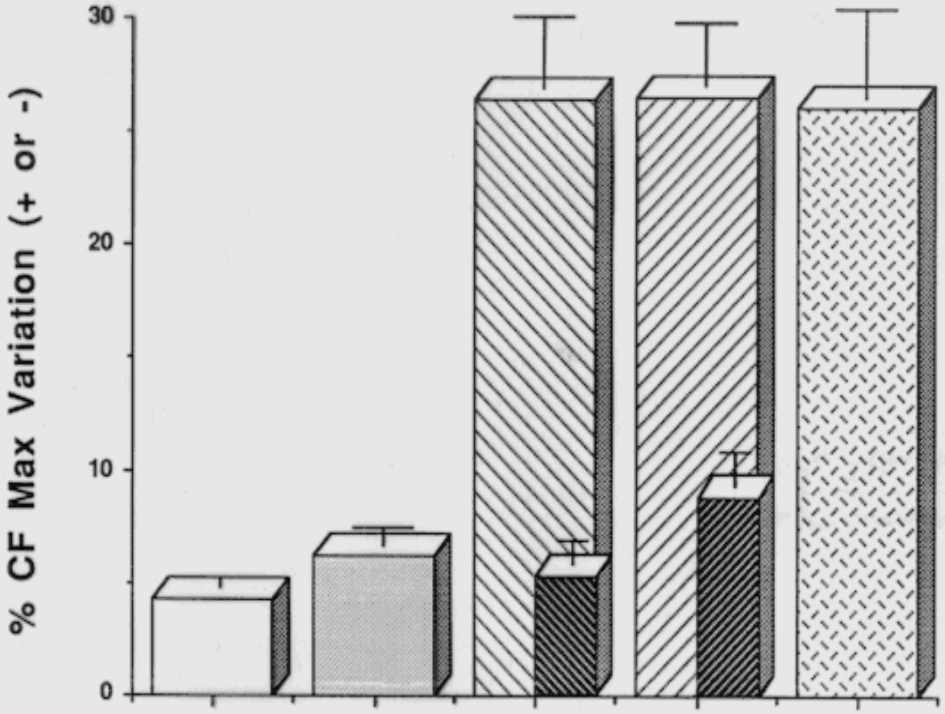
BIOLOGICAL ACTIVITY IN HOMEOPATHIC GRANULES (Histaminum 30CH vs Placebo 30CH)

BLIND EXPERIMENTS

(Guinea-pig isolated heart - % var. in coronary flow)

Placebo 30CH *Histaminum 30CH*	*6.4* *15.6*	*Open* *Open*	*CODE*
1)	4.3, 5.9	Blind	Placebo 30CH
2)	12.8, 17.6	Blind	Histam. 30CH
3)	4.9, 2.9	Blind	Placebo 30CH
4)	20.0, 18.7	Blind	Histam. 30CH
5)	15.8, 20.6	Blind	Histam. 30CH
6)	20.5, 9.1, 15.6	Blind	Histam. 30CH
7)	5.1, 5.8	Blind	Placebo 30CH
8)	5.1, 4.5, 2.3	Blind	Placebo 30CH

EFFECT ON CF OF "TRANSMITTED" AGONISTS
(GP immunized with Ova-Alum)



	C1	C2	Hist /Hist°C	Ova /Ova°C	Endo
n =	24	63	52 /13	57 / 6	39
Comp. w. C2 :	*	-	2.7e-10	1.6e-11	1.0e-8

C1 : naive water

C2 : water after transfer of water

Hist, Ova, Endo : water after transfer of histamine, ovalbumin, endotoxin respectively

Hist°C, Ova°C : water after transfer of histamine, ovalbumin respectively and heated for 2 hrs at 70°C

(in fact all samples added to the hearts are water)

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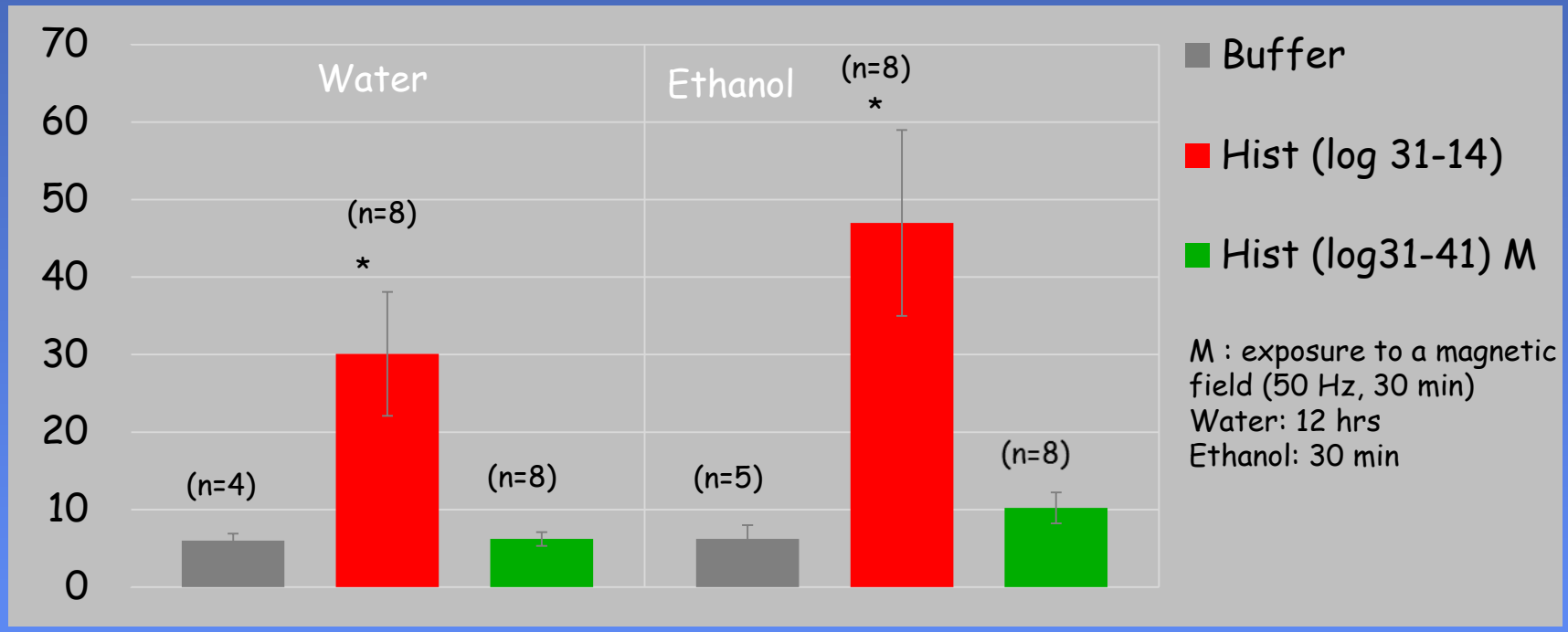
* ns at 0.05

Effect on CF of histamine (log31-41)

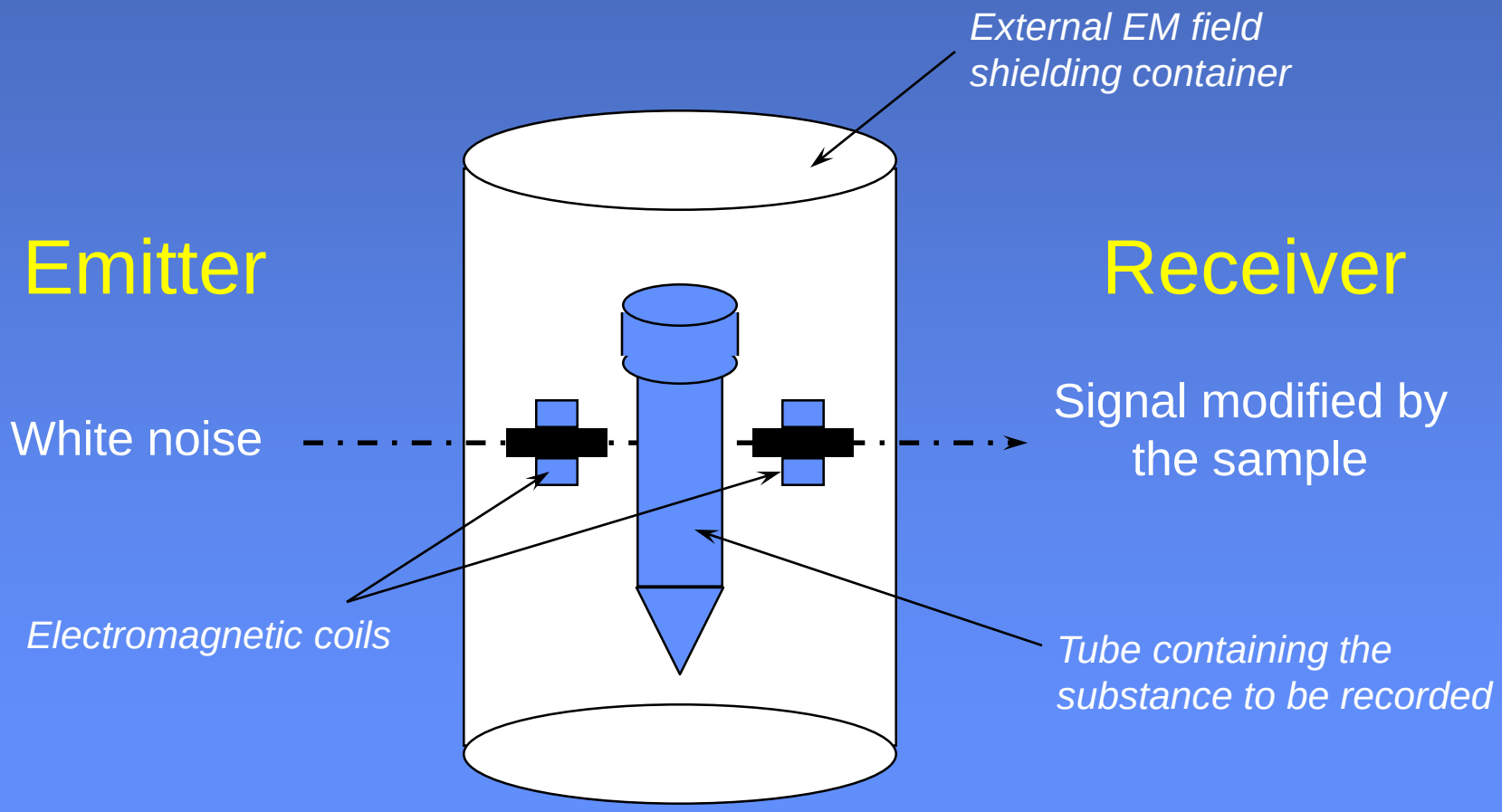
(Guinea-pig isolated heart - % var. in coronary flow)

Impact of magnetic field on HD



* p < 0,05 compared with buffer

Signal recording



Signal recording and transduction

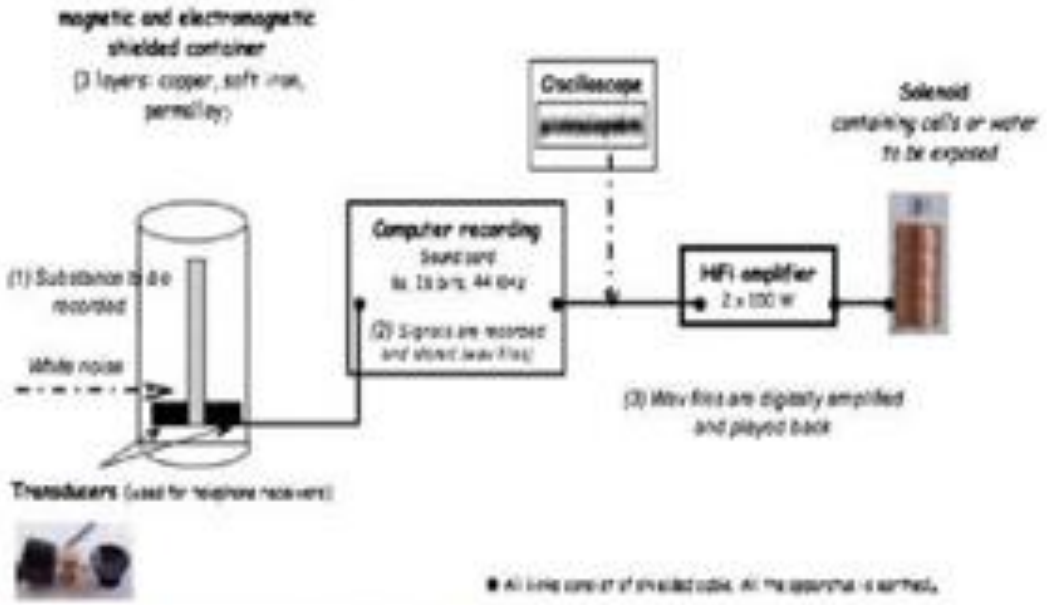
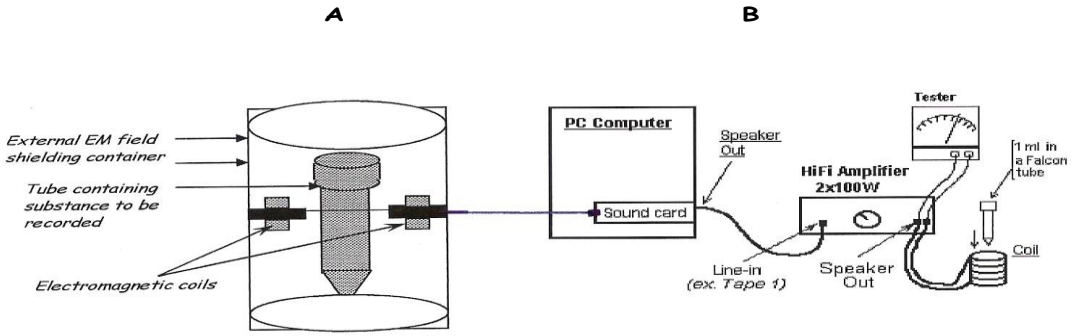
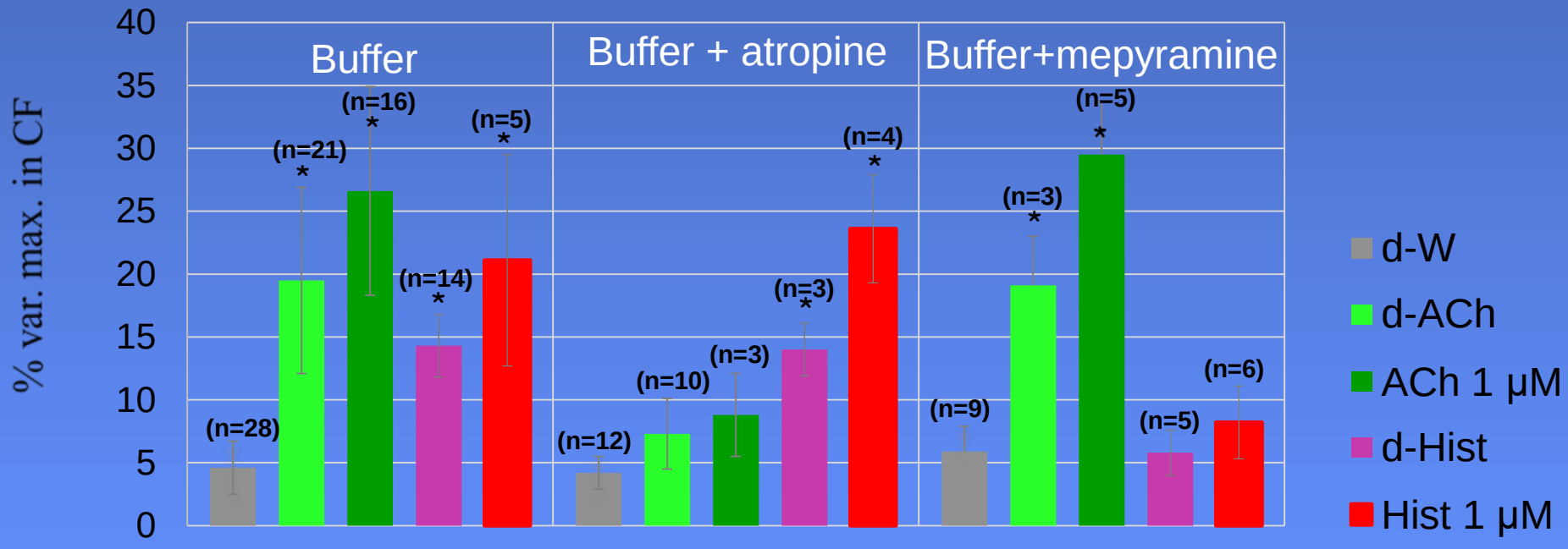


Figure 1 Schematic drawing of the computer-recorded signals: capture, storage and replay.
• Shielded cylindrical chamber composed of three superposed layers: copper, soft iron, permalloy, made from sheets 1 mm thick. The chamber has an internal diameter of 65 mm, and a height of 100 mm. A shielded lid closes the chamber. • Transducers: coil of copper wire, impedance 300 Ω, internal diameter 8 mm, external diameter 16 mm, length 6 mm, usually used for telephone receivers. • Multimedia computer (Windows OS) equipped with a sound card (5 KHz to 44 KHz in linear steps). • HiFi amplifier 2 × 100 watts with an "in" socket, an "out" socket to the speakers, a power switch and a potentiometer. Pass band from 10 Hz to 20 kHz, gain 1 to 10, input sensitivity 1 V. • Solenoid coil: conventionally wound copper wire coil with the following characteristics: internal diameter 50 mm, length 80 mm, R = 3.6 Ω, 3 layers of 112 turns of copper wire, field on the axis to the centre $44 \cdot 10^{-4}$ T/A, and on the edge $25 \cdot 10^{-4}$ T/A. All links consist of shielded cable. All the apparatus is earthed.

Figure 1


A
B
External EM field shielding container
Tube containing substance to be recorded
Electromagnetic coils
PC Computer
Sound card
Speaker Out
Line-in (ex. Tape 1)
HiFi Amplifier 2x100W
Tester
Speaker Out
1 ml in a Falcon tube
Coil

Effect on CF of digitally recorded (d) of His & Ach
(GP heart perfuded or not with atropine or mepyramine)
(% var. in coronary flow)



* p < 0,05 compared with d-water

Effect on CF of digitally recorded (d) of Arnica montana
(GP heart - % var. in coronary flow)

% increase in coronary flow	mean + 1 SD (n)
d-Arnica montana 15CH	19,4 ± 3,3 (5)*
d-Arnica montana 30CH	14,4 ± 1,4 (3)*
d-inert 30CH	2,9 ± 0,8 (3)

Arnica montana 15CH	16,0 ± 1,2 (4)*
Arnica montana 30CH	13,7 ± 8,7 (3)*
Inert 30CH	3,6 ± 3,0 (4)

* $p < 0,05$ compared with controls

Effect on CF of digitally recorded (d) coronary dilator drugs (Isolated guinea pig heart - % var. in coronary flow)

% increase in coronary flow	mean + 1 SD (n)	"Real" drugs (1μM)
d-Propranolol (β-blocker)	20,0 ± 9,1 (12)*	25,4/22,2
d-Nicorandil (K+ channel+)	20,4 ± 7,1 (13)*	16,0/21,6
d-Nifedipine (Ca2+ antag,)	13,3 ± 3,5 (6)*	23,1/19,2
d-Bradykinin (vasodilator)	19,7 ± 7,1 (9)*	20,0/20,0
d-water	5, 2,1 (9)	
White noise (EM signal)	6,1 ± 1,7 (17)	
Naive water	5,3 ± 2,1 (15)	

* $p < 0,05$ compared with d-water

Biological Systems (2)

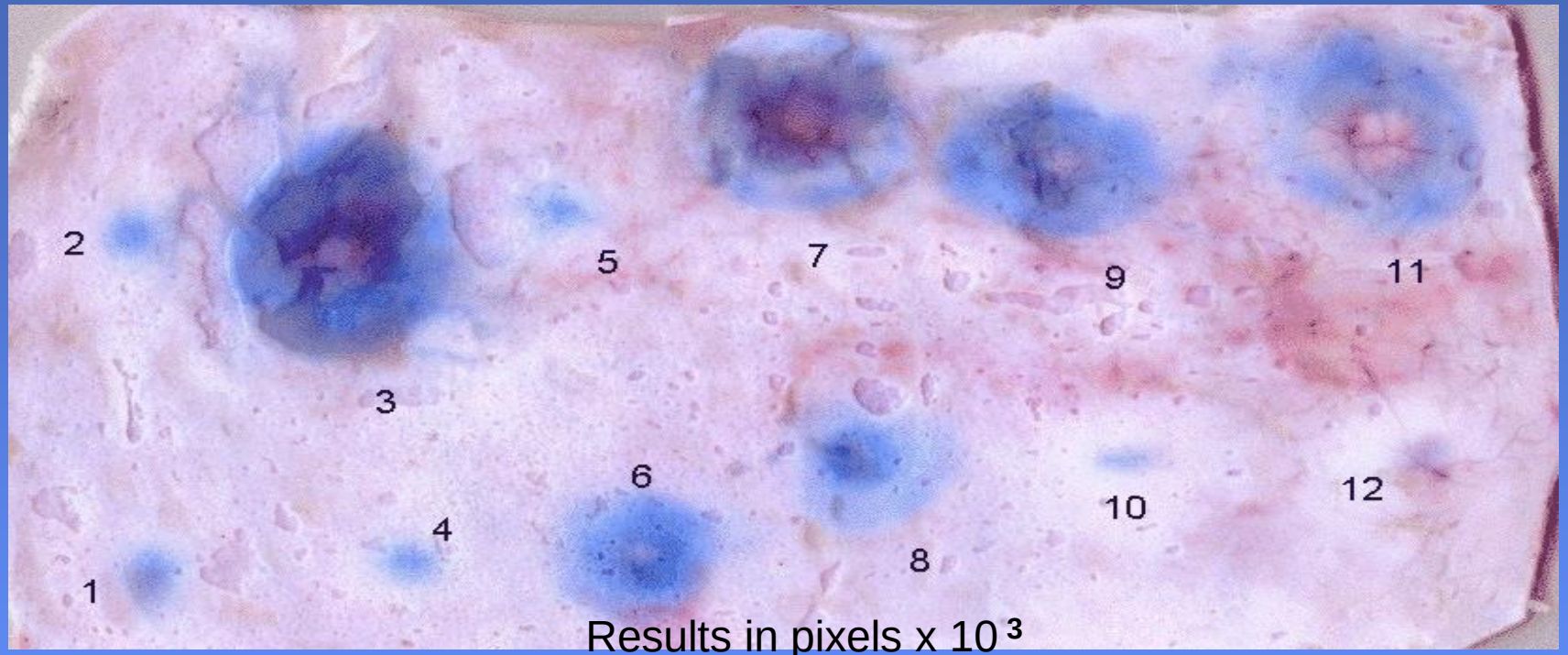
- 1997-1998 5) **Ag/Ab precipitation**. Detection of the recorded "signal" of bacteria (or of any antigen or antibody) by playing it to an immune reaction specific to this signal.

- 1998 6) **Skin test**. Intradermal injection to guinea-pigs or rabbits of water "informed" with the signal of vasodilators such as histamine, serotonin, acetylcholine, bradykinin induces local skin vasodilation inhibited by the specific inhibitor of the original molecule.

Biological system: skin test as "*in-vivo*" assay

- Intradermal injection to guinea-pigs or rabbits of water "informed" with the signal of vasodilators such as histamine, serotonin, acetylcholine, bradykinin induces local skin vasodilation inhibited by the specific inhibitor of the original molecule

Biological Sensitive System
Skin Test

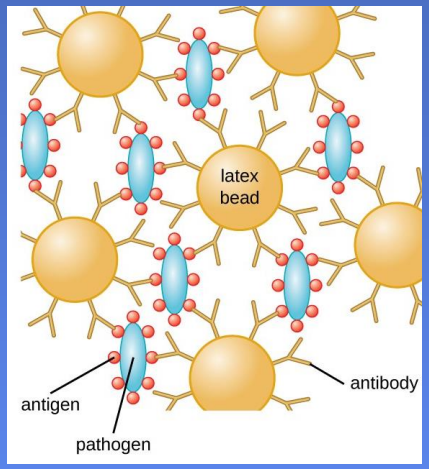


Results in pixels x 10^3

1 ACh -12 M vortexed in saline (68.2)
2 Same vortexed in 5 % glucose (59.5)
3 Same vortexed in water (1,949)
4 Same mixed in water (44)
5 Atropine + 3 (71)
6 Water + 3 (609)
7 ACh signal in water (1,294)
8 Same at low power (435)
9 Same as 6 (987)
10 Acetate+Choline as in 3 (25)
11 ACh 1 µg (1,154)
12 Atropine + ACh 1 µg (36)

Biological system: Antigen-Antibody agglutination as "*in vitro*" assay

- In the presence of a specific antigen, latex particles sensitized by the related antibody, undergo agglutination and form aggregates of various sizes
- In this work, the bacterial signal is electronically captured, digitized, stored in a computer and then applied to a sensitive biological system



Pathogens tested:
- E. coli
- Streptococcus

Biological system: Antigen-Antibody agglutination as "*in vitro*" assay

- The kit reagents consist of a latex particle sensitized with mouse monoclonal or rabbit polyclonal antibodies. In the presence of a sufficiently high concentration of antigen, the latex specific for the antigen present in the medium agglutinates on binding with the antigen and forms clumps visible to the naked eye

- We have intentionally lowered the antigen concentration so as to obtain aggregates of small size

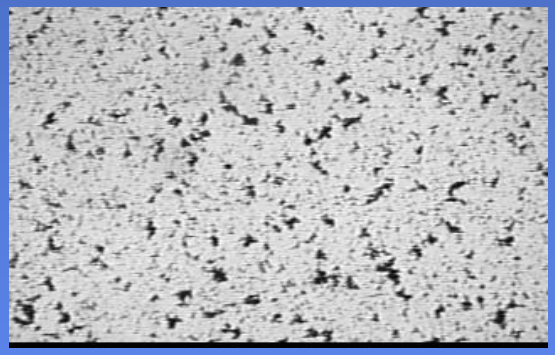
- If there is no specific antigen present, clumps do not form, and the latex retains its slightly milky appearance (low index)

- Applying the pathogen signal induces the formation of large aggregates

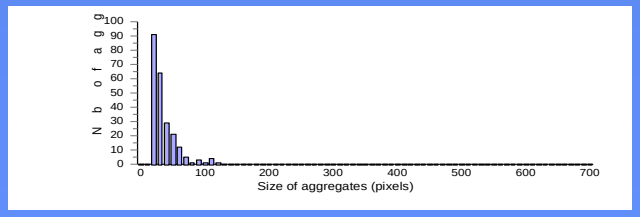
Biological system: Antigen-Antibody agglutination as "*in vitro*" assay

- Detection System: E. coli

  Transmitted Signal: Streptococcus

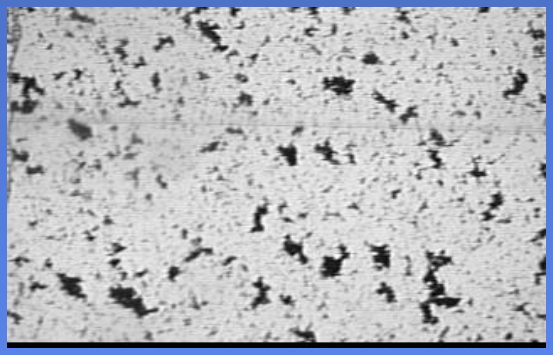


Aggregation index : 30

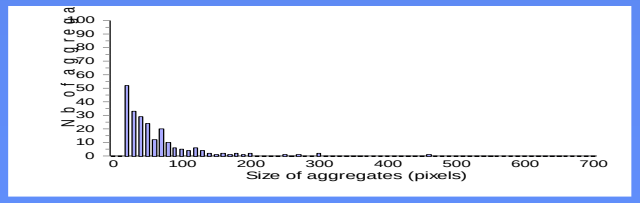


- Detection System: E. coli

  Transmitted Signal: E. coli



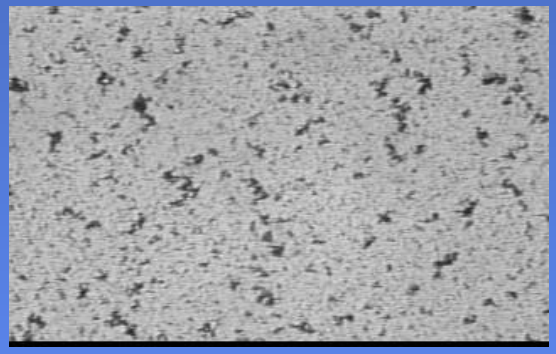
Aggregation index : 185



Biological system: Antigen-Antibody agglutination as "*in vitro*" assay

- Detection System: Streptococcus

Transmitted Signal: control



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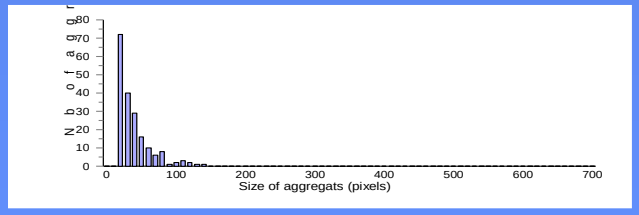
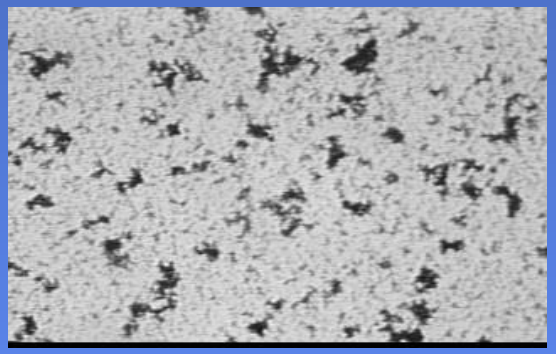
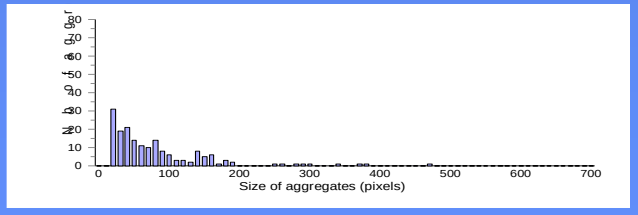


- Detection System: Streptococcus

Transmitted Signal: Streptococcus



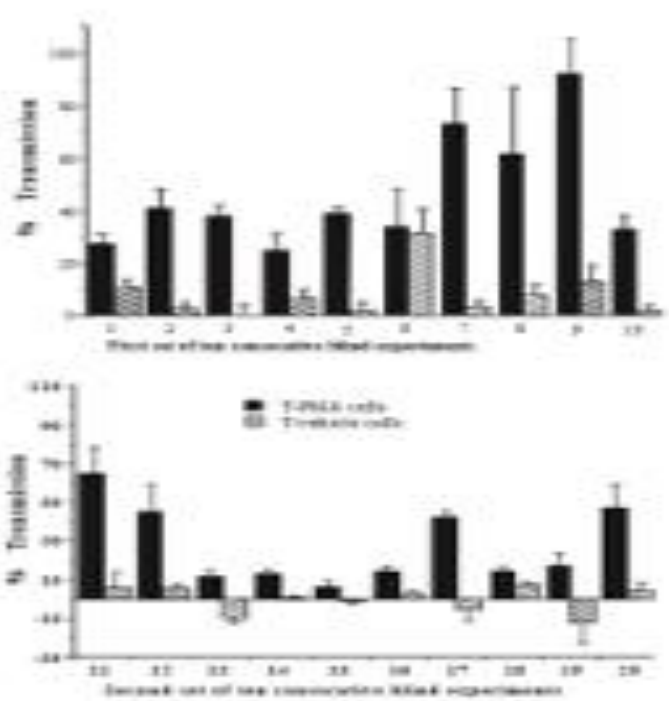
Aggregation index : 374

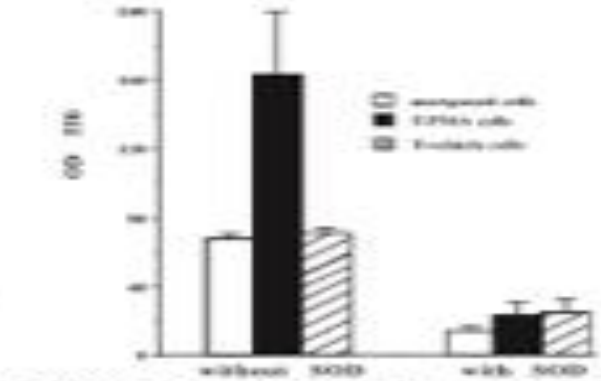


Biological system :
PMA activation of neutrophils
as "*in vitro*" assay

- We investigated whether molecular signals associated with phorbol-myristate acetate (PMA) could be transmitted by physical means, i.e. digital EMF signals, to human neutrophils to modulate reactive oxygen metabolite (ROM) production.

Biological system :
PMA activation of neutrophils as "*in vitro*" assay





T-PMA cells
T-vehicle cells

Fig. 1. Effect of transmitted PMA on neutrophil ROM production. Cells (1×10^6) were exposed at 37°C to either 'transmitted' PMA or 'transmitted' vehicle for 15 min. As an additional control, cells were placed 20 cm away from the output coil ('unexposed cells'). Incubation was continued for 45 min at 36°C before assessment of ROM production, as described in Materials and Methods. A first set of ten consecutive experiments (1–10) was performed at (redacted) CDT. A second set of ten consecutive experiments (11–20) was performed in a different laboratory (redacted) CDT, (redacted, Paris). In each experiment, 4 simultaneous transmissions were performed, using 4 source tubes (1 PMA and 3 vehicles). These 4 source tubes were prepared, labelled and blinded by coding at the beginning of each experiment. In case out of the second set of ten experiments, randomization and coding were performed by the head of the laboratory. One experiment was coded by a member of the laboratory. For each individual experiment, percent (%) transmission was calculated as defined in Materials and Methods. Each error bar corresponds to the standard error estimated from 4 OD values of exposed cell tubes.

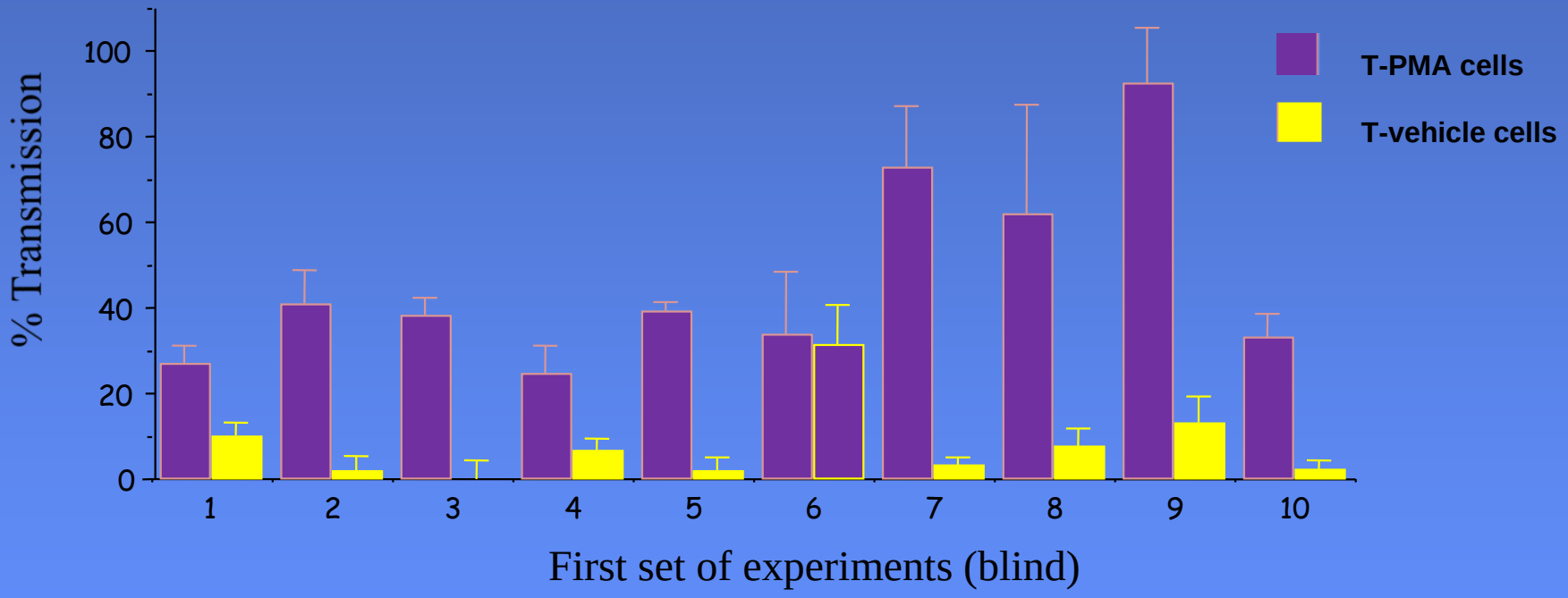
Fig. 2. SOD-inhibition of the effect of T-PMA on neutrophil ROM production. Cells (1×10^6) in the absence or presence of SOD (40 μg/ml) were exposed at 37°C to either 'transmitted' PMA or 'transmitted' vehicle for 15 min. For each experiment, four transmissions were performed simultaneously (two T-PMA and two T-vehicle). In each transmission, duplicate, with versus without SOD, cell-containing target tubes were placed side by side on the output coil. As an additional control, cells were also placed 20 cm away from the output coil ('unexposed cells'). Incubation was continued for 45 min at 37°C before assessment of ROM production as described in Materials and Methods. Data are mean OD values ± S.E.M. of three independent experiments. For the sake of simplicity, integer OD values were obtained by multiplying the measured absorption values by 1000.

cells treated with GF109203X (6 μM) or H-7 (20 μM) prior to PMA transmission were less effective at cytochrome-c reduction than untreated cells. GF109203X and H-7 did not affect cell viability.

A statistical summary is presented in Table 1 and Figure 4. At 60 min, T-PMA cells were associated with a 32.6 ± 3.4 % OD increase, in contrast to 2.3 ± 1.3% ($n = 50$ transmissions, $P < 10^{-5}$, Student's t-test) for T-vehicle, T-PDD and oscillator power off (T-PMA off). The PMA transmission effect is not only statistically different from other groups but is also larger by a factor of at least five 99%-confidence level). The overall result is highly significant even when calculated using a very conservative, binomial approach. In 50 of the 50 binary comparisons, mean OD values for T-PMA were above those obtained for T-vehicle, T-PDD or oscillator power-off ($P = 10^{-15}$). Note also that the OD variance for T-PMA cells is higher than for T-vehicle or other exposed cells,

Biological system :
PMA activation of neutrophils as "*in vitro*" assay

Effect of transmitted PMA on neutrophil ROM production



Effects on cell lines

1- Intoxication by heavy metals

The toxicity of cadmium (Cd) has been studied in human and murine lines. When the cells are cultured in the presence of 5 to 10 µM of Cd or Cd at high dilution or Cd recorded on a computer:

40 to 50% of mortality is observed, a fall in RNA synthesis and the induction of certain genes such as that which is involved in protection against intoxication by certain heavy metals.

2- Activation of Fibroblasts by Calcium Ionophor and PAF - acether

The synthesis and release of paf-acether by fibroblasts from normal human skin were studied in vitro. When fibroblasts from normal human skin in suspension were stimulated with ionophore calcium A23187 molecular or recorded on a computer.

A synthesis and a release of paf-acether are observed in vitro.

This synthesized material aggregated washed rabbit platelets and was inhibited by an antagonist and a specific paf-acether inhibitor.

Biological Sensitive System

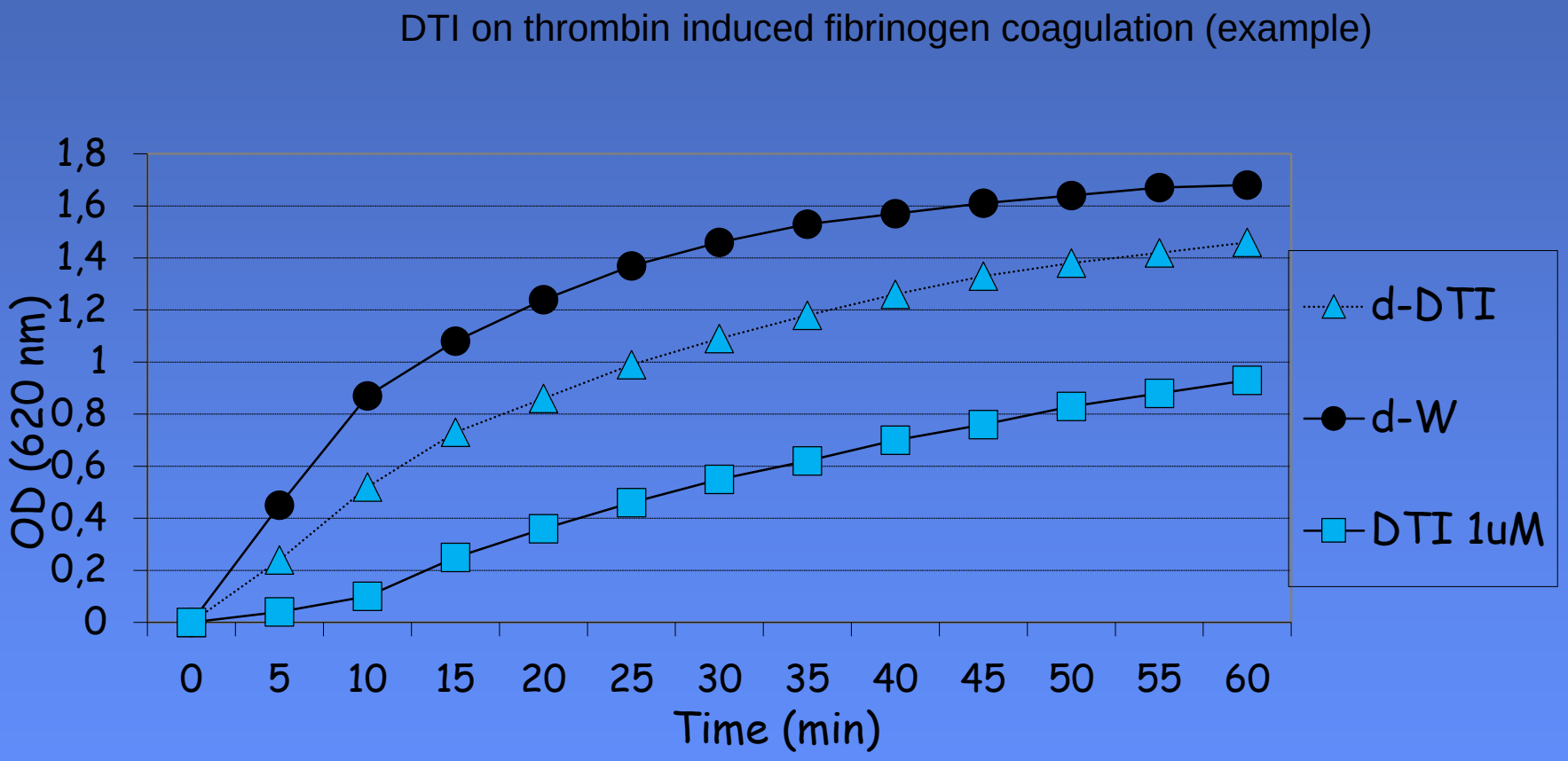
Delayed fibrinogen coagulation

1) Water containing thrombin is exposed to the hirudin (or water as control) signal.

2) The exposed water-thrombin tubes are mixed with fibrinogen and distributed in 96-well plates.

3) Coagulation is assessed by spectrophotometry and expressed as O.D.

Biological Sensitive System
Delayed fibrinogen coagulation



CONCLUSION

Here comes a milestone in the history of science. The transition from biology from the era of the structure of molecules to the era of digital information.

We can really switch to a completely electromagnetic medicine, the one where we can treat with waves and water. Obviously, this will not require more time, because the technical means are at our disposal.

Thank you

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