OFF-PRINT FROM

ADVANCED TECHNOBIOLOGY

edited by

BORIS RYBAK

Professeur à la Sorbonne nouvelle

SIJTHOFF & NOORDHOFF 1979
Alphen aan den Rijn — The Netherlands
Germantown, Maryland, USA

COMPLETELY OPEN FROG'S HEART ON EXTENSOMETER ELECTRICALLY AND MECHANICALLY MONITORED (TOPOELECTRONIC ELASTORESISTANCES)& MOLECULAR TECHNOLOGY OF AUTOMATICITY

B. Rybak

I.E.L.P.,Université de Paris III,F 75005 Paris

The specific physiology of a cavitar organ is concerned with the internal spaces it displays. I started with this conception in mind when I engaged some years ago - about 25 actually - in this field. Whereas any kind of vertebrate heart is relevant to this technique [according to adaptations linked to the anatomical restraints (= specificities)] -, figure 1 shows

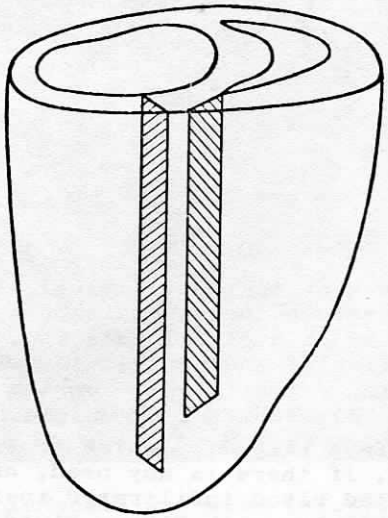


Fig. 1.

how the operatory tactic for opening a mammal ventricle is driven . I will demonstrate to you today :
1. the way to open a frog's heart,
2. how to transfer the pinned heart from the paraffin wax to the original extensometer (1) ,
3. how the suspended heart will contract regularly for hours without any perfusion thanks to the mechanical catalysis (2) of the six weights located according to chosen angles,
4. how, thanks to the original elastoresistances (3), the force $\mathcal{F}$ and the work $\mathcal{W}$ are recorded (4) and how one can record simultaneously local EKG.
In addition I will make some remarks on the biochemistry of automatogenic processes.

1. SURGICAL OPERATION :

The heart is extracted from the body of a double-pithed frog and immediately immerged in a free phosphate and free glucose Ringer's solution to permit the rapid release of the residual blood. The heart , lying down on its dorsal part, is then pinned up on paraffin wax with 2 sagittal pins at first, afterwards with 6 pins (Fig.2 a & b).After making a small transverse button-

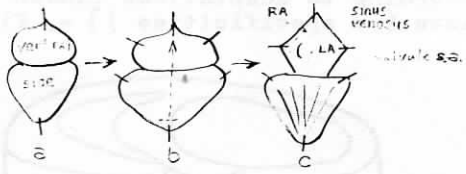


Fig.2 a,b & c .

hole, a branch of the ophtalmologic scissors is inserted into the ventricle through this button-hole and, with a minimum number of sagittal cuts, it is then possible to move the auricular and ventricular median edges in such a way that the former closed complex surface of the heart (Fig.2 a) becomes , by unfolding and stretching, an open surface (Fig.2 c) which contracts in a regular alternation. If there is any need, one can eliminate the coagulated blood infiltrated inside the muscular bundles by rinsing with Ringer fluid, eventually by

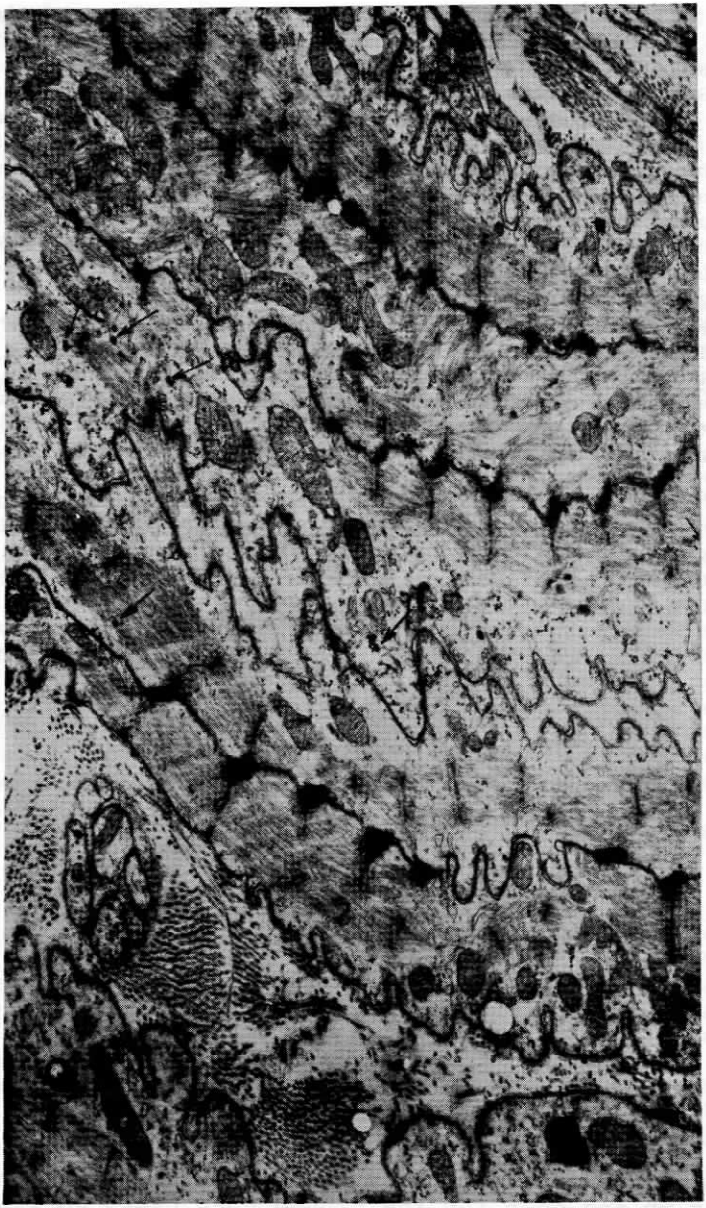


Fig.3. The arrows indicate granules (cf.5).

using a small cotton swab. At this point,the technique, quite simple, is usefull for high-school pupils. To keep the isolated open heart alive (working) for hours, one needs to put, from time to time,some drops of Ringer's fluid on the preparation. By elevating the open pinned heart from the contact with the wax, one can even cut out the sinus venosus and demonstrate then that ,under the influence of the mechanical catalysis, the heart mutilated from its princeps pacemaker, can still effectuate long-time regular revolutions. This "Advanced Technobiology" needs only the common material of a sewing-maid...

Fig.3 shows the electron-microscopic morphology of the sinus venosus (5) indicating notably a ratio : $\frac{\text{extent of the cell membrane}}{\text{mass of the contractile material}}$ in favour - if compared with the ultra-structure of a frog's ventricle - of the membrane surface.

2. and 3.OPEN HEART ON EXTENSOMETER

The mammal heart can advantageously be stretched on the extensometer (Fig.4) as we did (1) .This circular

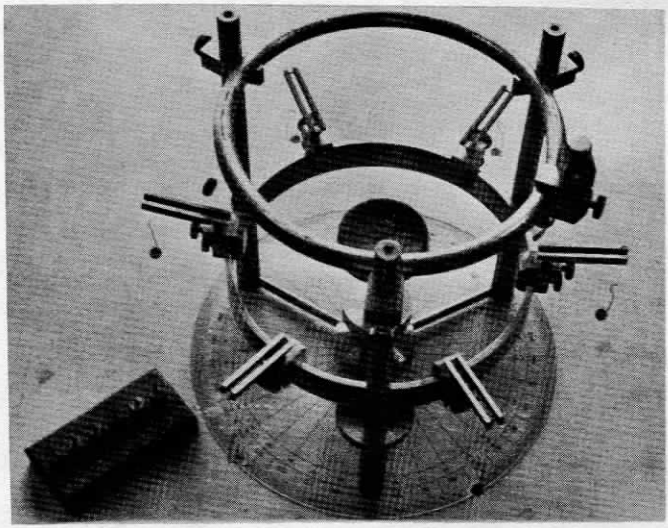


Fig.4.

extensometer is essentially built with a central pedestal moving up and down for the pinned open heart on its wax, a lower circle for six (or more) angularly moving brackets supporting the six hook-threads holding the heart

under definite weights (formally 6 x 1 g) when it is gently unpinned , and a higher circle as a stand for electrodes, sensors, etc(Ets Gauthier,Paris) .

To transfer the heart from the wax to its definitive situation when beating regularly in the air, one has just to fix one hook-string near each pin and then to remove the pins (do not forget to moisten the preparation and to avoid any thermic shock).

4. ELASTORESISTANCES CONTROL OF THE MECHANICAL PROCESSES

Topoelectronic is a new way for Electronics where, instead of doing solid state physics under rigid substrata, one uses deformable (elastic or plastic)substrates (3). We shall use here only elastoresistive devices attached to each of the six threads connected with the heart at each point and to the weights at the opposite ends.

First of all, some words about these elestoresistances.Those used today have a natural latex substratum strip-shaped(3/10 mm thickness) and they are coated in vacuum with gold using an AEI (Harlow,Essex) vacuum coating unit type MC9 working under 1.5 . 10^{-5} torr. The DC power supply (9V) is connected, through a resistance of$\sim$300 Ω , to the covered elastoresistance supplied with two silver connections stuck with a conductive glue, and to follow simultaneously the six mechanical cardiac events, the six topoelectronic sensors are plugged in AC (rapid changes) to a "Tektronix 5103 N" six-times commutated by a special set-up. To avoid noise the mobile contacts are manufactured with gold. As far as cardioelectrical events are to be recorded , fine wires are fixed to the silver elastoresistive connections to the six different mechanical channels so that the local EKG superimpose the local MKG.

In a preceding short paper (6)the thickness of the thin film (silver deposit) was calculated according to the classical resistivity equation $r = \rho 1/s$ in ohm-centimeter at 22 C. Our actual more accurate calculation is the following one : taking into account the gas removal from the latex when submitted to vacuum, the initial weight is m'_0 and m'_1 , after plating for gold weight m ; an identical piece of latex (the control) of weight m_0 is located in the upper part of the latex to be gilded, a paper sheet being interposed between the two pieces of latex; after gas removal $m_0 \longrightarrow m_1$, where $m_1 < m_0$. Then the hidden latex gives $\Delta_m = m_0 - m_1$, and the gilded latex gives $\Delta m' = m'_1 - m'_0$. Therefore:

$$m_{Au} = m'_1 - m'_0 \frac{m_1}{m_0} = \Delta m' + m'_0 \left(1 - \frac{m_1}{m_0}\right) = \Delta m' + \frac{m'_0}{m_0} \Delta m$$

Let us consider the experimental data : $S = 32.5\ cm^2$, $m_{Au} = 3.1 + \frac{0.8196}{0.6920}\ 3.8 = 7.6$ mg; then $e = \frac{7.6 \cdot 10^{-6} \cdot 10^{-3}}{19 \times 32.5 \times 10^{-4}} =$ 1230 Å.

For the moment any attempt to calculate the number of layers according to an atomic diameter of 2.68 Å for Au is subject to caution until experimental thin film patterns could give a topographical distribution of the atoms piled up orthogonally or in other ways. Moreover the calibration curves obtained from a virgin gilded latex for the same elestoresistance (here an original pressure transducer for catheterization)after taking its freshness off already indicates (fig.5) that

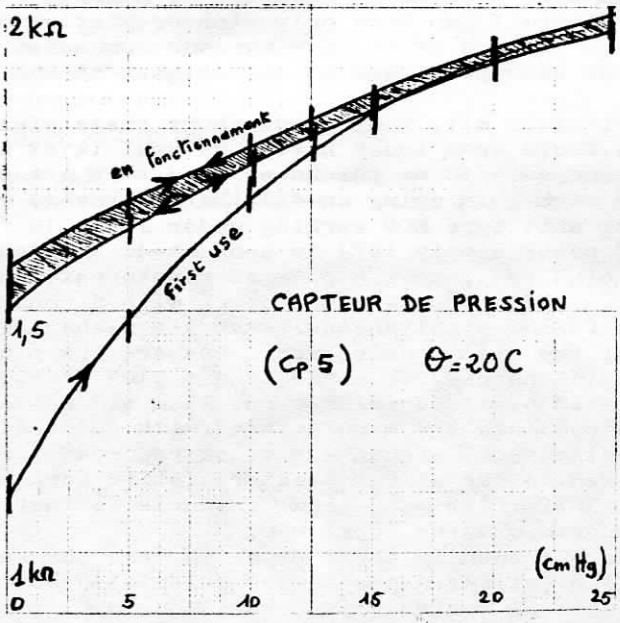


Fig.5

privileged stratifications could appear(as a mini-pressure sensor the linearity is located in a ± domain of validity corresponding to a hysteresis ∿ 4% when this catheter sensor is submitted to rather hypertensive cycle bulk stresses). In the near future the theory of finite deformations of elastic membranes should, in addition, be applied in connection with the behavior of the para-crystal structures of the thin metallic film.

Figure 6(7) is a "Polaroïd" photo showing the local EMG at the different angular summits of attachment to the open heart of a 52 g ♀ Rana esculenta (θ =20 C; $t_{left-right}$ = 500 ms/div.).

The full explanation will have to take into account the eventual ago- and antag-elastic interferences and resonances when the action potential spreads out(the auricular systole pulls passively the still mechanically inert ventricle) and, notably, when the preparation is aging. For the sake of clarity one will just indicate here the standardization method for describing force measurements (Fig.6). From this isomechanic level to the crest in each channel one obtains $\Delta\mathcal{F}$; the calibration being done, using gramme, 1 g-force = 981 dynes , therefore 1 dyne = 1.02 mg-force with an uncertainty of 6-10 %. Then, the work during a period is $W = \frac{1}{2}\Delta\mathcal{F}.\Delta L$ (L = length). According to what $\frac{\Delta L}{\Delta\mathcal{F}}$ = 0.25 mm/g in the case of the present elastoresistances.

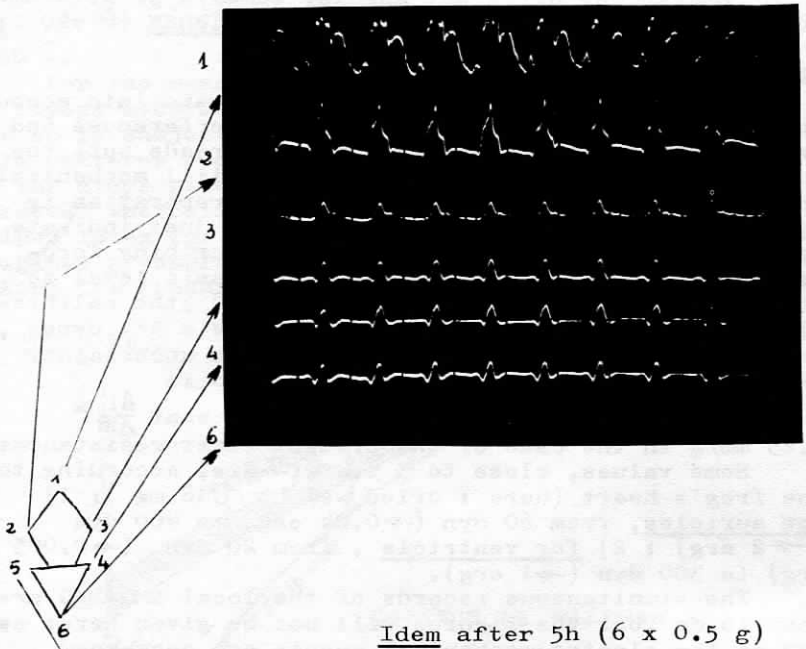
Some values, close to 5 %, of $\mathcal{F}_t W$ are, according to the frog's heart (here : dried weight $\sim$18 mg): 1) for auricles, from 60 dyn ($\rightarrow$0.04 erg) to 400 dyn ($\rightarrow$2 erg) ; 2) for ventricle , from 20 dyn ($\rightarrow$0.005 erg) to 300 dyn ($\rightarrow$1 erg).

The simultaneous records of the local EMG-EKG are easy to do ,but the figures will not be given here: as far as the electro-mechanical events are concerned , to establish their relationships, according to the technique described here, one needs a close examination of the concomitant wave forms which calls for a high-speed cinematography and, after what, a dynamical topology treatment. As these analyses are in progress, we will focus our attention now on point 5.

5. SOME REMARKS ON THE BIOCHEMISTRY OF THE AUTOMATOGENIC PROCESSES.

Mechanical catalysis means that for a certain mechanical stretch a mechanical threshold of restraints is reached, so that the heart - or even a ventricular fragment of a frog's heart (8) - can follow regular periodicity of diastole-systole revolutions. For the open frog's heart this threshold is located, depending on the size of the heart , around 6 x 50-100 mg at room temperature (these values are given just to figure an extent of evaluation). The natural equivalent of this mechanical stretch is the diastolic period, when the heart, filled by the blood, is submitted to a certain pressure stretching the muscle bundles. Now, classically

t_0= 1h after opening(6 x0.3g)



Idem after 5h (6 x 0.5 g)

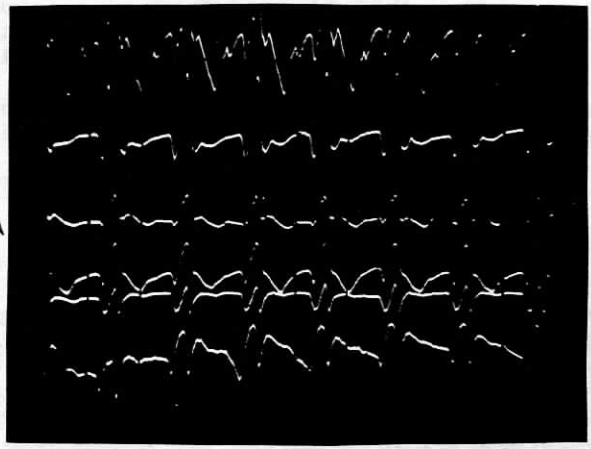


Fig.6.

diastole means rest. But diastole is in no way a pause : 1°) because of the mechanical catalysis, 2°) because it represents the period of re-synthetizing the energetic molecules involved in the next systolic phase; mechanical catalysis acts at two levels : as an automatogenic trigger - automatogenic contraction initiator and general contraction event. I started the biochemical analysis of these primeval effects in 1973 (9) when I became aware of the princeps role of cAMP : the plasma membrane nucleotidyl-cyclic machinery. Epitomizing, the main phenomena displayed during the pacemaker period, one can say for the moment, with the help of figure 7 (10) :

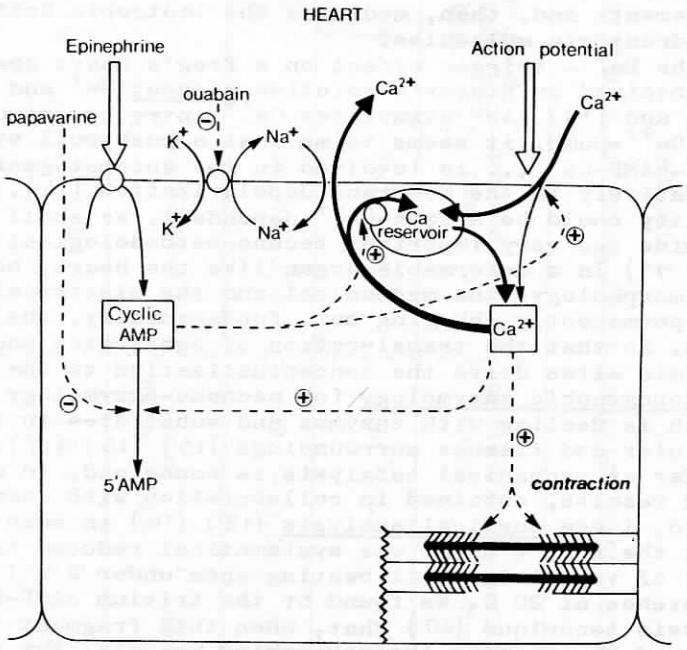


FIG. 7. Stimulus-contraction coupling in the heart. The action potential causes an influx of external calcium, most of which enters the calcium reservoir (sarcoplasmic reticulum) but some of which goes directly to the contractile elements. Calcium, which entered the sarcoplasmic reticulum during the previous beat, is also released and contributes to the build-up of free calcium responsible for initiating contraction. Relaxation occurs when this calcium level is reduced by being pumped into the sarcoplasmic reticulum or by extrusion across the surface membrane in exchange for sodium. Epinephrine exerts its positive inotropic effect via cyclic AMP, which modulates the ebb and flow of calcium during each heart beat. Cyclic AMP can either prolong the influx of calcium across the plasma membrane or enhance the uptake of calcium into the sarcoplasmic reticulum(10). (Michael J. Berridge; Raven Press NY)

1) While action potential leads to an incremential effect in the intracellular pool of Ca^{++}, what initiates the action potential is not indicated in Fig.7 , but Ca^{++} increases both the amount of cAMP under the automatogenic effect of $BaCl_2$ on the cardiac apices of the frog, and the duration of the Ba^{++} - induced cardiac apex automatism (11). Now :

2) cAMP activates protein kinase which catalyzes the transfer of a phosphate (the last one) of ATP to proteins of the plasma and sarcotubular membranes where is also located the catecholamine sensitive adenyl-cyclase enzymes of entrance (11) ;

3) the diffusion of Ca^{++} through the sarcolemma is prompted during the action potential, and Ca^{++} could intervene in the Ca^{++} - bound and Ca^{++} - free (available) movements and, then, mediates the inotropic action of β-adrenergic molecules,

4) the Ba^{++}- trigger effect on a frog's heart apex can be obtained in Ringer's solution without Na^{+} and K^{+} (12) and (13) cAMP stimulates Ca^{++} entry by stimulating the Ca^{++}-pump, it seems to me that a push-pull system Ca^{++}-cAMP-Ca^{++}... is involved in the automatogenic effet, tentatively in the membrane depolarization (14). This ability could be ATPase-Ca^{++} dependent. We shall now examine two very important techno-methodological points.

1°) In a deformable organ like the heart, not only the morphology, the mechanical and the electrical events are permanently changing but, fundamentally, the metabolism. So that the translocation of substrates and active enzymic sites drive the conceptualization to the notion of topographic enzymology (or mechano-enzymology in situ) which is dealing with enzymes and substrates in their cellular and tissues surroundings (15) (16) (17).
As far as mechanical catalysis is concerned, in unpublished results, obtained in collaboration with Jean-Claude David, I use surgical analysis (18) (19) in such a way that the frog's heart was systematical reduced to a fragment of ventricle still beating when under 2 x 1 g stretches at 20 C. We found by the tritium cAMP-binding protein technique (20) that, when this fragment is stopped by removing the stretching weights, the amount of cAMP falls to $\sim$3.5p mole/mg total P_i while it was $\sim$ 9.75 pmole/mg total P_i when beating under stretch, and rises again to $\sim$ 7.1 pmole/mg total P_i when it restarts thanks to the initial stretch.

2°) This is in good agreement with previous results concerned with Ba^{++}- frog's heart apex (11) (21). But, as we underlined (11), an important aspect of analytical enzymology à savoir que dans des homogénats et les

TABLEAU 1

Détermination des contenus auriculaire et ventriculaire en cAMP

	Tissus	Nombre de pièces par collection	pmoles / g de tissu frais	pmoles/mg P_i total	pmoles/mg protéines totales (260nm/280nm)
Fonctionnels	oreillette branchiale	4	53,4 ± 2,4	2,6 ± 0.1	0,155 ± 0,005
	ventricule branchial	8	14,6 ± 2,4	0,73 ± 0,09	0,042 ± 0,007
Non Fonctionnels	oreillette branchiale	1	non détectable	non détectable	non détectable
	ventricule branchial	1	3,45	0,19	0,09

TABLEAU II

Détermination des contenus auriculaire et ventriculaire en cAMP en présence, au moment du broyage, de 100 µM isopropylméthylxanthine

Tissus	Nombre de pièces par collection	pmoles / g de tissu frais	pmoles : mg protéines totales	
			260nm/280nm	Lowry & al.
Oreillette branchiale fonctionnelle	4	364 ± 28	1,43 ± 0,15	1,79 ± 0,23
Ventricule branchial fonctionnel	4	76 ± 6,5	0,46 ± 0,07	0,51 ± 0,05

surnageants correspondants, ce que l'on dose comme substrat est toujours le résultat d'une optimalisation entre l'enzyme de biosynthèse (ou enzyme d'entrée) et l'enzyme hydrolytique (ou enzyme de sortie). This means that a molecule, like cAMP, is located in a black box between the AMP-cyclase and the AMP-phosphodiesterase (exit enzyme). So that if we do not block the entrance and the exit enzymes the value for the molecule titrated will be, depending on the speed and dexterity of operators, on the right lines but at an uncertain level. In other unpublished results, obtained with J.C. David, using the Myxine's branchial heart we were able to test the validity of this concept :

Using still the same techniques for titration of cAMP of Tovey et al. (20) we re-examine previous results obtained with the Myxine's branchial heart (11). Our quantitative conclusions are the following : the auricle is richer in cAMP than the ventricle in confirmation of what was found at first (11), but the scale is different as Table I and II demonstrate, - the phosphodiesterase specific inhibitor is the isopropylmethylxanthine according to (22).

REFERENCES

1. B.Rybak , C.R.Acad.Sc.Paris,1960,250,3391.
2. B.Rybak:a) Nouvelles perspectives en physiologie cardiaque,Conférences Palais Découverte Paris, Série A,n°226,1957;
   b) A Medicina Contemporânea,Lisboa,1958, 76,n°7,295.
3. B.Rybak , L'Onde électrique,1977,57,n°6-7,455.
4. B.Rybak , M.Morel & A.M.Le Camus, J.de Physiol. 1976,120 A.
5. B.Rybak , C.Ruska & H.Ruska, C.R.Acad.Sc.Paris, 1964,258,5073.
6. B.Rybak , Aerospace Med.Ass.1974 Ann.Sc.Meeting Washington,D.C.,Preprint ASMA/AIAA panel:Biotechnology,Res.& Devel. 95.
7. B.Rybak , unpublished data.
8. B.Rybak,Arch.Mal.coeur,1965,n°5,711.
9. B.Rybak , Proc.Can.Fed.Biol.Soc.1973, 16,58.
10. For the School this resulting figure is to consider inside the full set of major related publications in this field I recommend to study :
    a) Advances in Cyclic Nucleotide Research,Raven Press,New York, specially vol.V,1975 (F.E.Bloom & al.; P.Greengard ; S.Kakiushi & al.; A.M.Katz & al.; J.P.MacManus & al.; J.H.Wang ; B.Weiss);
    b) idem,vol.VII,1976 (D.L.Friedman & al.);

c) idem,vol.IX,1978 ; 3rd Int.Conf.on Cyclic Nucleotides, New Orleans,Louisiana ,July 1977, P.Greengard & G.A.Robison ed.;
d) E.Carafoli & al.ed.;Calcium Transport in Contraction and Secretion,North Holland,Amsterdam, 1975 (A.Margreth & al.; M.Tada & al.);
e) Methods in Cyclic Nucleotides Research,Dekker, New York.

11. B.Rybak & M.Simon,Acta physiol.scand.1974,90, 501.
12. E.B.Babskij, E.A.Donskikh & M.R.Mukum,Dokl.Akad. Nauk.SSSR ,1974,216,n°1,229.
13. D.B.Mc Namara Jr.,Canada Physiologie,1973,4,163.
14. J.R.Williamson & S.Schaffer, Recent Adv.in Studies on Cardiac Structure and Metabolism. Vol.9 .The Sarcolemma,Univ.Park Press,Baltimore,1976,205.
15. B.Rybak ,J.de Physiol.1972,65,163 A.
16. B.Rybak , Encyclopaedia universalis,Paris,Universalia n°2,1975,197.
17. A.M.Le Camus & B.Rybak ,Biochimie,1976,58,1003.
18. B.Rybak , Life Sc.,1962,5,201.
19. B.Rybak , Pathol.Biol. 1963,11,639.
20. K.C.Tovey, K.G.Oldham & J.A.M.Whelam, Clinica Chim. Acta,1974,56,221 .
21. B.Rybak & J.C.David , Bioch.Biophys.Res.Comm. 1977,79,n°4,1065.
22. M.Chassin & D.N.Harris in:Methods in Cyclic Nucleotides Research,Dekker,New York,1972,3,169.

DEMONSTRATION AND DISCUSSION

The Lesson was a typical Lecture since during the conference demonstrations were performed.

A T.V.transmission permitted to the audience to follow the different operations on the frog's heart (Rana esculenta) : opening of the heart, mounting in the air (moisted from time to time by Ringer's fluid), 6-channels local MKG recording. The reversible iterative extention of elastoresistances showing a 20 % stretch from initial relaxed lengths was demonstrated, as well as elastocapacitances developing 5-7 pF under stretch.

Many questions were asked notably by J.D.Degos, N.H.C.Hwang, S.Ji,J.V.Jennings , T.Durali,J.C.de Oliveira,A.Abdelgawad, A.Limoge, W.E.Moore,E.Noël,A. Rialland,B.Gautheron ,J.F.Vibert,D.Caille,M.A.Boumendil, with B.Rybak and M.Morel explanations and comments.

This Lesson was delivered July 1,1978,at 10^{15} a.m.