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LEFT THORACOTOMY OF THE RABBIT AND SPECTRO-FLUOROMETRY WITH THE BRITTON CHANCE'S LIGHT GUIDES TIME SHARING APPARATUS *

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The left thoracotomy of a mammal displaying a complete mediastinum -as it is the case for the rabbit- was developed some years ago(1)(2). It permits to experiment: quantitatively,with a mammal heart, working,in situ,without extra-corporeal blood circulation and artificial ventilation - both assistances meaning constraints. Quantitations were already obtained with this original technique (3)(4)(5)(6)(7)(8)(9)(10). It was notably shown that the CO_2 induces a chemoreflex developed at the trigeminal nasal branches of the rabbit (11)(12)(13)(14)(15)(16)(17)- with a dyspnoea or, even, an apnoea followed by a polypnoea and, after,a return to the normopnoea - is characterized by: 1)an immediate drop of the blood plasma P_{O_2} associated with a deoxygenation of HbO_2, followed by overshoots of both and a return to normoxia according to a chronology closely related to the cardio-ventilatory macro-events, 2) a quasi-simultaneous time course of the intracellular $NAD \rightleftharpoons NADH$ - and it was the first time that a macro-reflex was shown to have its precise image at an intracellular molecular level-,3) a first rise of the peripheric blood pressure followed by a decrease of it and then,often,by a comparable re-increase of it before the return to the normotensive state, 4)a definite change of the ventricular action potential unit recorded with a succion electrode, the monophasic aspect of the action potential displaying a transitory biphasic morphology (splitting of the "MAP" pattern contemporary of the pro-

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nounced bradycardia).

Figure 1 shows the sudden ventilatory changes of a urethane (2g/kg) anaesthetized adult rabbit (vertical

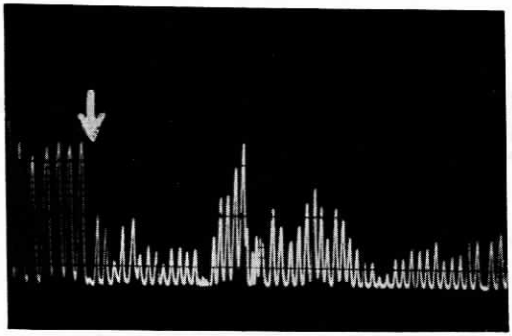

Fig.1

arrow : CO_2 at once). The left thorax is open between the 3rd and 4th ribs at room temperature; the record ($\vec{t}$ = 2s/division) was obtained from the original ventilometer described in this volume (cf. ventilomètre portatif) and specially designed for the head of a rabbit. The stabilization of the rhythm and the amplitude happens after 1-2 mn.

Figure 2 shows the simultaneous records of the ventilation -lower trace- and of the heart revolutions- upper trace- using the original ventilohygrometer and an

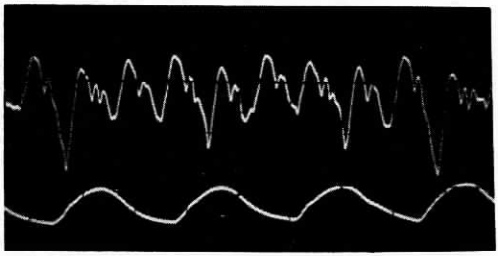

Fig.2

original elastoresistance adapted to the string located at a fixed point and, by a hook, at the cardiac apex ($\vec{t}$ = 250 ms/division).

The first physico-chemical results we obtained at the Johnson Foundation (1) used the combined fluorometer and double-beam spectrophotometer for reflectance measurements (18). In the present study we (19) use the time-sharing multichannel spectrophotometer, reflectometer and

fluorometer (20) owing to the greater versatility of the present apparatus, while positioning the light guide makes a little more delicate the work with a moving organ like the heart. With a cardiac system beating slowly the kymographic effect (9) - Fig.3 - is not to be neglected but, with a fast beating heart (the rabbit's heart for instance), the ratio signal/"noise" makes the measurements reliable.

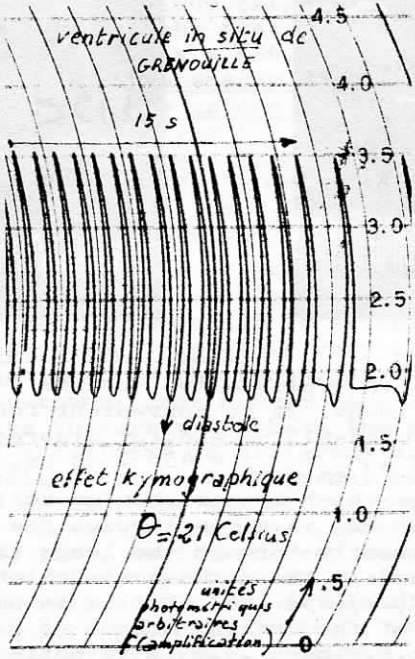

Fig.3

Figure 4 shows for the rabbit heart :at the upper part the reflectance signal, at the lower part the amplitude frequence time-course of the ventilation while the medium trace is related to the fluorescence of the NAD-NADH redox system ($\bar{t}$= 5 s/division). This figure is dealing with the calibration of reflectance (R) and fluorescence(F) using the quenching device of the apparatus in such a way that the zeros (R_o and F_o) are represented by the respective horizontal traces, allowing then to evaluate the procentual changes of R and F in function of the respective differences of amplitude. The calibrations represented in figure 4 belong to the left atrium at the left part of the figure and to the left ventricle at the rignt one. Notice that, for R and F, the atrium

displays a small but greater, while negligible, kymographic effect than the ventricle if reported to the ven-

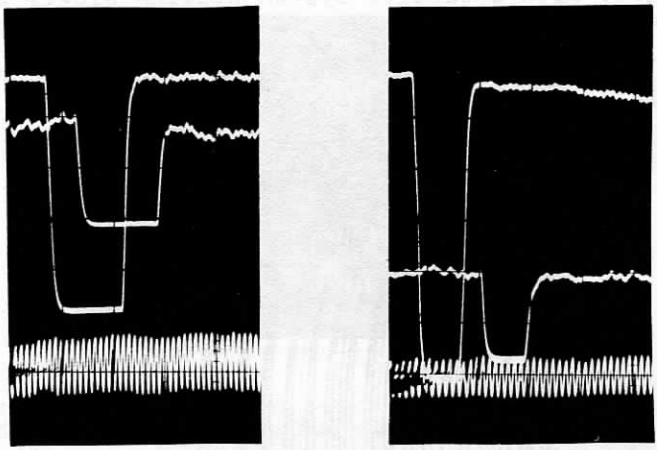

Fig.4

tilatory trace. The calibration must be done before and after any experiment. It is convenient for reflectance to operate at λ 543-615 nm and, for fluorescence at $\lambda_{exc.}$ = 366 ± 20 nm with a $\lambda_{em.NADH}$ = 450 ± 30 nm.

The changes which appear when an acute or sub-acute blow of O_2, N_2 or CO_2 is given through the nose or, via tracheotomy, directly through the lungs (21)(22)(23) affect conspicuously the acido-basic balance, the neurohumoral agents (24)(25) (26) what is to put in relation with the texts of the present School of D.Caille, J.Costa, A.Hugelin and J.F.Vibert . It is to notice that young animals withstand better than adults hypoxia even asphyxia; as well < = or > circadian biothythms should to be taken into consideration for any exhaustive explanation of the totality of the CO_2 chemoreflex. Then the intracellular electron transport and energy dependent phenomena correlated with a translocative reticular feedback where intracellular thermodynamics is adequatly in homorhesis with the macro-events we are treating as surgeons, pharmacologists, organ-physiologists, technobiologists, including the inherent interdisciplinary logic of living systems I would say (27) . Before moving to the field of the elements to take into consideration, when treating the thermodynamics of the polyphasic intracellular redox which constitutes the CO_2 chemoreflex, I would like to give some bibliographical informations

concerned with the ASI presentations of Pr A.Carpentier, and mainly, of Dr J.N.Fabiani: (28)(29)(30)(31)(32)(33). Now let us consider the classical thermodynamics of the CoI following (34).

The global reaction $NAD_{red.} + 1/2\ O_2 \rightarrow NAD_{ox.} + H_2O$ presents a $\Delta G = -52$ Kcal/mole."The process of electron transport from $NAD_{red.}$ to oxygen can then account for about 12 x 52,000 = 624,000 cal/mole of glucose oxidized. If we recall that the free energy of combustion (sic!cf.35) of glucose is - 686,000 cal/mole, it is clear that almost all the free energy decrease in the biological oxidation of glucose occurs during the enzymatic transport of electrons from the first electron acceptor along the respiratory chain to molecular oxygen"(34). In addition , "because the formation of three moles of ATP.requires... at least 3 x 7,000 cal and the oxidation of $NAD_{red.}$ delivers 52,000 cal, we can deduce that the oxidative phosphorylation of three moles of ADP conserves 3(7,000 / 52,000)100 = 41 percent of the total energy yield when one mole NAD is oxidized by oxygen" (34). This theoretical aspect is very important because it shades some light on the technique we are dealing with since the transitions $NAD \rightleftharpoons NADH$ look like the most attractive to currently check the evolution of the energetics of aerobiosis in different organs in situ as for this peculiar aspect. I would not be able here to stress, as it should, the definitive usefulness of this technique for biomedical purposes, mainly in surgery -, but let us add that, as it had been recognized in the field of the respiratory chain by my friend Britton Chance ," observation of flavoprotein fluorescence requires a blood-free perfused system"(what is also interesting to observe is that, probably often, with the $FMN\text{-}FMNH_2$ co-enzymes, there is an intermediary free radical formed). So, in terms of quantum chemistry according to the henceforth classical L.C.A.O. approximation (meaning: linear combination of atomic orbitals) the two candidates offering a great electronic affinity are precisely NAD(H) and $FMN(H_2)$ since the energy of the highest occupied orbitals (meaning : the capability to give electron) is $\sim$ 1.03 for NAD ,$\sim$ 0.3 for NADH ,$\sim$ 0.5 for FMN and $\sim$ -0.1 for $FMNH_2$ while the energy of the lowest free orbital (meaning : the capability to accept electron) is $\sim$ -0.35 for NAD, $\sim$ -0.91 for NADH ,$\sim$-0.34 for FMN (not far from NAD) and $\sim$ -0.95 for $FMNH_2$. When a substrate S is respired the immediate dehydrogenation by the system apodehydrogenase-CoI involves a E'_o=-0.32 V while the E'_o of the cytochrome - oxidase is + 0.81 V

at pH 7.4, resulting in the thermodynamic cascade to "O^{-}"; if the oxygen supply is lowered (hypoxia) or stopped (anoxia) a more or less reversible intoxication by electrons occurs (cf.35). Now it is to point out - as Dr Neely as well as Dr Opie did some years ago during a discussion with Dr Chance, Dr Williamson and others on the "border zone" for oxygen and glucose - that "glycolysis is inhibited in severely ischemic tissue contrary to what one might expect... compared to anoxic tissue, the degree of glycolytic inhibition increases with greater restriction in coronary flow. A 60 % reduction in flow results in a glycolytic rate approximately one-half that seen in anoxic tissue... One can inhibit the anoxic acceleration of glycolysis by adding either H^{+} or lactate to the perfusate in amounts which decrease cellular pH or increase lactate... the effect of lactate does not depend on changes in cellular pH but rather appears to be mediated either directly by lactate or indirectly through changes in other cellular constituents". This is obviously to be related to the "non-reflow phenomenon", and an additional reference should help because it is revealing the existence of "island anoxias" over the surface of the left ventricle (of rat or rabbit) in coronary artery occlusions, arteriole blockage and low flow ischemias. Complete blockage of regional coronary circulation by coronary artery occlusion resulted in a region of uniformely high NADH fluorescence with well defined sharp border indicating the abrupt change in

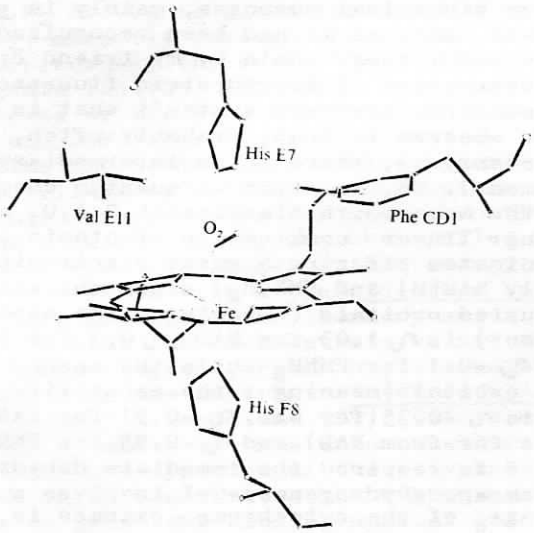

Fig.5

oxygen tension from the normoxic to the anoxic zone (36).

For further precisions regarding the calculation of the ΔG when the CO_2 chemoreflex described thereby developed, one should keep in mind that : 1) myoglobin is an excellent reservoir for oxygen supply when P_{O_2} drops (Figure 5 shows the most recent model of the structure of oxymyoglobin ; cf. 37) ; 2) the changing diameter of the coronary vessels may act upon the reflectance (Dr M.Kessler , through Dr S.Ji,personal information).

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DEMONSTRATION

Left thoracotomy was practised by B.Rybak showing the steady ventilation of the preparation as well as the heart movements. A transitory blow of 100 % CO_2 was repeatedly given through the nasal route displaying each time the described chemoreflex. On a question of Pr Degos, after the apnoea , instead of removing the CO_2 stream, this gas was still given : a deep slow ventilation resumed then and, after elimination of the CO_2 stream, the normal right-lung ventilation rate reappeared . Pr Carpentier,Pr Akkas, Dr Ji,Dr Fabiani,Dr Jessel,Dr Schamaneche went into different questions and comments.

July 3, 1978 , 5 p.m.