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EFFECT OF HEAVY METALS ON THE ELECTRICAL RESPONSE OF ISOLATED MUSCLE FIBERS OF EEL HEART.

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Engaged in the research program on marine pollution since the 15th of October 1971 the following account will evidently give no results but deal with the purpose of the experiments we plan to carry out.

Our study will be done in close collaboration with Mr Bouquegneau who investigates the sublethal effect of Hg on the physiology of different organs of intoxicated eels, especially the gills which play an important role in ionic equilibria (active transport).

We are trained in an electrophysiological technique which can be used to show the mechanisms which are fundamentally responsible for the action potential in muscle fibers (Marcq 1971)

We will therefore try and study the effect of heavy metals at the tissue level and attempt to show the role of sublethal quantities of heavy ions as Hg^{++}, Zn^{++}, Cu^{++}, Pb^{++} and Cd^{++} in the generation of the action potential in the cardiac fibers of eel.

At rest, the membrane of a nerve cell is relatively permeable to K^+ ions and not very permeable to extracellular cations like Na^+. When activated, the membrane becomes permeable to extracellular cations and this brings the membrane potential to a value near the Nernst equilibrium potential of the concentration cell corresponding to these cations. This hypothesis involves the existence of passive ionic currents through the membrane but also supposes that there is an active pump which eliminates the Na^+ ions and restablishes the intracellular ratio of K^+ ions.

This scheme proposed by Hodgkin and Huxley (1952) has been applied to other excitable tissues as the skeletal muscle fiber (Ildefonse et Gargouil 1963, Kao and Stanfield 1967, Adrian et al 1968) and cardiac muscle (Düddel et al. 1967., Reuter 1967-1968, Rougier et al. 1968-1969).

To observe the ionic currents, one must use a <u>voltage-clamp technique</u> : the potential across a prescribed area of the cell membrane is held at a known value. Conversely to measure the membrane potential changes one holds the current constant (<u>current clamp</u>).

The use of intracellular electrodes is difficult in tissues as thin as cardiac fibers and it is easier to work with extracellular electrodes and to use the <u>Technique of the "double sucrose gap"</u> (Julian et al 1962b, Rougier et al. 1968).

I. Physiological equipment and method.

A. Experimental chamber

The experimental chamber consists of 5 compartments as shown in fig 1.

The muscle fiber is stretched between the compartments 1 and 5 which contain an isotonic Kcl solution. The compartment 2 and 4 contain isotonic saccharose and compartment 3 is continually flushed with a physiological solution. (Test compartment).

The experimental chamber is connected to the electrical apparatus with agar bridges and calomel electrodes in compartments 1, 3, 5.

B. Membrane potential recorded in current clamp.

Fig.2.

To record an action potential, a constant current is applied to the preparation by the following procedure.

The operationnal amplifier is connected between compartment 5 and ground potential.Because its infinite gain there is no potential difference between the two inputs of the amplifier and point "S" (inside the fiber) is always maintained at ground potential.

Rectangular current pulses are delivered by the stimulator connected between ground and the compartment 1.

The current flows through the resistances RiI and Rm (variable resistance of the membrane) and elicits a potential difference at the ends of Rm. The voltage changes are recorded by the oscilloscope (Vo) between ground and the output of the amplifier. They are photographed and show the time course of the potential between the two sides of the membrane across which a constant current is maintained.

C. Membrane current recorded in voltage clamp.

Fig. 3.

The experimental chamber is the same, but the connexions are inverted in compartments 1, 3, 5.

II. Physiological preparations.

The fibers which are used, are isolated from the heart's atrium because their structure and dimensions are similar to the sinoauricular fibers of frog which have been well studied by Rougier et al (1968) using the double sucrose gap technique.

The heart of fish is made up of what appear to be typical vertebrate cardiac muscle fibers. Jensen (1965) reported that the diameter of atrial fibers in the hagfish's heart were 6,1u while atrial fibers in the dog's heart are 10 u (Hoffman and Cranefield 1960).

III. Research plan

We shall first try and define the shape of the action potential in atrial fibers to compare with the data of the litterature. According to Randall (1968), the ventricular resting

potential of the trout's heart is about 60 to 70 mV and the shape of the action potential is similar to the one recorded from other vertebrate hearts (amplitude about 85 mV and duration about 620 msec.).

When the conditions to otbtain a stable electrical response will have been established ionic currents will be investigated using the voltage clamp method.

As shown by many authors in some vertebrate hearts there are two components in the inward current. In frog's heart Rougier et al. (1968) have shown that the fast inward current of Na^+ is followed by a slow current of Na^+ and Ca^{2+} which explains the prolonged action potential.

The use of specific inhibitors of the Na^+ and Ca^{2+} permeability allows to investigate the possible contribution of Ca^{2+} ions to the depolarizing current in the cardiac cell membrane.

The different parameters having been well established in normal cardiac fibers we will then try and observe the alterations of the action potential in physiological solutions containing heavy ions. Finally, we will use fibers from eels intoxicated during a definite period in contaminated see water to compare their electrical responses with the responses obtained by direct intoxication.

It will then be possible to show the eventual role of heavy metals in the generation of the action potential "in vivo" and to define the threshold value at which the effect becomes detectable.

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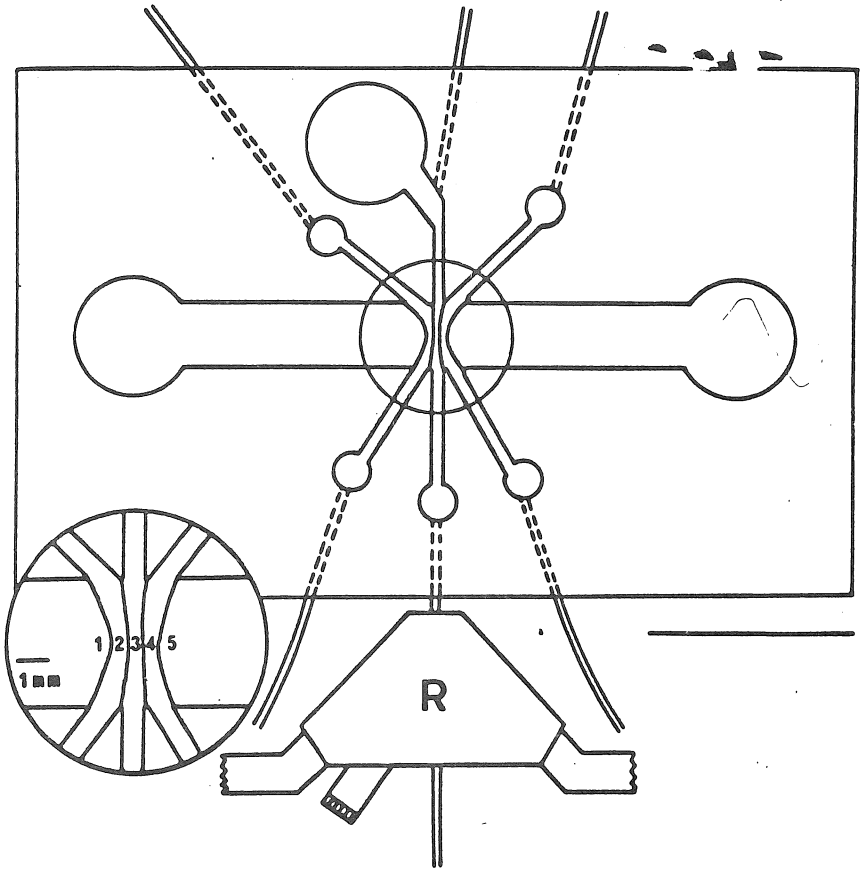

Fig. 4 SCHEMA DE LA CUVE EXPERIMENTALE.

Dans le médaillon, la partie centrale a été agrandie.

R. = Robinet
1- 5 = compartiments de référence
2- 4 = compartiments de saccharose largeur 400 à 600 microns
3 = compartiment test largeur 200 microns.

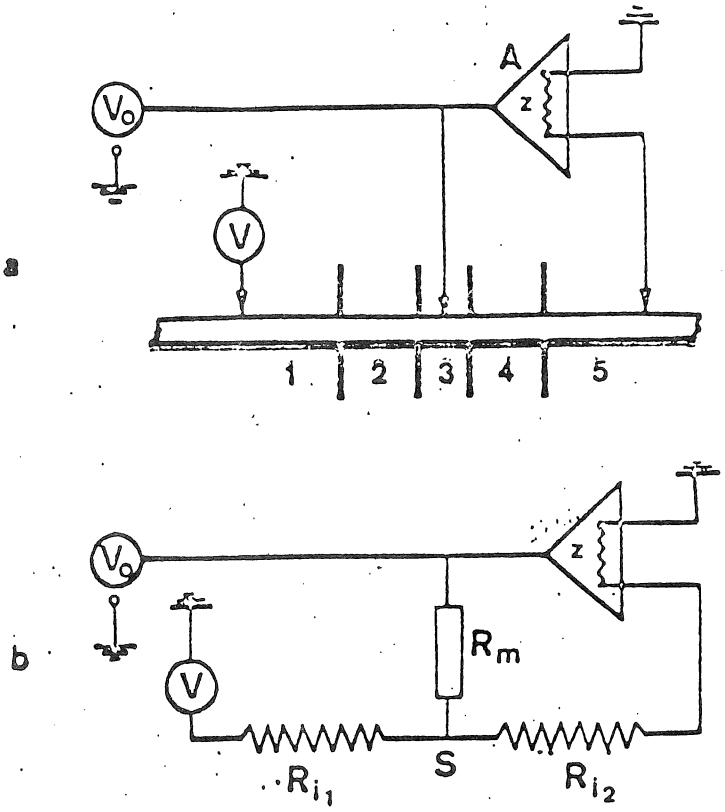

Figure 2 : Enregistrement du potentiel de membrane en courant imposé :

a : Schéma du montage : I,2,3,4 et 5 : compartiments précédemment définis;
A : amplificateur différentiel à courant continu, d'impédance Z;
V : générateur d'impulsions rectangulaires; Vo : oscilloscope cathodique.

b : Circuit équivalent. R_m = résistance de membrane du compartiment 3;
R_{i1} et R_{i2} = résistance série correspondant essentiellement à la résistance intracellulaire dans les compartiments 2 et 4.

Le point de sommation S, qui correspond à l'intérieur de la fibre dans le compartiment 3, est constamment maintenu au potentiel "de terre".

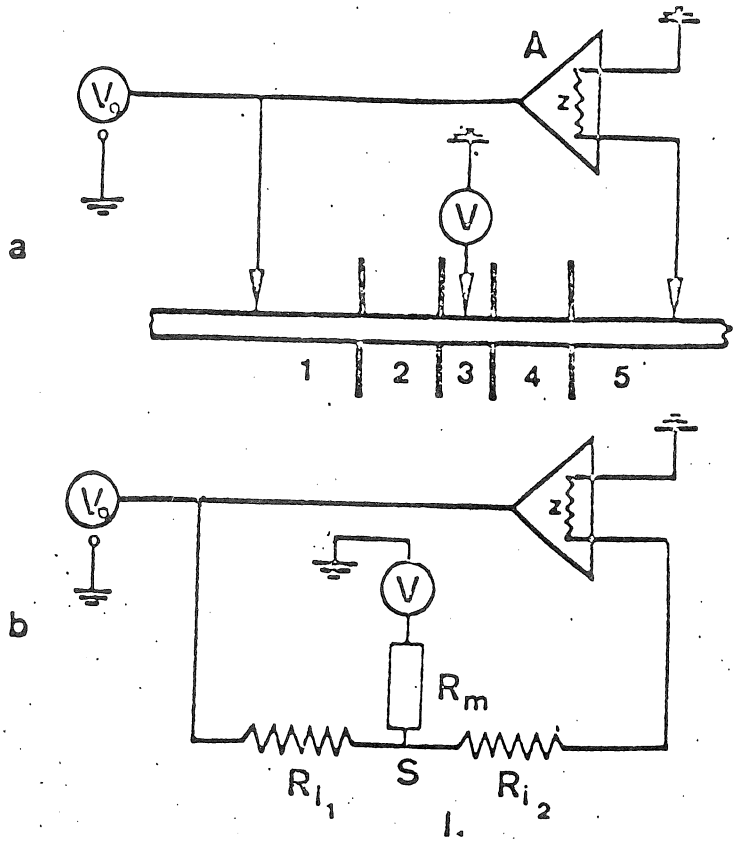

Figure 3 : Enregistrement du courant de membrane en voltage imposé.

a : Schéma du montage. I, 2, 3, 4 et 5 : compartiments précédemment définis; A : amplificateur différentiel à courant continu, d'impédance Z; V : générateur d'impulsions rectangulaires; V_o : oscillographe cathodique.

b : circuit électrique équivalent. R_m : résistance de membrane du compartiment 3; R_{i1} et R_{i2} : résistance série correspondant essentiellement à la résistance intracellulaire dans les compartiments 2 et 4.

Le point de sommation S est constamment maintenu au potentiel de terre.