

**Results and discussion.** From the sympathetic nerve of 18 cats, in local anaesthesia, we have isolated 12 units influenced by different light intensities. The discharge frequency of the tonically active fibres showed lower values (0.2–5 imp/sec) during illumination and higher values (0.5–18 imp/sec) in darkness. Other units were phasically active, only showing activity in darkness or at low values of light intensity. The same units exhibited spontaneous discharge changes, at times even shifting from tonic to phasic activity (figure): however, an inverse relationship between light intensity and discharge frequency was always present. A highly significant correlation, probably linear, was found in our experimental conditions, between the discharge frequency in the light and in the darkness, independently from the type of activity of the fibres ( $r = 0.758$ ).

The pattern of discharge frequency of the fibres, shifting from light to darkness, consisted mostly in an initial peak of frequency, then the firing frequency settled down at lower values, but higher than those presented during the previous illumination. Occasionally the phasic units, after the initial peak, became progressively silent. When shifting from darkness to light the unitary discharge showed a sudden complete inhibition lasting 2–4 sec, followed by a gradual return of the firing frequency at a level that depended on the light intensity. These fibres could never be evidenced in preliminar experiments in which the animals underwent different levels of general anaesthesia whether Nembutal, Urethane-Chloralose or Chloralose were used. Very small doses of Nembutal (5 mg/kg) administered in unanaesthetized cats greatly decreased the resting discharge of the sympathetic light-responsive units and completely abolished the response to darkness.

An increase or decrease of the discharge rate always coincided with pupillary dilatation or constriction both in the normal and in the sympathectomized eye. Such preganglionic fibres are evidently concerned with pupillary dilatation, since no other ocular structures are reported to have such a functionally strict relationship with light intensity<sup>8</sup>. The presence of light-responsive

sympathetic fibres indicates that the sympathetic nerve not only maintains a tone in the dilatator pupillae muscle but also modulates finely its discharge activity in order to adjust, together with the parasympathetic nerve, the pupil size in the reflex response to changes of illumination. In most of our trials, the per cent variation in frequency was higher when shifting from light to darkness than vice-versa and it was particularly evident in those phasic fibres which, after a few minutes of darkness, exhibited a progressive decrease till complete inhibition and no change of activity to the successive illumination occurred. These data suggest that the sympathetic system plays a bigger role in pupillary response to darkness than to light. Furthermore, the parasympathetic activity recorded from the short ciliary nerves in the cat showed that the discharge enhancement during the light reflex is bigger than the decrease in the reflex to darkness<sup>9</sup>. These results confirm the Duke-Elder hypothesis<sup>10</sup>, according to which the reflex to light is primarily parasympathetic whereas the reflex to darkness is primarily sympathetic.

The study of the role of the sympathetic output in the direct and consensual light reflex showed that the variations of discharge frequency of light-responsive fibres were much larger when the same change of illumination was performed on the ipsilateral than on the contralateral eye. This fact gives electrophysiological demonstration that the sympathetic reflex to light is mainly ipsilateral in the cat, notwithstanding numerous crossings of both afferent<sup>11,12</sup> and efferent<sup>12</sup> pathways in the brain stem and upper cervical segments.

8 H. Davson, in: *Physiology of the eye*. Churchill Livingstone, Edinburgh and London 1972.

9 I. Nisida and H. Okada, *Jap. J. Physiol.* 10, 64 (1960).

10 S. Duke-Elder, in: *System of Ophthalmology*, vol. 12. Ed. S. Duke-Elder. Harry Kimpton, London 1971.

11 M. H. Evans, *J. Physiol.* 158, 560 (1961).

12 H. Okada, O. Nakano, K. Okamoto, K. Nakayama and I. Nisida, *Jap. J. Physiol.* 10, 646 (1960).

## The potential independent series resistance in rat ventricular fibres<sup>1</sup>

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**Summary.** Current clamp experiments performed in rat ventricular fibres revealed the presence of a resistance  $R_s$  (between 14.5 and 15.5  $K\Omega$ ) in series with the membrane capacity.  $R_s$  behaved as a lumped resistance and its value remained constant throughout the action potential repolarization phase.

**Introduction.** Since the results of Hodgkin et al.<sup>2</sup> on the squid axon, the presence of a resistance ( $R_s$ ) in series with the membrane capacity ( $C_m$ ) was observed in various cardiac tissues<sup>3–5</sup>. There has been evidence that in voltage clamp experiments the presence of  $R_s$  causes deviations of the transmembrane voltage from the command pulse and shifts the voltage-current relationships<sup>6,7</sup>. In the squid axon,  $R_s$  is usually attributed to the Schwann cell layer, while in cardiac muscle it may represent the cleft resistance<sup>8</sup>.

During a cardiac action potential, Na, Ca and K ions move through the cell membrane. If the cleft narrowness

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2 A. L. Hodgkin, A. F. Huxley and B. Katz, *J. Physiol., Lond.* 116, 424 (1952).

3 H. A. Fozzard, *J. Physiol., Lond.* 182, 255 (1966).

4 G. W. Beeler and H. Reuter, *J. Physiol. Lond.* 207, 165 (1970).

5 M. Tarr and J. Trank, *J. Gen. Physiol.* 58, 511 (1971).

6 F. Ramon, N. Anderson, R. W. Joyner and J. W. Moore, *Biophys. J.* 15, 55 (1975).

7 J. W. Moore, F. Ramon and R. W. Joyner, *Biophys. J.* 15, 11 (1975).

8 E. A. Johnson and J. R. Somner, *J. Cell. Biol.* 33, 103 (1967).

acts as a diffusion barrier, the extracellular ion content might change in the course of the electrical activity. This suggests that the extracellular space conductivity and consequently the  $R_s$  could deviate from their resting values.

The purpose of the present paper is to confirm the presence of a series resistance in the cardiac tissue being submitted to the experiment and to study the  $R_s$  value at different membrane potentials levels.

**Material and methods.** Right ventricular fibres of about 2 mm long and 100  $\mu\text{m}$  diameter were isolated from rat hearts. They were laid in a double sucrose gap perfusion chamber in which a small segment (100  $\mu\text{m}$ ) was electrically isolated from the ends by 2 high resistance isotonic sucrose flows<sup>9,10</sup>. The compartments were separated by vaseline seals, which gives a better voltage control than liquid partitions<sup>11</sup>. The normal solution was a modified Tyrode solution of following composition (mM): Na 143, Ca 2.16, Mg 0.25, Cl 155, tris 5, glucose 11, pH 7.4. The sucrose solution (300 mM) contained also

$\text{CaCl}_2$  ( $10^{-5}$  M) in order to keep the internal longitudinal resistance ( $R_i$ ) more constant<sup>12</sup>. The 2 ends of the fibre were soaked in an isotonic KCl solution. All the liquids were saturated with pure oxygen and thermostatically regulated at  $18^\circ\text{C} \pm 1^\circ\text{C}$ .

Figure 1 shows a simplified diagram of the electrical circuit associated with the double sucrose gap fibre arrangement. The potential changes ( $V$ ) were measured at the output of a dc-feedback amplifier<sup>10</sup> connected to the central gap. In the results, only experiments in which the short circuit factor was 0.8 or more were considered. A subthreshold depolarizing current step ( $I$ ) is assumed to generate the consecutive membrane potential transients. At time 0, the membrane resistance ( $R_m$ ) is short circuited by the membrane capacity ( $C_m$ ). The current passes then entirely through the  $C_m R_s$  branch and induces a step magnitude  $I R_s$  in the voltage response<sup>13</sup>. Later on, the membrane potential deviates exponentially toward a steady value  $V_m = (R_m + R_s) I$  with a time constant which will not be discussed here.

**Results and discussion.** Depolarizing current steps are applied every 10 sec through the fibre and the potential changes between the central pool and the KCl pool (A) are recorded. As predicted by the theory, the membrane potential presented 2 inverted steps of the same magnitude upon switching the current on and off (figure 2). The potential steps increased proportionally to the current amplitude (figure 2, A–D).

There are, however, considerable factors against a lumped series resistance in a multicellular preparation. The cells in the core of the bundle have probably a much higher series resistance than those near the outside of the fibre. Moreover, near the core of the preparation, all along the central gap, the boundary between the Tyrode solution and sucrose solution will not be accurate. In this connection, the initial potential step was studied at fast sweep speed. The figure 3 shows 2 initial potential drops recorded on a rat ventricular fibre (left part) and on an equivalent circuit showing very similar electrical characteristics (right part), in response to the same current pulse. In the 2 cases, the apparent time to peak for the

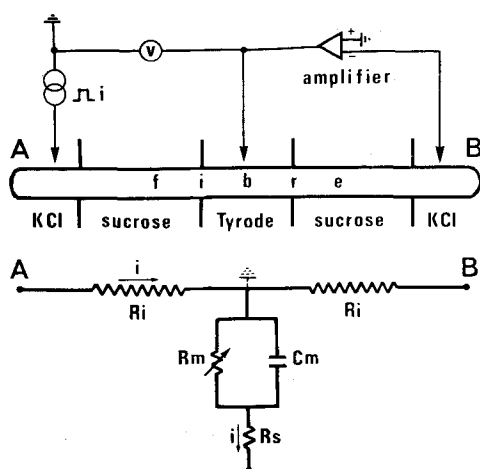


Fig. 1. The fibre (AB) arrangement for current-clamp recordings (upper part) and the associated equivalent electrical circuit (lower part). (See symbols in material and methods.)

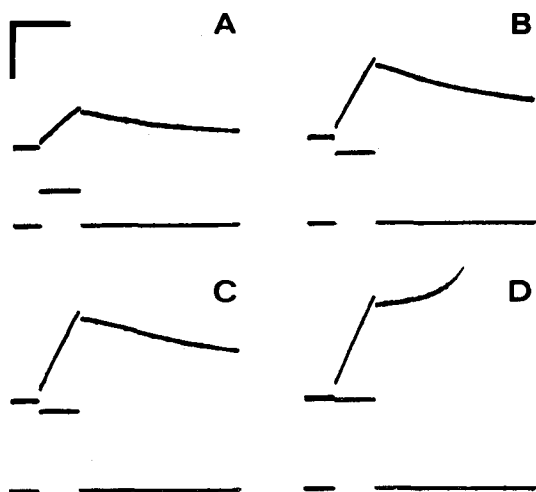


Fig. 2. Recording of membrane potential changes (upper traces) in response to dc-current pulses (lower traces) increasing from A to D. The potential steps appearing upon switching the current on and off increased proportionally to the current amplitude. Horizontal scale 10 ms, vertical scales: 100 nA and 10 mV.

- 9 R. Stampfli, *Experientia* 10, 508 (1954).
- 10 O. Rougier, G. Vassort and R. Stampfli, *Pflügers Arch. Ges. Physiol.* 307, 91 (1969).
- 11 A. De Hemptine, *Pflügers Arch.* 363, 87 (1976).
- 12 A. Kleber, *Pflügers Arch.* 345, 195 (1973).
- 13 R. D. Keynes, E. Rojas, R. E. Taylor and J. Vergara, *J. Physiol., Lond.* 229, 409 (1973).

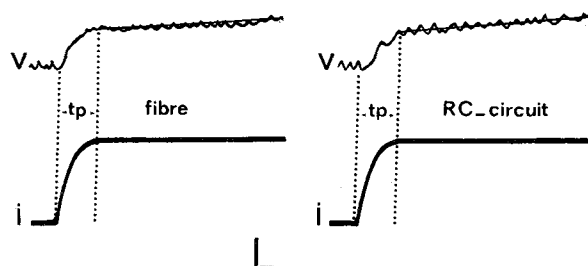


Fig. 3. High speed recorded initial potential steps ( $V$ ) elicited by a depolarizing current ( $I$ ) of 175 nA, in a rat fibre (left) and in an equivalent electrical circuit (right). In the rat fibre the following parameters have been calculated  $R_i = 830 \text{ K}\Omega$ ,  $R_m = 500 \text{ K}\Omega$ ,  $R_s$  (fibre + electrode + liquid) =  $13.7 \text{ K}\Omega$ ,  $C_m = 23 \text{ nF}$ . In the RC-circuit:  $R_i = 820 \text{ K}\Omega$ ,  $R_m = 510 \text{ K}\Omega$ ,  $R_s = 14 \text{ K}\Omega$ ,  $C_m = 22 \text{ nF}$ . The time to peak for initial potential ( $t_p$ ) is very similar in the 2 cases. Horizontal scale: 20  $\mu\text{s}$ , vertical scale: 2 mV.

potential step (tp) is very near from the rising current. However, in both the rat fibre and the equivalent circuit, the time for half potential peak is 5–10  $\mu$ s longer than the time for half rising current. This delay may probably be attributed to the recording equipment's time lag, since the amplifier bandwidth was about 40–50 kHz under current clamp conditions. The initial potential step and the current intensity follow the Ohm's law and a global  $R_s$  of  $20.5 \text{ K}\Omega \pm 2.11 \text{ K}\Omega$  has been obtained from 8 experi-

ments. In the central gap, the resistance of the calomel electrode and external liquid was 5 to 6  $\text{K}\Omega$ , so that the actual series resistance of our preparations may be estimated to be between  $14.4 \text{ K}\Omega \pm 2.06 \text{ K}\Omega$  and  $15.4 \text{ K}\Omega \pm 2.06 \text{ K}\Omega$ . Fast potential steps (5–10 mV) in response to depolarizing currents were recently reported in rat papillary muscle flooded with Na free solutions<sup>14</sup> (figure 2). Unfortunately,  $R_s$  could not be calculated since the size of currents was not shown in the recordings.

In a second series of experiments, 2 identical steps of suprathreshold depolarizing current were applied through the membrane. The first current pulse generated an action potential. The second pulse, imposed during the repolarization phase permitted us to determine  $R_s$  value at different potential levels. A typical experiment is shown in the figure 4. The initial potential step remains constant either at the resting potential or at all membrane potentials between 10 and 90% of the action potential repolarization phase.

From our experiments carried on rat ventricular fibres, it can be pointed out that: a)  $R_s$  behaves as a pure lumped series resistance, b)  $R_s$  is independent of the membrane potential. The latter result suggests that ionic movements involved in a single cardiac action potential are unable to change appreciably the global conductance of extracellular spaces. This work does not preclude the cable problem in multicellular preparations since the anatomical features of  $R_s$  (clefts, tubules, intercellular spaces)<sup>13</sup> authorizes us to assume that each cell in the bundle has probably its own  $R_s$ . However, the potential independent behaviour of  $R_s$  may become important later on when electronic compensation of this parameter is attempted.

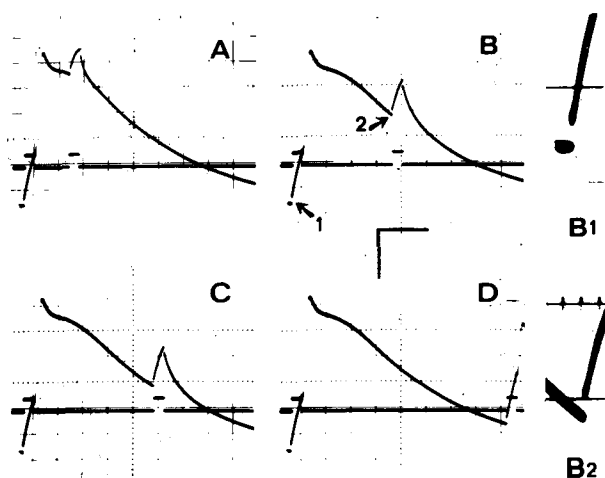


Fig. 4. From A to D: Identical initial potential steps in response to a constant current pulse applied at the resting potential or throughout the repolarization phase of the action potential. The steps 1 and 2 (panel B) were magnified respectively in B 1 and B 2 in order to emphasize this similitude. Scales from A to D; horizontal line: 20 ms, vertical line: 40 mV. B 1 and B 2 are enlarged 3.8 times.

14 G. W. Mainwood and J. S. McGuigan, *Experientia* 31, 67 (1975).

## Repercussions de l'hyperthyroïdie sur le contenu total en ADN du cervelet de rat âgé de 6 et 35 jours. Effets comparés de la LT3 et de la DLT4

### Repercussions of hyperthyroidism on the total cerebellar DNA contents in the rat at 6 and 35 days of age. Comparative effects of LT3 and DLT4

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**Summary.** The total cerebellar proteins RNA and DNA contents from DLT4- and LT3-treated rats was studied at 6 and 35 days of age. The effect of injections of 5  $\mu$ g/j of DLT4 is comparable to that of 25  $\mu$ g of LT3 at birth, followed by 0.5  $\mu$ g every 2 days. On the other hand, injection of 0.5  $\mu$ g of LT3 every 2 days does not induce any significant modification of the total DNA contents in the cerebellum.

L'injection d'hormones thyroïdiennes depuis la naissance à de jeunes rats, accélère la maturation histologique<sup>1-3</sup> et biochimique<sup>4-6</sup> du cervelet. Toutefois, les études concernant le contenu en ADN de cet organe<sup>4,7</sup>, ne donnent pas des résultats concordants. En effet, les animaux étudiés reçoivent de la LT3 avec une forte dose à la naissance dans une expérience<sup>4</sup> et de la DLT4 à doses beaucoup plus faibles dans l'autre<sup>7</sup>. Cette constatation nous a conduits à étudier l'influence de la nature et des doses des hormones thyroïdiennes administrées.

- 1 J. Legrand, *Archs Anat. microsc. Morph. exp.* 56, 205 (1967).
- 2 A. Rebière et J. Legrand, *Archs Anat. microsc. Morph. exp.* 61, 105 (1972).
- 3 J. Tusques, *Biol. méd. Paris* 45, 395 (1956).
- 4 R. Balázs, S. Kovacs, W. A. Cocks, A. L. Johnson et J. T. Eayrs, *Brain. Res.* 25, 555 (1971).
- 5 J. Dainat et J. Legrand, *C. r. Séanc. Soc. Biol.* 169, 1377 (1971).
- 6 J. Dainat et A. Rebière, *J. Neurochem.* 26, 935 (1976).
- 7 J. Gourdon, J. Clos, C. Coste, J. Dainat et J. Legrand, *J. Neurochem.* 21, 861 (1973).