Histology. — *"An Inquiry into the Distribution of Potassium-compounds in the Electric Organ of the Thorn-back (Raja Clavata)."* By M. W. Woerdeman. (Communicated by Prof. H. Zwaardemaker).

(Communicated at the meeting of October 30, 1920).

On Prof. Zwaardemaker's suggestion I have latterly examined different tissues and organs microchemically on the presence of potassium-compounds. I intend to discuss the method and the results of this inquiry more in detail; for the present I will publish only my experience in the investigation of the electric organ of the Thorn-back (Raja clavata).

Zwaardemaker's researches established that the function of organs, perfused artificially with a salt-solution, largely depends on the potassium-content of that solution, and that the potassium, being a weakly radio-active element, plays such a prominent part in the origin of the organic actions, on account of its very radio-activity. It may be supposed, therefore, that the potassium compounds which are normally to be found in the organs of animals or plants, take an important part in the normal functions of these organs. We presume, therefore, that information concerning the presence or the absence of potassium-compounds in certain cells, tissues, or organs will be gladly received.

Now, through chemical examination the quantum of potassium, contained in various organs, has already been determined with great accuracy. From this examination we do not learn, however, where in the organ the potassium-compounds are located. Macallum namely has detected that in a number of tissues and organs the potassium-salts are not distributed at random and irregularly, but that they often occur there at definite places, bound to quite definite structures. Macallum's[1]) reagent on potassium-compounds is a modification of the mixture used for the first time by De Koninck[2]). Macallum's mixture of cobalt-salt and sodium-nitrite, added to a potassium-salt

[1]) A. B. Macallum. On the distribution of potassium in animal and vegetable cells. Journ. of Physiol Vol. XXXII, 1905 and Die Methoden und Ergebnisse der Mikrochemie in der biologischen Forschung. Ergebn. der Physiologie, Jrg. 7 1908, p. 552.

[2]) de Koninck. Zeitschr. f. analyt. Chemie. Bnd. 20. 1881, p. 390.

solution precipitates Fischer's crystalline salt (a potassium-cobalt sodium-nitrite). Macallum puts sections of frozen material or teased out preparations of fresh tissue in his cobalt-nitrite-sodium-nitrite mixture. In the tissues and in the cells the potassium-precipitate can now be formed. After being thoroughly washed with distilled water, by which the reagent is washed away, the tissue is subjected to an after-treatment with ammonium sulphide, which renders Fischer's salt black (formation of cobalt-sulphide). Wherever microscopical examination reveals black precipitates or black decoloration, we may decide upon the presence of potassium-compounds.

If e.g. voluntary muscle-tissue is treated after Macullum's method, it will be seen that the potassium-compounds occur almost exclusively in the doubly refracting discs of the muscular fibres. The discs of single refraction do not contain any potassium. Now, because the electric organs of the so-called electric fishes are developed (with a few exceptions) from voluntary muscular tissue, and since nobody has as yet studied the distribution of potassium-compounds in this metamorphosed muscular tissue, we considered an inquiry into the distribution of potassium compounds in the electric organs of some consequence. I regret to say that, although Dr. C. Kerbert, Director of Natura Artis Magistra offered his kind assistance, the strong electric fishes could not be put at my disposal. But also the Thorn-back (Raja clavata), occurring at the Dutch North Sea-coast, has an electric organ, which, though its action is weak, largely resembles in structure the organs with stronger action. I, therefore, applied to the Zoological Station at Helder, where, through the kindness of Dr. Redeke and Dr. van Goor I was enabled to perform the potassium-reaction in the living electric organ of a thornback. The tissue was put in the reagent and afterwards made into sections with an ice-microtome (spray from liquid carbon dioxide). Although it is better to make the sections first and to treat them immediately after with the reagent, I followed the other way, because there was no freezing microtome at the Station. This altered working-method, however, did not lessen the value of the results. I took care to put very small pieces of tissue in the reagent in order to allow the reagent to permeate the tissue as effectually and as rapidly as possible. As I do not see any difference between the outer and the central parts of the preparations, the diffusion appears to have succeeded well. Before reporting the results of this inquiry I may as well remind the reader that the electric organ of the Thorn-back is situated within the lower two-thirds of the tail. There it lies at the side of the dorsal and ventral tail-muscles. It consists of a large number of so-called electric platelets disposed in rows running parallel to the axis of the body. Each platelet (see fig. 1) consists of 1. a so-called anterior cortical layer composed of a single layer of cells; 2. an inner layer composed of numerous twisted lamellae

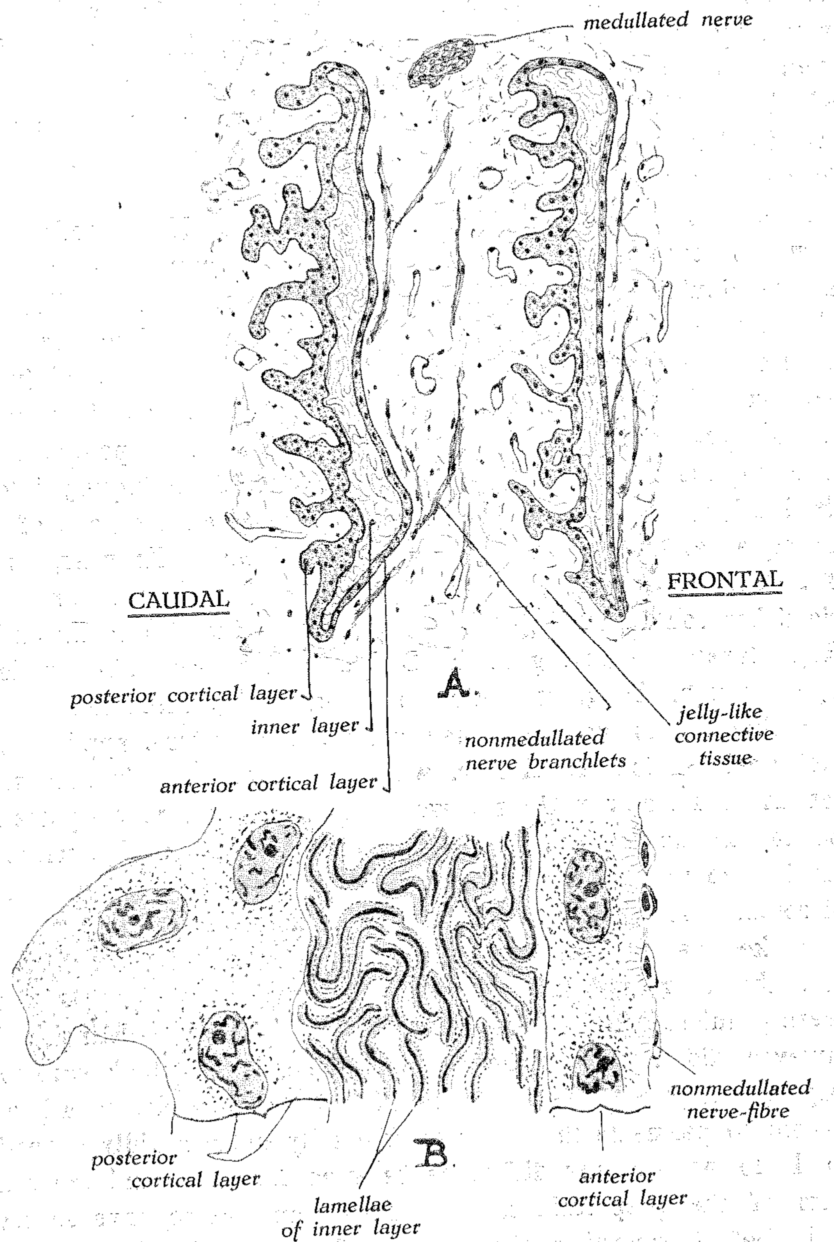

Fig. 1. A. Sagittal section through electric organ of thorn-back. (only two electric platelets have been represented).
B. Part of an electric platelet, more enlarged.
(Diagrams, made after picture of frozen sections).

and 3. a posterior cortical layer. The latter consists, like the anterior layer, of a single layer of cells, but this layer is folded in so many ways, that in sections the impression is excited as if the posterior cortical layer is provided with fringe-like prolongations. The entire platelet is enveloped by a very thin homogeneous membrane (the electrolemma). A very fine network of non-medullated nerve-fibres lies against the anterior cortical layer. Where the fibres reach the cells of this layer, those cells present peculiar rods. The inner layer is the most complicated. To realize its structure we should bear in mind that each electric platelet must be considered as a modified muscular fibre.

The lamellae of the inner layer are derived from the anisotropous- and isotropous-discs of the striated muscle-fibre (see also ENGELMANN's researches in "Onderzoekingen Physiol. Lab. Utrecht 4e Reeks III, p. 307).

The doubly refracting layers become thicker, they lose their faculty of double refraction, while the isotropous discs remain visible as finer, dark stripes. The layers are sinuously disposed and thus originates the complicate structure of the inner layer of the electric platelet.

BABUCHIN could distinguish in young living rays the gradual transformation of the muscular fibres into electric platelets and was able to demonstrate that electric stimulation still elicited contractions in fibres which had not yet undergone a complete transformation. The electric plate once being formed, contractility is lost, but the generation of electricity, which is also a property of the muscular fibre, has far more become a principal function. So the electric organ may justly be looked upon as a highly interesting object with a view to potassium-researches.

Let it be finally observed that all electric platelets are located in a jelly-like connective tissue.

In successful preparations, treated after MACALLUM, I was now enabled to establish that the electric platelets contain a great many potassium compounds, whereas the jelly in which they are lying is almost destitute of potassium (see fig. 2). Whereas in the medullary sheath of the medullated nerves a distinct reaction is found, by which the neurokeratin-reticulum is disclosed, the non-medullated fibres appeared to be entirely potassium-free. It follows that in most preparations nothing is to be seen of the nerve-network which lies against the anterior cortical layer. This confirms MACALLUM's finding that no potassium-reaction occurs in the axis-cylinders of the nervefibres. The electrolemma is colourless and therefore apparently potassium-free. In the electric platelet itself the reaction reveals itself most distinctly in the inner layer (Fig. 3). In the anterior and posterior cortical layers we observe fine-granular, black precipitates, above all round the nuclei. In the nuclei themselves there is no reaction. Indeed, MACALLUM could never observe a potassium-precipitate in a nucleus. This justifies the assumption that the nucleus is potassium-free, since so-called masked potassium-compounds (such as iron in haemoglobin) are unknown.

Cell-boundaries between the various nuclei I did not detect, consequently I would rather describe the anterior and posterior cortical layer as a syncytium. It is very striking that there is a considerable accumulation of black grains in that portion of the anterior syncytium which leans on the inner-layer of the platelet.

A regular granular layer exists on the boundary between the anterior syncytium and the inner layer. On the boundary between the inner layer and posterior syncytium my preparations do not reveal that accumulation of potassium-compounds. Although in some parts of the anterior syncytium also rods could be distinguished, they did not show any black coloration. The inner layer of the electric platelet is highly potassium-rich and it is remarkable that here also dark and light stripes occur, just as in voluntary muscular tissue. The dark stripes are diffusely black; I did not see any grains. — In pieces of voluntary muscular tissue taken from the tail of the ray, which were also treated with the potassium-reagent, the anisotropous discs were also diffusely black and the isotropous layers completely colourless. Now, because the inner layer of the platelet has arisen from the fibrillary part of a voluntary muscular fibre, we can hardly be mistaken in conceiving the alternation of dark and light stripes in the inner layer of the electric platelet as a remainder of the alternation of anisotropous, potassium-bearing and isotropous, potassiumfree discs of the muscular fibre.

In the jelly between the platelets I found only potassium deposits in the protoplasm of the starshaped connective-tissue cells. They, however, are poor in potassium and so the whole quantity of potassium-compounds in the jelly is very small; anyhow, strikingly small compared with the potassium-rich electric platelet. The same pictures recurring in various preparations as described above, I may be justified in considering the above-mentioned distribution of the potassium-compounds to be not a casual, but a typical phenomenon.

According to MACALLUM the forms under which potassium occurs in the tissues are the following:

1st. as a local precipitate;

2nd. as a series of local sharply outlined deposits;

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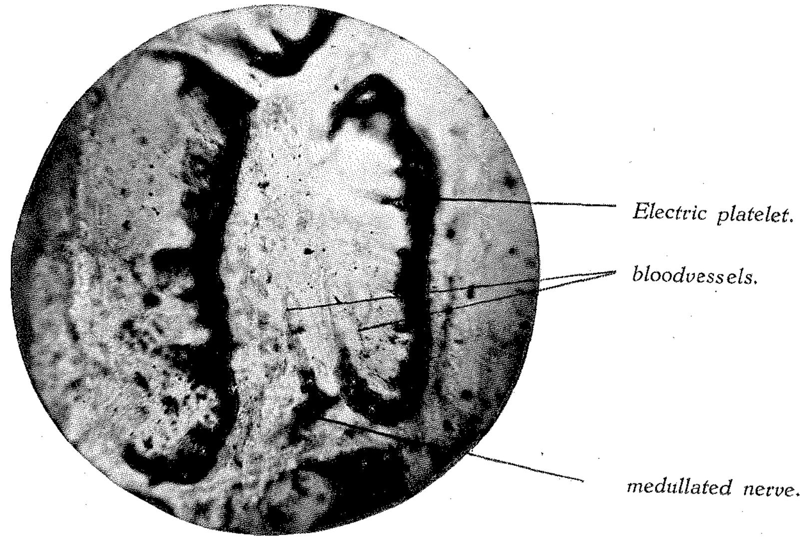

Fig. 2. Microphoto of two electric platelets of Raja punctata. (Section of frozen organ, treated with potassium-reagent) Exhibits a very black precipitate in the electric platelets and little or no precipitate in the jelly-like connective tissue between the platelets.
(Reichert. Objective 4. Ocul. 2).

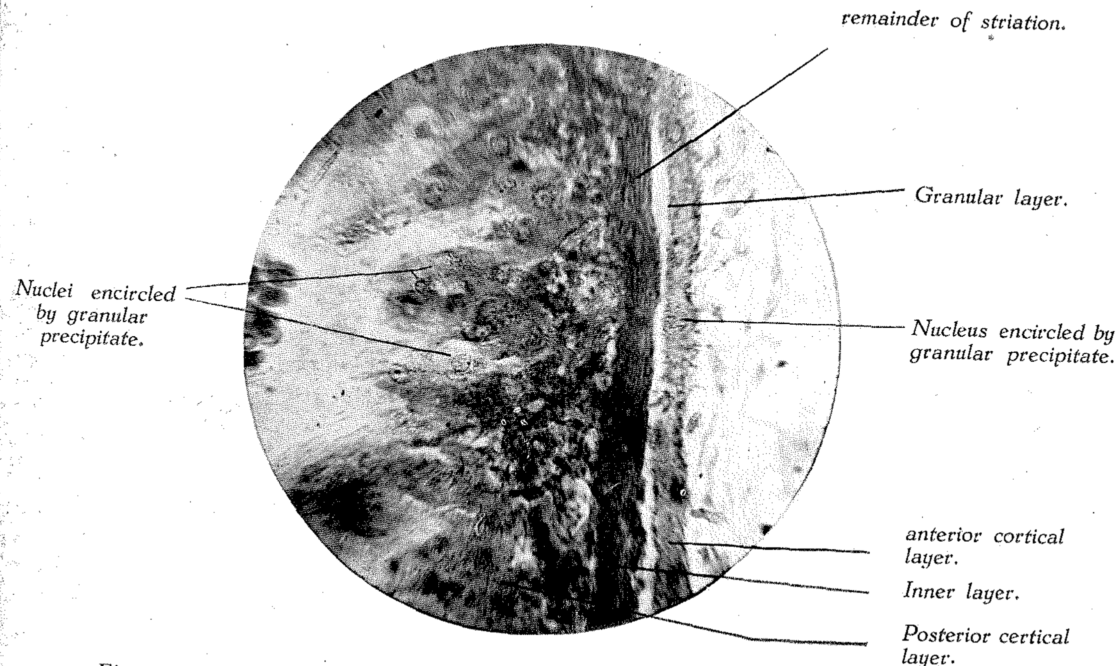

Fig. 3. Microphoto of an electric platelet of Raja punctata. (frozen section, treated with potassium-reagent).
(Oil-immersion 1/12 Leitz. Oculair 2).

3rd. as a so-called bio-chemical condensation.

According to this differentiation potassium occurs in the anterior, and the posterior cortical layer as a local precipitate, in the inner layer, however, in biochemical condensation.

The local precipitate is to be found especially round the nuclei of the two cortical layers and on the boundary of the anterior cortical layer and the inner layer, while in the latter the potassium has been condensed in the originally doubly refracting discs.

The most interesting facts brought to light by this experimentation are, in my judgment, 1st. the potassium-richness of the electric platelets and the slight quantity of potassium in the surrounding jelly; 2nd. the occurrence of a large precipitate of potassium on the boundary between the anterior cortical layer, and the inner layer, and 3rd. the fact, that in the inner layer the peculiar distribution of the potassium-compounds, found in voluntary muscular tissue, has been maintained. The physiological explanation of these facts will, as I hope, be given by those who are competent to do so.

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