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HISTOCHEMICAL INVESTIGATIONS CONCERNING KISS' MULTIPOLAR CELLS

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In 1932, F. Kiss recognized in the cerebrospinal ganglia of mammals certain dark cells which he named multipolar cells and demonstrated with his own method of prolonged osmic acid impregnation.

These cells can be made conspicuous also with toluidine blue (Bacsich; Kiss, Gellért and Bacsich, 1932) ; silver impregnation and pyridine (Blair and Davies, 1933 ; Blair, Bacsich and Davies, 1935) ; acid fuchsin and light green after fixation in Regaud's fluid combined with postchromization (D. Picard and Mme Chambost, 1949).

Previously [3,4], we have demonstrated Kiss' multipolar cells in the spinal ganglia of fowls and various mammals by means of ferric haematoxylin (Haidenhain), azocarmine (Azan, Figs. 4, 5), orange G (either haematoxylin-eosin-orange G, or aniline blue orange G) and acid fuchsin (Figs. 1—3). The stains were applied to specimen fixed in Regaud, Orth, 10 per cent formaldehyde or Tupa,[1] followed by warm postchromization, and also to specimens fixed in Aoyama and subjected to warm postchromization with 1 per cent cadmium chloride.

During the course of these investigations we had the opportunity of convincing ourselves of the clearly multipolar character of Kiss' cells. Characteristic pictures have been obtained particularly from the spinal ganglia of fowls.

There are, however, numerous authors who still recognize exclusively pseudo-unipolar cells in the spinal ganglia. In 1953 E. G. Burghardt reviewed the literature on the subject, and investigated histochemically phosphatase activity in the spinal ganglia but failed to detect there multipolar cells. The phosphatase reaction was made use of also by other authors (Polumbei, Roskin and others) but neither of them found differences between the cells of the spinal ganglia.

In the following, we wish to report on our histochemical studies involving also reactions for complex lipoids of the cells of the spinal ganglia. The electivity

[1] $Cr_2O_7K_2$, 3% ..... 80 ml
Formalin..... 20 ,,
NO_3U..... 1 g

of Kiss' multipolar cells to osmic acid and azo-dyes (azocarmine and orange G) has induced us to employ the above methods, as well as the fact that these dyes are taken up also by specimens which are treated with cadmium and chromium salts.

Methods

The material was obtained from fowl (turkey, geese, ducks and poultry) and small mammals (guinea pigs, rabbits, cats and dogs) which were bled to death for this purpose.

Serial sections were made and embedded in paraffin, with the technique described in detail in our preceding studies [3,4].

After removal of the paraffin the specimens were subjected to treatment with (i) acid haemateine (Baker), (ii) Sudan III, (iii) Sudan Black B, (iv) Smith—Dietrich, (v) periodic acid — Schiff.

Stains 1, 3 and 5 were applied also after pyridine extraction at +60° C for 24—48 hours, as well as after extraction by means of alcohol-ether for 24—48 hours.

Results

(i) With haemateine the best preparations were obtained on prolonged staining (8—12 hours) at +58° C. Differentiation was made under microscopical control. With acid haemateine Kiss' multipolar cells were stained electively. Haemateine was intensively absorbed by the nucleus and by the cytoplasm of the pericarion. The reaction was positive also for the processes of the multipolar cells which indisputably proves their multipolar character. The dark brown colour of the multipolar cells was easily distinguished from the bluish shade of the myelinated fibres. After intensive differentiation the cells showed a granular aspect similar to Nissl's corpuscles (Figs. 6, 7, 8, 9).

After cold and warm pyridine extraction (+ 60° C) for 24—48 hours, or after alcohol-ether extraction for the same period, the acid haemateine reaction remained positive.

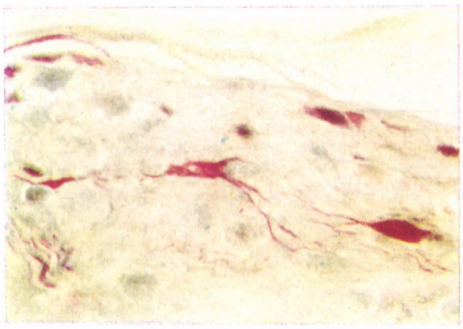
(ii) Sudan III did not differentiate the multipolar cells. Warm post-chromization for several days did not change the aspect of the cells.

(iii) With Sudan Black B the multipolar cells show a dark blue shade more intensive than that of the myelinated fibres. By this method the cells can be easily compared with the neighbouring pseudo-unipolar cells which do not stain with the same technique. The stain is diffusely absorbed by the cytoplasm of the pericarion (Fig. 10).

Extraction by pyridine or alcohol-ether, as mentioned under point (i), did not change the Sudan Black staining of the multipolar cells.

(iv) With Kultschitsky's haematoxylin, multipolar cells are demonstrated electively only by prolonged staining for several days, or by previous supplementary chromization of the specimens (Fig. 11).

(v) With PAS (McManus-Hotchkiss) multipolar cells show a more intense shade and they can be easily distinguished from the pseudo-unipolar cells.



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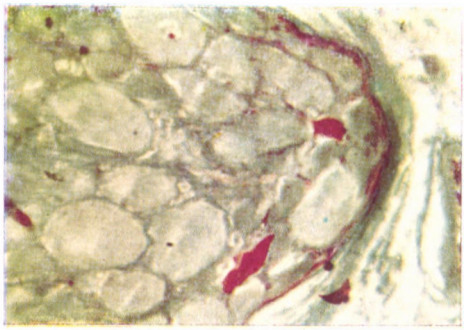

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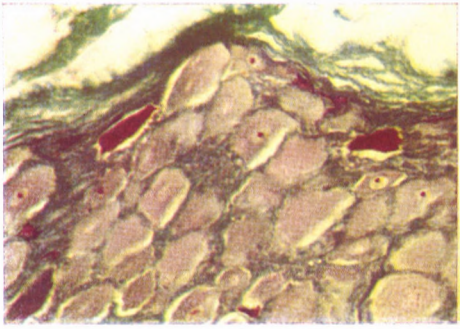

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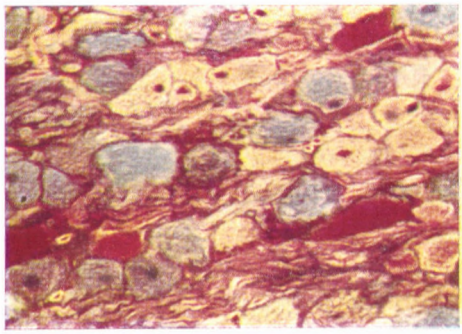

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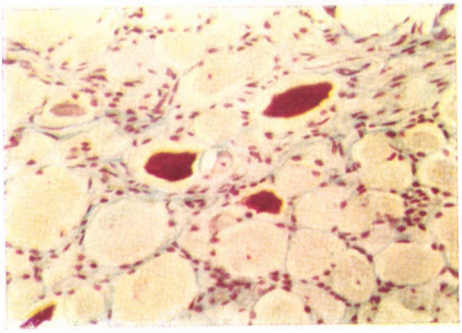

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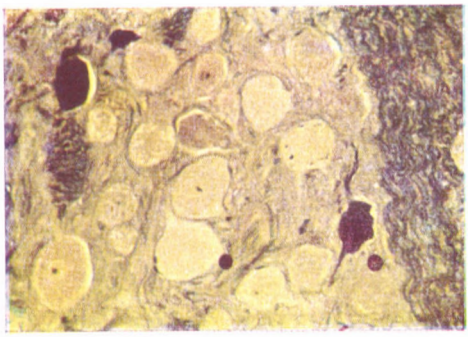

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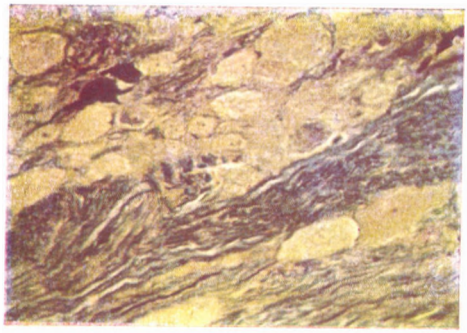
Fig. 1. Spinal ganglion from turkey (1 : 36) Fix. Regaud. Color. Fuchsin — green light
Fig. 2. Sp. gangl. from dog (1 : 144). Fix. Regaud. Color. Fuchsin — green light
Fig. 3. Sp. gangl. from turkey (1 : 144). Fix. Aoyama. Color Fuchsin — green light
Fig. 4. Sp. gangl. from turkey (1 : 144). Fix. Regaud. Color Azan
Fig. 5. Sp. gangl. from dog (1 : 144). Fix. Formol. Color Azan
Fig. 6. Sp. gangl. from turkey. Fix. Regaud. Color Haem. Baker



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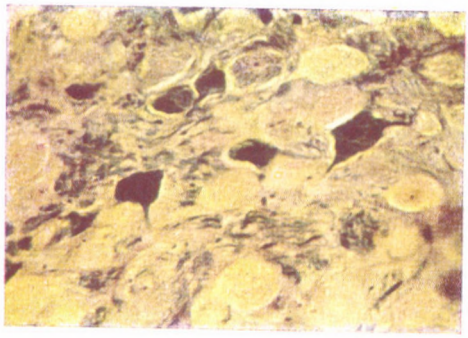

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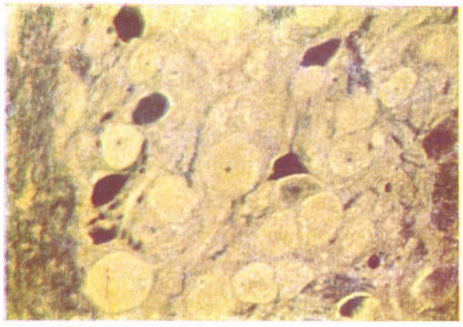

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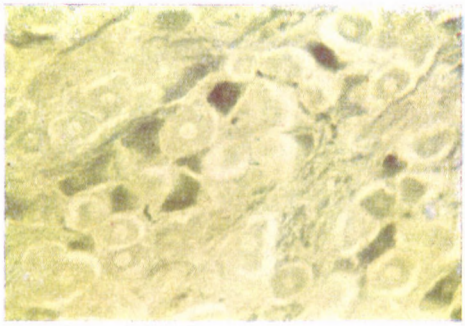

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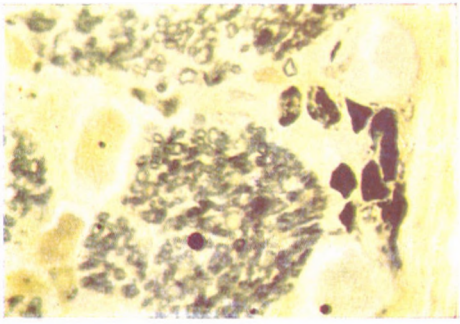

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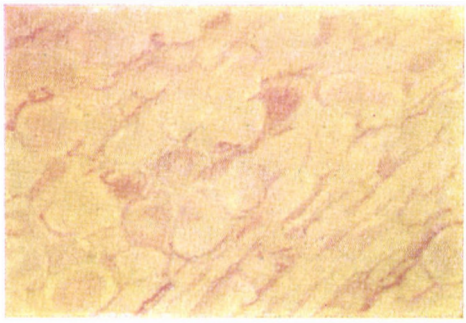

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Fig. 7. Sp. gangl. from turkey. Fix. Regaud. Color. Haem. Baker
Fig. 8. Sp. gangl. from turkey. Fix. Regaud. Color Haem. Baker
Fig. 9. Sp. gangl. from dog. Fix. Regaud. Color Haem. Baker
Fig. 10. Sp. gangl. from turkey. Fix. Regaud. Color Sudan black
Fig. 11. Sp. gangl. from dog. Fix. Regaud. Color Haem. Kultschitzky
Fig. 12. Sp. gangl. from turkey. Fix. Regaud. Color P. A. S.

The intensity of the reaction of multipolar cells is similar to that of the connective tissue in the capsule of the ganglia (Fig. 12). The reaction is positive also after extraction with pyridine or alcohol-ether.

In contradiction with dyes 1, 3 and 4, the PAS reaction was positive in postchromized specimens fixed in Helly's liquid.

(vi) In our microphotographs the multipolar character of Kiss' cells may be clearly seen, especially in the ganglia of the fowl (Figs. 1, 6, 8, 10, 12).

Topographically, the cells are scattered all over the ganglia forming groups at the periphery of the ganglia. It seems that in birds they occupy one pole of the spinal ganglion, giving the impression of a genuine vegetative ganglion.

Discussion

(i) The diffuse staining of the multipolar cells with Sudan Black (Fig. 10) indicates the presence of a lipoidic component in the cytoplasm. Furthermore, Kultschitzky's haematoxylin and, especially acid haemateine suggest the presence of phospholipids or galactolipids (Figs. 6—9, 11).

(ii) Chromium and cadmium salts create stable lipoprotein complexes. These are responsible for the fact that these cells take up specific lipoid reagents (Sudan Black, Kultschitzky's haematoxylin, acid haemateine). Azo-dyes (azocarmine and orange G, see 1 and 2 in the bibliography) give the same results. The presence of a protein component in the complex is proved by its taking up Heidenhain's ferric haematoxylin (1 and 2).

The insolubility of lipoids in pyridine at $+60°$ C, and in alcohol-ether, is another evidence of their becoming bound to a protein component under the action of cadmium and chrome.

(iii) The intense PAS reaction of the multipolar cells might be attributed to the presence of a hydroxil and an amino group in the neighbourhood of the double bond in the fatty acid (WALMAN's hypothesis [17]), while galactoses in the galactolipids (LILLIE [16]) show that galactolipids may produce a PAS reaction.

The first hypothesis does not explain the difference between the multipolar and the pseudo-unipolar cells, since CHU [2] has shown that unsaturated fatty acids are present in all nerve cells and axis cylinders.

On the other hand, the fact that the PAS reaction of the multipolar cells is positive both in specimens (fixed in Regaud, Aoyama, etc.) which give the lipoid reaction, and in those (fixed in Helly's fluid) which give a lipoid reaction also on postchromization is another evidence in favour of the hypothesis that Schiff's reagent acts with the aldehyde formed at the levels of galactoses.

Consequently, the PAS reaction appears to be sufficient evidence of the galactolipid content of the multipolar cells.

Conclusion

(i) The multipolar cells of the spinal ganglia are electively demonstrated by acid haemateine (Baker), Sudan Black B, Kultschitzky's haematoxylin and the periodic acid Schiff reagent, after fixation in 10 per cent formaldehyde, Orth, Regaud, Tupa or Aoyama, and postchromized or postcadmiumized.

(ii) The reaction of multipolar cells is positive in specimens treated with periodic acid Schiff reagent, also in those fixed in Helly's fluid.

(iii) The histochemical basis of the electivity of multipolar cells is their galactolipid content. The galactolipid forms complexes under the prolonged action of osmium (Kiss), of chromium and of cadmium (DICULESCU and coll.).

(iv) Morphological differences, as revealed by various prolonged methods and histochemical differences prove that Kiss' multipolar cells are constant elements of the spinal ganglia of fowls and mammals.

Summary

The existence of multipolar cells in the spinal ganglia has been confirmed by histochemical methods in postchromized or postcadmiumized specimens fixed in formaldehyde, Orth, Regaud, Tupa and Aoyama.

The multipolar cells react with acid haemateine, Sudan Black, Kultschitzky's haematoxylin and the periodic acid Schiff reagent. These reactions are positive also after extraction with pyridine or by alcohol ether.

The different histochemical character of Kiss' multipolar cells is due to their galactolipid content.

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ГИСТОХИМИЧЕСКИЕ ИССЛЕДОВАНИЯ ПОЛИГОНАЛЬНЫХ КЛЕТОК КИШША

И. ДИКУЛЕСКУ, Г. БОРДА и З. ПАСТЕА

Авторы проводили гистохимические исследования мултиполярных клеток спинномозговых ганглий. Они фиксировали ганглии в формальдегиде, в смесях Орта, Тупа, Рего и Аойама, а затем обрабатывали их солями хрома, или же кадмия (в нагретом состоянии).

Мултиполярные клетки реагируют (окрашиваются) кислым гематеином, судановым черным В, гематоксилином Кульчицкого и ПАСК. Эти реакции положительны даже после экстрагирования теплым пиридином, или же спирто-эфиром. Реакция ПАСК положительна даже в случае веществ, фиксированных в смеси Гелли.

На основании этих наблюдений авторы пришли к тому заключению, что ганглиозные клетки содержат галактолипоиды, которые не извлечимы и которые на действие осмия, хрома и кадмия выпадают.

HISTOCHEMISCHE UNTERSUCHUNGEN DER KISS-SCHEN POLYGONALEN ZELLEN

I. DICULESCU, G. BORDA und Z. PASTEA

Die multipolaren Zellen der Spinalganglien wurden histochemisch untersucht. Die Ganglien wurden in Formaldehyd, in Orth-, Tupa-, Regaud- oder Aoyama-Gemisch fixiert und danach mit Chrom bzw. mit Cadmiumsalzen (warm) behandelt.

Die multipolaren Zellen färben sich mit saurem Hämatoxylin, mit Sudan-Schwarz B, mit Hämatoxylin Kultschitzky und mit PAS. Die Reaktionen sind auch nach Extraktion durch Ätheralkohol bzw. warmes Pyridin positiv. Die PAS-Reaktion ist auch nach Fixierung in Helly-Gemisch positiv.

Auf Grund dieser Beobachtungen wurde gefolgert, dass die Ganglienzellen Galaktolipoide enthalten, welche nicht extrahiert werden können und auf Wirkung von Osmium, Chrom und Cadmium gefärbt werden.

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