A STUDY OF MITOCHONDRIA IN EXPERIMENTAL POLIOMYELITIS.

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INTRODUCTION.

Although many observations have been made on mitochondria in normal tissues, both adult and embryonic, the study of these structures in pathological material has been relatively limited and the results in some cases have been conflicting. The information regarding this type of cell granule has been summarized by Cowdry,[1] but in view of the fact that several observers have recorded changes in mitochondria, often occurring quite early in certain lesions,[2–13] it seemed possible that some such results might be obtained in the case of poliomyelitis, which would throw light on the pathology of that condition.

Spinal ganglia were employed for the study, because they show typical lesions in monkeys dying of experimental poliomyelitis, and because, of the structures showing these lesions, they could be most

[1] Cowdry, E. V., *Am. J. Anat.*, 1916, xix, 423.
[2] Barrett, J. O., *Quart. J. Micr. Sc.*, 1913, lviii, 214.
[3] Beckton, H., *Arch. Middlesex Hosp.*, 1909, xv, 182.
[4] Beckton, H., and Russ, S., *Arch. Middlesex Hosp.*, 1911, xxiii, 59.
[5] Bensley, R. R., *Tr. Chicago Path. Soc.*, 1909–12, viii, 78.
[6] Ciaccio, C., and Scaglione, S., *Beitr. path. Anat. u. allg. Path.*, 1913, lv, 131.
[7] Goetsch, E., *Bull. Johns Hopkins Hosp.*, 1916, xxvii, 29.
[8] Homans, J., *J. Med. Research*, 1915–16, xxxiii, 1.
[9] Regaud, C., and Favre, M., *Compt. rend. Soc. biol.*, 1911, lxviii, 658.
[10] Regaud and Favre, *Compt. rend. Soc. biol.*, 1912, lxix, 328.
[11] Romes, B., *Anat. Anz.*, 1913–14, xlv, 1.
[12] Scott, W. J. M., *Am. J. Anat.*, 1916, xx, 237.
[13] Strongman, B. T., *Anat. Rec.*, 1917, xii, 167.

successfully obtained and fixed. Cowdry[14] has given a full account of the mitochondria occurring in normal ganglion cells of vertebrates.

EXPERIMENTAL.

In a few instances injection fixation was employed. Here the method described by Cowdry[1] was used with the following variations. The formalin bichromate mixture was more successful when used in half rather than in full strength. 2 to 3 feet of gravity pressure of injection fluid were found to give less edema and distortion of the tissues than the higher pressure (4 to 6 feet), so that while the saline infusion was given at the higher pressure for the first 5 to 10 minutes to insure complete washing out of clots, the lower pressure was maintained throughout the remainder of the period. The femoral vein of one side was cut, rather than the vena cava, as the latter gave too rapid an outflow of fluid. The cardiac, mesenteric, and opposite iliac arteries were clamped. The injection of fixative was continued for 1 hour and the best results were obtained when the animal was kept lying on the board back down, for $\frac{1}{2}$ hour longer before autopsy.[15] Injection was less successful in poliomyelitic monkeys and in the lumbar region of operated rabbits than in normal animals, possibly owing to vascular lesions in the former.

The ganglia, however, were quite as well fixed by simple immersion in fixing fluid. The animals were usually chloroformed and the ganglia taken at once. In a few cases the animals died and were autopsied within 2 hours of death. The specimens were placed in formalin bichromate mixture (3 per cent potassium bichromate 4 parts, neutral formalin 1 part, and water 5 parts) for 4 days and then transferred to half strength ($1\frac{1}{2}$ per cent) potassium bichromate for 5 days. This procedure was found to give better results than the solution ordinarily used. Full strength solutions and the acetic-osmic-bichromate mixture[16] (2.5 per cent bichromate 8 cc., 2 per cent osmic acid 2 cc., and glacial acetic acid 1 drop) in full strength or diluted to half strength were apt to give good fixation only in the

[14] Cowdry, *Am. J. Anat.*, 1914–15, xvii, 1.
[15] Schirokogoroff, J. J., *Anat. Anz.*, 1913, xliii, 522.
[16] Bensley, *Am. J. Anat.*, 1911–12, xii, 297.

case of superficial cells. The osmic acid mixture also darkened the whole section to such an extent that it dimmed the contrast between mitochondria and small Nissl bodies.

At first the use of chloroform in embedding was tried, but in many cases the mitochondria disappeared under this treatment. In one case the tissue was first embedded by the xylol method and sections were cut, then reembedded by the chloroform method.[17] The former sections showed mitochondria, but none was present in the latter. This may have been due to rehandling, however. Embedding was done as described by Cowdry,[14] except that absolute alcohol-xylol for 1 hour, xylol 1 hour, paraffin 3 hours, was found to be sufficient and less liable to destroy the mitochondria. Both the acid fuchsin-methyl green, and the iron-alum-hematoxylin (Regaud and Favre[9]) methods were used for staining.

Rabbits.—In order to compare cell changes in another form of paralysis a series of rabbits was used, in some of which ischemic paralysis of the hind legs was produced by the Stenson operation (Fredericq,[18] Ehrlich and Brieger[19]). The animals were etherized. An area about 3 inches wide, extending from ensiform process to symphysis pubis, was shaved and cleaned with alcohol. Aseptic technique was employed. An incision was made in the midline, and the intestines were covered with cloths wet with warm saline. The abdominal aorta was exposed and a soft bulldog clamp placed on it about $\frac{1}{2}$ inch below the renal arteries, the effectiveness of the clamp being tested by palpation of the vessel below it. The intestines were replaced and the animal was kept under light ether anesthesia, the clamp being left in place for $\frac{1}{2}$ or $\frac{3}{4}$ hour. The longer period was found to give certain results while the former failed in some cases to give paralysis. Care was taken to keep the animal warm during this period. At the end of the period the clamp was removed, the abdominal wall sewed with silk, and the animal allowed to recover.

In almost every instance, flaccid paralysis of the hind legs was evident as soon as the animal recovered from the ether. In two rabbits

[17] Mallory, F. B., and Wright, J. H., Pathological technique, Philadelphia and London, 6th edition, 1915, 284.

[18] Fredericq, L., *Arch. biol.*, 1890, x, 131.

[19] Ehrlich and Brieger, *Z. klin. Med.*, 1884, vii, Supplement, 155.

in which the shorter compressions were used, the hind legs were spastic, with convulsive twitchings, which gradually disappeared, the animal recovering the full use of the legs.

The animals were chloroformed at various periods after the ligation and fixed by the injection method. The lumbar region of the cord did not always take the fixative so well as did the cervical region, or the lumbar region of normal animals, and in those animals killed 12 or more hours after the clamping, the cords remained extremely soft, and were uncolored by the chromate and very difficult to handle.

Material.—A series of ganglia was obtained from fourteen monkeys with experimental poliomyelitis.[20] Six were either in the preparalytic stage, without definite lesions in the ganglia, though having shown such symptoms as irritability, etc., or if they showed paralysis, the particular ganglia used failed to show typical lesions. The remaining ten, taken from the 1st to the 7th day after the onset of paralysis of some muscle group had been noted, all showed typical lesions of the cord and ganglia, including those used for mitochondria, as shown by examination in gross or of sections stained by hematoxylin and eosin.

Five monkeys were used as controls. Two of these showed lesions of tuberculosis at autopsy. One was an apparently normal monkey which died while being etherized, and the viscera showed no gross abnormalities. The other two monkeys had received poliomyelitis virus intranasally but showed no symptoms either during life or post mortem.

Results.—The ganglia from the five control monkeys and those from the six poliomyelitic monkeys presenting no lesions in the ganglia used showed mitochondria similar to those described by Cowdry in the normal animal. Great variations were found in the number of mitochondria and the intensity with which they took the stain, and several showed many cells in the chromatophilic state.[21] In one, considerable postmortem degeneration had occurred.[22] One showed a large amount of typical lipoid. All, however, were cells similar to those described by Cowdry as normal cells. This was true also

[20] Flexner, S., and Lewis, P. A., *J. Exp. Med.*, 1910, xii, 227.

[21] Cowdry, Contributions to embryology, *Carnegie Institution of Washington, Publication No. 224*, 1916, Contribution No. xi.

[22] Ciaccio, *Centr. allg. Path. u. path. Anat.*, 1913, xxiv, 721.

of the ganglion cells from the normal rabbits and those from the cervical region of the operated rabbits. In these rabbit sections, no chromatophil cells were found.

In the tissues from the poliomyelitic animals also, many cells were normal in appearance. In many, moreover, the mitochondria appeared to be even more clearly shown than in normal cells. This appearance may have been due to disappearance of Nissl substance, which was reduced in these cells. In normal cells it was often hard to differentiate between mitochondria and small Nissl bodies as the granules were of nearly the same size and in some preparations tended to take the fuchsin stain. Many cells contained much particulate lipoid and many were in the chromatophilic state, but in these particulars they did not seem to exceed normal limits.

In the cells which showed marked neurophagocytosis, mitochondria-like threads could often be seen, even though only a small remnant of protoplasm remained. They appeared as minute reddish threads or dots, or larger masses, even to fairly large rods, lying in the usual bluish background and in spaces between the invading cells. They did not have the globular shape characteristic of lipoid, and they seemed to have some of the chemical reactions of the mitochondria, for they were not found in slides in which the mitochondria had been lost through poor fixation. In cells in which the destruction had been less complete, typical mitochondria were found to persist in an apparently normal ratio to cell substance.

A similar, though less marked persistence of mitochondria was also noted in the lumbar ganglia of the rabbits. 1 hour after the production of the anemia, a few darkly staining cells were found, showing a few reddish threads against a dark purplish background. At 7 hours almost all the cells had this chromatophilic tendency, a few still showing the reddish threads, but in the majority there was only a shrunken, irregularly stained protoplasm. At 12 hours, the position of a few remaining cells was indicated by dark, indefinite spots.

DISCUSSION.

If these red-staining threads occurring in the invaded cells in poliomyelitis are mitochondria, the mitochondria in this condition

at least outlast any other cell structure now recognized. This is remarkable in view of their usual tendency to disappear under slight changes, such as acidity or temperature. Since the stain is not absolutely specific, and we lack means at the present time of differentiating mitochondria with certainty from other lipoidal structures, it may be that these threads, or rods, are merely part of a coagulum of some different nature. Yet, since typical mitochondria persist after the disappearance of typical Nissl substance, and various gradations can be traced up to the stage of almost total replacement of the original ganglion cell, it would seem safe to call these mitochondria under the present use of the term.

It would be desirable to have some means of quantitative estimation of the mitochondria for these purposes, but the one so far described (Thurlow[23]) does not prove applicable to the rod-like forms.

CONCLUSION.

Typical mitochondria can be found in the spinal ganglion cells of monkeys with experimental poliomyelitis, even when typical Nissl substance has disappeared, and mitochondria-like structures are found in the remaining protoplasm in the latest stage of neurophagocytosis.

[23] Thurlow, M., Contributions to embryology, *Carnegie Institution of Washton, Publication No. 226*, 1917, Contribution No. xvi.