HISTOLOGICAL CHANGES INDUCED IN SYMPATHETIC, MOTOR, AND SENSORY NERVE CELLS BY FUNCTIONAL ACTIVITY. (*Preliminary Note.*)

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(Read before the Scottish Microscopical Society, May 18th, 1894, under the title: "What alterations are produced in nerve cells by work?")

WITHIN the last few years several endeavours have been made to determine whether the functional activity of the nervous system is accompanied by demonstrable histological changes in nerve cells, and the respective investigations have resulted in a confirmation of the surmise.

The chief observations were made by F. C. Hodge [1] in America, who stimulated with an induced current the intervertebral ganglia on the posterior roots of spinal nerves, and who, in addition, also studied the phenomena of daily fatigue by comparing the cephalic ganglia of bees, swallows, &c., which had been placed in fixing solutions in the morning, after a night's rest, with those fixed in the evening after a day's labour. The changes he observed in nerve cells stimulated faradically, fixed and stained in 1 per cent. osmic acid, are these:—In the resting nerve cell the nucleus is paler than the protoplasm, and it possesses a smooth outline, while in the fatigued cell the nucleus is darker than the protoplasm, crenated, and markedly decreased in size. The protoplasm of the fatigued cell shows a slight shrinkage in size;—lessened power to stain or to reduce osmic acid and vacuolation. In the cell capsule he further noticed a decrease in size of the nuclei.

[1] C. F. Hodge, (*a*) "Some effects of stimulating ganglion cells" (Prelim. Comm.), *Amer. Journ. Psych.*, vol. i. p. 479, 1888, Baltimore; (*b*) "Some effects of electrically stimulating ganglion cells" (Dissertation), *Amer. Journ. Psych.*, vol. ii. p. 376, 1889; (*c*) "The process of recovery from the fatigue occasioned by the electrical stimulation of ganglion cells," *ibid.*, vol. iii. p. 530, 1891, Worcester; (*d*) "A microscopical study of changes due to functional activity in nerve-cells," *Journ. of Morphol.*, vol. vii., 1892, November, No. 2, p. 1 [(*e*) "Die Nervenzelle bei der Geburt und beim Tode an Alterschwache, *Anat. Anzeiger*, vol. ix. No. 23, 1st August 1894, pp. 706–710. This reference has been added since the paper was read.—G. M.]

In his most recent communication (footnote 1, *e*) Hodge has compared ganglion cells of newly-born animals with those taken from very old animals (man and bee), and finds in advanced age (92 years) that the nucleoli of the spinal ganglionic cells are not stained with osmic acid, while they readily stain in newly-born infants. With age the nucleus becomes shrivelled, but does not stain more deeply than the protoplasm, as it does in the case of fatigue;—the protoplasm of the cell becomes pigmented [an observation I can fully confirm.—G. M.] and vacuolated. He noticed also that in the human cerebellum and in the cerebral ganglia of bees there goes hand in hand with advancing age a destruction of nerve cells amounting to 25 per cent. and more of the original number.

Friedrich Vas[1] then experimented on the superior cervical sympathetic ganglion of the rabbit. He stimulated the sympathetic nerve on one side of the animal, 3 cm. below the ganglion, for 15 minutes; the ganglion on the other side serving as a control.

Vas found in the stimulated ganglion an increase in the size of both the cells and their nuclei, and stated that the chromatin granules which surround the nucleus of the resting cell move in the stimulated ganglion to the periphery of the cell, and that thus a clearing of the central part of the cell is brought about. He did not suggest that the amount of chromatin diminishes during the activity of the cell. He further states that the nuclei go to the periphery of the cell.

The experiments of Vas were repeated by Lambert[2] on rabbits and cats, and the changes in the chromatin interpreted in the same way, but the French observer failed to make out an undoubted increase in size of the cells or air nuclei.

The great difference between the statements of the above observers, Hodge and Vas, necessitated further observations, and I therefore commenced my inquiry by repeating the experiments of Vas. The superior cervical sympathetic ganglia of rabbits

[1] Friedrich Vas, "Studien über d. Bau d. Chromatins in d. sympathischen Ganglienzelle" (1 pl.), *Arch. f. mik. Anat.*, vol. 40, 1892, Heft 3, pp. 375–389.

[2] Lambert, "Note sur les modifications produites par l'excitations électrique dans les cellules nerveuses des ganglion sympathiques" (Note prélim.), *Compt. rend. hebd. des séances de la Soc. de Biol.*, 1893, No. 31, Neuvieme série, Tome 5, pp. 879–881 (November).

and cats were fixed in various osmic acid mixtures, but preferably in my watery fixative, viz. :—

Saturated solution of $HgCl_2$ in $\frac{3}{4}$ per cent. NaCl = 100 cc.
Picric acid, ... 1 gr.
Tannin, ... 1 „

or simply in a saturated solution of $HgCl_2$ in $\frac{3}{4}$ per cent. NaCl. As stains I used Toluidin blue, Ehrlich's Sulphuric acid Methylen blue, Methyl blue, and water-soluble Eosin, all obtained from Dr Grübler. Bordeaux red, Safranin, Congo red, Gentianviolet, and Methylviolet; Bismarck brown and Tropaeolin; Ehrlich's triacid mixture and Ehrlich-Biondi's fluid; M. Heidenhain's iron-alum-hæmatoxylin, Ehrlich's acid hæmatoxylin, and Delafield's hæmatoxylin.

The Methyl-blue I employ as follows :—

The ganglia should be fixed in $HgCl_2$, be taken through the paraffin process and sections be cut not thicker than $2\frac{1}{2}$ μ.

The sections are fixed to the slide by my albumen method[1] and then placed, after the paraffin has been removed, in the following staining solution :—

1 per cent. Methyl-blue (Grübler), water soluble, and almost quite insoluble in Alc. absol. . . = 35 cc.
1 per cent. Eosine, water soluble (Grübler), . 45 cc.
Aq. distill., ... 100 cc.

(1) Stain for 24 hours. (2) Remove superfluous stain with water. (3) Dehydrate with Alc. absol.
(4) Place slide in a glass vessel containing Alc. absol. = 30 cc.
1 per cent. NaOH in Alc. absol., . . 4 drops.
(5) Leave in this mixture till the dark blue section has become reddish (1–5 minutes).
(6) Wash off all traces of caustic soda with Alc. absol.
(7) Place sections into a vessel with tap water; bluish-red clouds will be given off. When no more clouds are given off, (8) transfer slide to a vessel containing water which has been acidulated with 2–3 drops of Acetic acid. Leave section in this water for 3 minutes to neutralise all traces of NaOH, to fix the Eosine and to deepen the

[1] Gustav Mann, *Anat. Anzeig.*, Jahrg. viii. (1893), p. 442.

Methyl-blue in colour. (9) Dehydrate with Alc. absol. and mount in Turpentine-balsam. If sections are still too blue the process will have to be repeated. In properly stained sections, the red blood corpuscles should be red, everything else blue with the exception of the nucleoli which are red or purplish. This method is the best I know of for the nuclear chromatin of nerve cells.

Sections of the ganglia were varied in thickness from $\frac{1}{2}$ to 8 μ, and fixed to the slides by my albumen method.[1]

A sympathetic nerve cell has a very complicated structure (figs. 1, 2); it is multipolar, and in the rabbit possesses, as a rule, two nuclei, although one to four may be found. The protoplasm of the cell consists of a ground substance with a great number of fibrils embedded in it, which run in bundles, and may be traced from one process past the nucleus into another process. *In no case do the nerve-fibrils spring from the nucleus.* Lying between the fibrils (fig. 10), I have been able to demonstrate by the above Methyl-blue and Eosine staining method, bodies which show characteristic staining reactions, and which up till now have not been described. Each of these bodies (there are four to ten in one cell) consists of two dumb-bell shaped elements placed side by side, and may therefore be called a "*bigeminal body*," and it is of such size as to be readily recognised when magnified 300 times.

Embedded in the ground-substance ("interfibrillar substance"), figs. 1 and 2, and lying most abundantly between the bundles of fibrils and at the very periphery of the cell, are numerous short rod-shaped chromatin granules, which were first described by Vas. It is these granules which Vas believes to move to the periphery of the cell after 15 minutes' electrical stimulation of the ganglion.

A repetition of Vas' experiments has shown me that an increase in the size of the ganglion cells and their nuclei does take place, but I could not convince myself of a shifting of the granules from the centre towards the periphery of the cell. The centre of the cell certainly becomes clearer (compare figs. 1 and 2), but this is due, firstly, to absorption of lymph by the central part of

[1] Gustav Mann, *Anat. Anzeig.*, Jahrg. viii. (1893), p. 441.

the cell, and secondly, due to either a change in the composition of the stainable material, rendering it unstainable, or to the using up of the chromatin rods.

The changes in the nucleus are these: In the resting nucleus the nuclear hyaloplasm is stained by my Methyl-blue eosin method, while it remains quite colourless in the stimulated cells. The true nuclear chromatin, which shows a specific affinity for Methyl green OO, is slightly diminished, and not increased as stated by Vas.

The nucleolus also enlarges and the endo-nucleolus becomes more evident, thus rendering the nucleolus paler. The linin threads are now readily recognised.

A further difference between the stimulated and control ganglion, best seen under a low magnifying power, is the following:—In the control ganglion the lymph spaces around the cells appear as clear lines, while the stimulated ganglion has a homogeneous look, for the spaces have disappeared because of the increase in size of the ganglion cells.

On stimulating a ganglion intermittently for 6–9 hours a further change may be observed (fig. 3):—The nuclei begin to darken slightly and to shrivel; this collapse being frequently restricted to one side of the nucleus. The chromatin rods of Vas are greatly diminished in number and very pale, while the "bigeminal bodies" are deeply stained, and stand out sharply.

The extreme stages of fatigue, as described by Hodge, namely, the nucleus becoming darker than the cell, I was unable to obtain.

So far, then, my results were not a simple confirmation of those obtained by Vas, for I found that the chromatin of the cell does not shift its position during activity, and that it actually undergoes diminution.

Starting with this idea, I passed to other portions of the nervous system, to see whether or not analogous changes could be found after ordinary normal stimulation.

On comparing the motor areas in the cerebra of two dogs, one of which had been resting, while the other had been doing ten hours' muscular work, I found the following changes:—

In the large pyramidal cells of the resting brain, fixed by injecting a warm saturated solution of $HgCl_2$ in $\frac{3}{4}$ per cent.

NaCl solution from the aorta, and stained for 5 minutes in a ½ per cent. watery solution of Toluidin-blue; then rapidly dehydrated in Alc. absol.; cleared in xylol; and mounted in turpentine balsam,—Nissl's chromatic spindles lying between the nerve fibrils are intensely stained. The interfibrillar ground-substance may also be deeply stained by the above detailed method of using Methyl-blue.

In the worked brain Nissl's spindles are less deeply stained by Toluidin blue; they may show a jagged outline, or may be altogether absent, while also the interfibrillar substance can no longer be stained with Methyl-blue.

Hence in the fresh brain the nerve cells appear as deep blue bodies on a light background, while in the worked brain they are very pale or quite colourless, and appear as light figures on a darker background.

The nuclei of the worked cells were swollen and the nuclear hyaloplasm colourless, the latter in the resting brain being distinctly stained with Methyl-blue.

In the lumbar region of the *spinal cord* (figs. 4, 5) in the fresh dog, the nuclei of the motor cells were paler or of the same intensity of colour as the protoplasm, with distinct separate chromatin granules, and the outline of the nucleus smooth, while in the fatigued dog the nucleus was darker than the protoplasm, stained homogeneously and distinctly shrivelled.

Hence the appearance first described by Hodge in the spinal ganglia, as resulting from prolonged electrical stimulation, can be confirmed as occurring normally as the result of excessive fatigue.

Nissl's spindles were further much less deeply stained and fewer in the fatigued cord as compared with the resting one.

Seeing the above marked changes in motor cells, the question naturally suggested itself to me, Can similar changes be seen in sensory cells? The special sense which I have investigated up till now is that of vision, and the effects of light on the retina and the optic centres of the brain are the following:—In four dogs (figs. 6, 7) which were allowed to run about for twelve hours with one eye covered up while the other eye was exposed, and the brains and retinæ of which were fixed by inject-

ing $HgCl_2$ from the aorta, I notice in the retina kept in darkness (fig. 6) that the nuclei of the rods are very rich in chromatin, the individual chromatin segments being globular, spherical externally, and facetted where in contact with one another, while in the exposed eye the chromatin segments are greatly shrunken and quite stellate. The nuclei of the ganglion cells of the dark retina are smaller than those in the exposed one, and in the latter the nuclear hyaloplasm is no longer stained with Methyl-blue.

In the nuclei of the external geniculate bodies, the corpora quadrigemina and occipital lobes (figs. 8, 9) corresponding to the dark eye, the ganglion cells are much richer in chromatin and the nuclei smaller than in those cells in connection with the exposed eye. In the Rabbit I have found the same changes, not so well marked in the retina, but more evident than in the case of the Dog on the lower aspect of the occipital lobe, but the investigation of the optic centres is not as yet completed. I hope, however, to be able to exactly localise the vision-areas in the case of the Rabbit, Dog, and Monkey by observing the changes the brain undergoes when stimulated by light.

In figs. 8, 9 there will be seen in several of the nuclei of the large pyramidal cells, peculiar deeply stained crescentic bodies, which may be the homologues of the centrosomes to be found in the nuclei of sympathetic nerve cells (compare fig. 1_7).

Another point worth mentioning is this. The cell chromatin to disappear last lies in many cases at that pole of the nucleus pointing towards the molecular layer, and there forms a distinct cap or pyramid. The reason for this being that the bundles of nerve fibrils coming from the basal processes and the axis-cylinder process have to sweep past the nucleus, that they converge only a considerable distance above the nucleus, and thus leave immediately above the nucleus an area free of fibrils, which is made use of for the deposition of food in the form of chromatic material.

My investigation has thus shown—

1. That DURING REST, several chromatic materials are stored up in the nerve cell, and that these materials are used up by it during the performance of its function.

2. That ACTIVITY is accompanied by an increase in size of the cells, the nuclei, and the nucleoli of sympathetic, ordinary motor and sensory ganglion cells.

3. That FATIGUE of the nerve cell is accompanied by shrivelling of the nucleus and probably also of the cell, and by the formation of a diffuse chromatic material in the nucleus.

In this preliminary note I feel indisposed to venture any speculation in explanation of the phenomena observed, but I shall endeavour to do so when my completed paper, accompanied by photographic illustrations, tables of measurements, and a fuller account of the literature, will make its appearance.

In conclusion, I have to express my deep obligation to Professors Rutherford and Munk, in whose laboratories the above researches were conducted.

DESCRIPTION OF PLATE I.

Figs. 1–3. Three cells from superior cervical sympathetic ganglion of the rabbit.

Figs. 1 and 2 fixed in my watery picro-corrosive fluid. Stained in Sulphuric acid Methylen-blue and Eosin.

Fig. 1. From the control ganglion, showing a resting nerve-cell, with (1) peripheral, (2) interfibrillar, (3) intrafibrillar chromatin rods, (4) the nuclear chromatin, (5) the nuclear hyaloplasm, (6) the nucleolus, with its endo-nucleolus, (7) centrosomes (?) lying in the nucleus.

Fig. 2. Cell from ganglion stimulated faradically for thirty minutes, showing diminution in the amount of chromatin, aggregation of lymph in the centre of the cell, &c.; (8) the enlarged nucleolus and endo-nucleolus, with delicate radiating fibrils of linin.

Fig. 3. Cell from ganglion stimulated nine hours. Fixed in equal parts of a saturated solution of $HgCl_2$ and 1 per cent. Osmic acid solution. Both nuclei collapsed and somewhat deeply stained.

Figs. 4 and 5. Two motor cells from lumbar region of spinal cord of dog. Fixed in $HgCl_2$, and stained in Toluidin blue. Fig. 4 from the fresh dog—(1) pale nucleus, (2) dark Nissl's spindles, (3) bundles of nerve-fibrils.

Fig. 5. From the fatigued dog, with (4) dark shrivelled nucleus and (5) pale spindles.

Figs. 6 and 7. V.S, retinæ of dog fixed in corrosive sublimate, stained in Toluidin-blue. (*a*) layer of nuclei of cones (1) and rods (2), (*b*) middle ganglionic layer.

Fig. 6 from eye kept dark.

Fig. 7 from eye exposed for twelve hours to ordinary daylight on a sunny day.

Figs. 8 and 9. V.S, from lower aspect of occipital lobe of rabbit. Fixed in sublimate, stained in 1 per cent. watery solution of Eosin for 1 minute and Toluidin-blue $\frac{1}{2}$ per cent. for 15 minutes. After treatment as stated above, p. 105. a = molecular layer; b = layer of cells, first described by Ramon y Cajal, for which I suggest the name "submolecular"; c = layer of small pyramidal cells; d = layer of large pyramidal cells. 1. Intra-nuclear chromatic crescents (centrosomes ?). 2. Chromatic material on apex of nucleus.

Fig. 8 corresponds to eye kept dark.

Fig. 9 corresponds to eye exposed for two hours to flashes of light.

Fig. 10. A sympathetic nerve-cell stained with Eosin Methyl-blue to show (1) nuclear hyaloplasm, (2) nuclear chromatin, (3) centrosomes (?), (4) nucleolus with endo-nucleolus, (5) five "bigeminal" bodies.

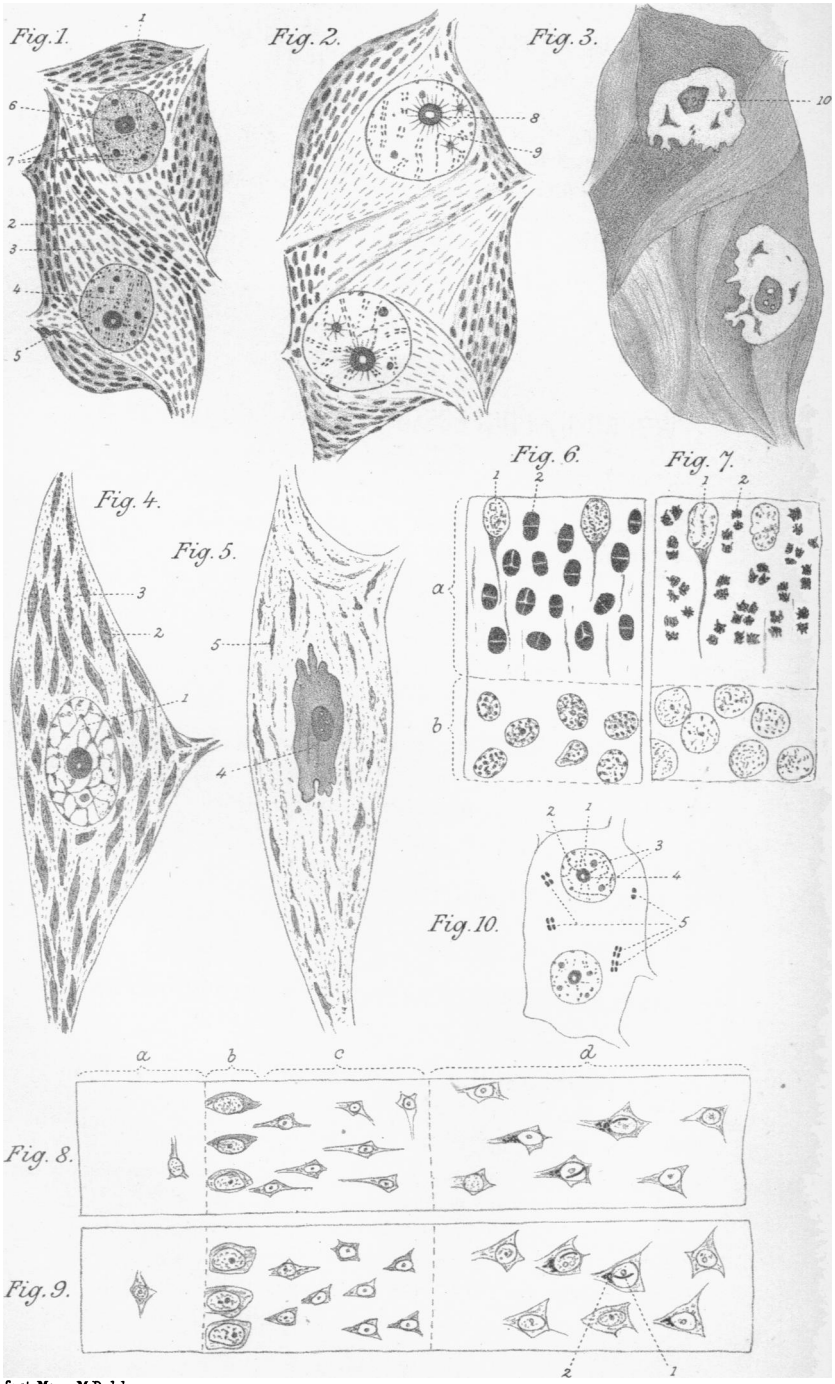
Fig. 1.
Fig. 2.
Fig. 3.
Fig. 4.
Fig. 5.
Fig. 6.
Fig. 7.
Fig. 10.
Fig. 8.
Fig. 9.
a
b
c
d