ON THE PRIMARY STAINING OF THE RAT'S BRAIN BY METHYLENE BLUE.

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ONE of the most curious and important features in neurohistology is the extraordinary affinity which methylene blue has for the elements of the nervous system, especially in regard to what Bethe terms its primary staining capacity, *i.e.*, the staining of fresh material, not previously acted on by chemicals, with basic dyes.

By different ways of applying the stain very different results are obtained; thus, at one time it picks out certain details and neglects all others, at another time these details fail to show and quite different ones are revealed.

Ehrlich was the first to apply the stain directly to the tissues, which he did by injecting it during life into an animal. In this case the great bulk of the cytoplasm of the pyramidal cells is scarcely coloured at all, but other cells, scattered irregularly about in the cortex, stain very darkly. There is shown also around some of the pyramidal cells a network of fine, beaded fibrils. My method, which gives a similar, but more detailed picture, enables us to obtain this reaction in dead tissues. This I have called a pseudo-vital reaction, and up to the present time have only succeeded in getting it occasionally, and, as a rule, in small isolated parts of the tissue.

Apäthy, Bethe, Dogiel and others, by a different procedure, succeed in staining the neurofibrillæ of the ganglion cells, but apparently their methods do not differentiate the darkly-stained cells above mentioned.

Under still different conditions the dye seizes on other details in the ganglion cells, and stains the so-called Nissl-bodies (which are probably structures not directly concerned

with nerve conduction), and leaves the rest of the cell body uncoloured. Bethe applies the term "primary staining" to tissues which have been dehydrated in alcohol or ether before staining, and therefore regards the colouring of the Nissl-bodies as primary, and Lenhossék and I have shown that perfectly fresh tissues acted on only by the dye clearly show them.

As a rule, in primary staining by methylene blue, it is one or other of the nervous elements which are especially selected. The glia is largely neglected. However, during my attempts to get the pseudo-vital reaction in animals' brains, I have found that occasionally the dye specially picks out certain of the glia cells and structures which, I believe, are also derivatives from these cells.

I propose to give, in the following pages, a short account of these results, and also of some in which I have succeeded in obtaining the pseudo-vital reaction in the brain of the rat.

I shall deal first with the pseudo-vital reaction which I have been able to get in only one or two cases after keeping the dead animal for twenty-four hours in the oven, at 25° to 30° C., or else by taking out its brain and leaving this organ under similar conditions in a closed glass vessel. The staining mixture, consisting of 40 cc. of 1 per cent. methylene blue, to which 2 drops of lactic acid has been added, and 4 cc. of peroxide of hydrogen (10 vols.) was slightly warmed, and the brain put into it in a covered vessel, and left in the oven (25° to 30° C.) for six or seven days, then fixed for twelve to twenty-four hours in 10 per cent. molybdate of ammonium, washed, dehydrated, embedded in paraffin and cut. The successful results having been limited to very small areas in the cortex and striate body of two or three brains, I purposely give a very cursory account of the procedure, as I am still experimenting, and hope before long to be able to arrive at more constant and more widespread results. After a great number of trials it is evident (1) that the reaction does not occur in perfectly fresh tissue; (2) that the addition of a trace of lactic acid to the staining fluid somewhat facilitates matters (with this observation we must connect the fact that lactic acid is developed in

dead nervous matter); (3) any signs of putrefaction due to aerobic organisms prevent a successful result, and at the same time, by setting free ammonia compounds, give an alkaline reaction to the tissue.

During these investigations I found if the brain was subjected to a certain heat, when immersed in the staining fluid, that a very different result from that sought after was obtained. Scattered irregularly throughout the cortex and white matter were small, multi-branched cells which stained absolutely black, and which appeared to be glial. Furthermore the apices of the pyramidal cells frequently stained deeply and showed numerous fine, short, lateral projections, ending in knobs; and, lastly, around many of the nerve-cells beautiful Golgi nets were shown.

(A) PSEUDO-VITAL REACTION.

Cortex cerebri (median aspect adjacent to the corpus callosum).—The cytoplasm of the pyramidal cells (including the cells of the second layer) and the spindle-cell layer, stain a pale sky-blue and quite homogeneously; the nucleus is somewhat darker and densely granular; except the apex, the other protoplasmic processes are practically not shown. Scattered here and there throughout *all* layers of the cortex are small (an average size being $14 \times 9 \mu$) multi-branched cells, not definitely orientated, staining nearly black and giving off extremely long, delicate processes, which spring abruptly from the cell body and pursue a very sinuous course. These processes are not regular in contour, but present a number of fine beads or more elongated thickenings, lying close together connected by finer strands, and with high magnification the thicker parts do not appear quite homogeneous, but have a loose structure, something like cotton wool, and in favourable cases they show numerous delicate, lateral twigs, similar to those on the processes of certain of the striate body cells. The processes ramify freely within the first layer (fig. 1). The pericellular beaded network is very scanty; indeed, scarcely more pronounced over the cells than in the intermediate regions. Its fibrils are

extremely fine and the black beads are generally about $2\ \mu$ apart, but the distance between them varies considerably as in human brains. So far I have not seen anything approaching the dense fibrillar felt work such as exists between the cells in the human cortex. The fibrils forming the pericellular net work appear very similar to the finest branches of the dark cells: in both cases the structures are beaded, but usually the dendrites appear less homogeneous and sharply cut than the fibrils of the network.

FIG. 1 ($\times$ about 500 diameter).

Small, darkly-stained cell in the second layer of the cortex. Its branches are extremely fine, and pursue a sinuous course for very long distances, retaining the same calibre. Note how they spring abruptly from the cell-body, and that some of them pass up into the first layer. X probably represents its axon passing up into the first layer.

Contrary to my universal experience with human brains, the first layer in rats, I find, takes on the reaction and shows considerable fine structural details. Fairly numerous extremely fine black fibrils run in it at all depths parallel to the surface. They have a clean-cut aspect and present at irregular intervals minute swellings, sometimes beads. Similar fibrils pass up into this layer from beneath, and

bend over to one side or the other. A fair number of nerve-cells are met with, staining the same light-blue colour as the cells in the lower layers. Occasionally also here and there the apices of pyramidal cells which have stained black are seen traversing this layer and bifurcating in it; sometimes they reach nearly to its outer margin, and it is to be noted that along their sides are closely clustered black dots, which join to the dark process by a delicate pedicle. In the few cases where I have been able to trace these dark apices to their parent cell the lateral offshoots were not apparent over the cell body, and only began to show at some little distance from it on the processes. These side twigs have very much the appearance of those on the dendrites of the striate body cells, but are shorter and more thickly clustered.

Cornu ammonis.—My remarks refer to the long pyramidal cells and the stratum radiatum. Only the bodies of the pyramids show, and they stain of an uniform pale blue, their nucleus not much darker. The ground work of the stratum radiatum and the spaces between the cells, where they are not so closely packed together, stain a dull pale gray, and coursing in the groundwork, usually in a direction more or less direct from the bodies of the pyramids to the granule layer, are a number of very fine, dark processes which originate from darkly-stained cells lying here and there among the pyramids or in their immediate neighbourhood. These dark cells are similar to those already described, but are, as a rule, somewhat larger (18μ), and often more or less bipolar.

In the places where the bodies of the pyramids lie closely packed together they are marked off from each other by a darkly-stained beaded line, which, in places, gives off branches which pass over the cell bodies. Where the pyramidal cells do not lie quite so close together the fibrils course sparingly throughout the intermediate substance (see fig. 2).

Striate body.—This shows a structure very similar to the human caudate nucleus. Its cells are nowhere definitely orientated; the pale variety, much the more numerous, are chiefly indicated by their nuclei, as the cytoplasm is only faintly coloured just around these bodies. A few angular

cells stain deeply and give off comparatively short rectilinear processes, which dwindle rapidly and pass from view, and these I am inclined to regard as cells belonging to the pale

FIG. 2 ($\times 780$)

Darkly-stained cell, lying in amongst the long pyramidal cells of the cornu ammonis (which are represented in outline only). Fine, beaded fibrils invest the pyramidal cells sparsely, and in some cases appear to take origin from the dendrites of the dark cell.

category, which have, for some unknown reason, coloured darkly.

The dark cells are exactly like those I described and figured in the human caudate nucleus (BRAIN, Autumn,

FIG. 3 ($\times 780$).

A dark cell from the striate body. Delicate lateral twigs ending in a bead are given off from the more distal portions of the dendrites. At X a long, beaded fibril appears to arise from a dendrite.

1903). Their long, slender, sinuous processes form a coarse felt-work with each other, and generally in their distal parts

they give off numerous small, but distinct, lateral twigs ending in a knob a little distance from their origin, or else long fibrils with small heads on them at short intervals (fig. 3). Not all the dark cells show these "thorns"; the processes of some present a very irregular, moniliform, or coarsely-beaded appearance.

Numerous bundles of fine axons traverse this body on their way to the cortex, and where individual fibres can be detected they are of a pinkish hue and do not show definite beads, although their contour may not be perfectly smooth.

REMARKS.

Although the results so far obtained are meagre, they show that the "dark" cells occur in both the first and second layers of the cortex and in the cornu ammonis, in which places I have not yet succeeded in showing them in the human brain. The presence in the first layer of the dendrites of these cells, especially of those cells which lie just below it, and which send up processes to ramify therein (*vide* fig. 1), might be supposed to militate against the idea that the beaded pericellular network is a derivative from these dendrites, because this layer is poor in cells. But in the new-born infant and in full-grown cats, as I have frequently observed, it does contain a considerable number of cells, the chromophilic elements of which are fairly well marked, and arranged in a similar manner to those in the pyramidal cells lying lower down in the cortex, a characteristic which at all events appears to mark them off as distinct from the dark cells. Very likely also in rats' brains these chromophilic cells are persistent throughout life, as in the cat.

I believe that not only do these dark cells form an intermediary system between the end afferent stations and the first efferent (*e.g.*, in the cortex), but that they are met with at afferent stations of different levels (*e.g.*, Clarke's column nuclei, gracile and cuneate nuclei, optic thalamus, &c.), interposed between the terminals of one level and the cells of the next; and that in all probability they will be met

with similarly disposed at the efferent stations of different levels, both cerebral and cerebellar.

Golgi long ago described cells to which he gave the name of intercalary; very probably these are included with others (notably Martinotti's cells) in my dark-cell system, and therefore I propose to call it the intercalary nerve-cell system.

Besides their staining peculiarities they have other well-marked characteristics by which they can be identified, and which, I submit, afford reasonable grounds for regarding them, wherever situated, as of like nature.

These are four in number, viz. :—

(1) *Dark staining*.—This, although a useful character by which their presence is readily revealed, is not alone to be depended on, for although, so far as I am aware, they always stain deeply, yet occasionally cells of the pyramidal system take on a dark colour also, and as this occurs very often in the brains of presumably healthy rats, especially in the cornu ammonis, it probably does not necessarily depend on a pathological condition, but possibly on some chemical alteration accompanying the functional activity of these cells.

(2) *Shape*.—Although in this respect they vary very much, yet they are extremely rarely pyramidal. They are usually square, pentagonal or oval, but whatever form they assume their protoplasmic processes spring from them abruptly and do not pass off by insensible degrees as, *e.g.*, the apical process of a pyramidal cell; sometimes, especially in the oval forms, a thick process springs from the cell in such a manner that one is unable to determine exactly where it leaves the cell body, but in such cases, after a very short course, it divides into two much smaller branches, which run for very long distances without alteration in calibre.

(3) *Dendrites*.—The way in which they arise has just been described. They run for very long distances without marked diminution in calibre, and are irregular in outline, with either moniliform swellings or lateral twigs. Their course is sinuous, and not, as is almost universally the case with dendrites of the pyramidal cells, rectilinear. This last feature I look upon as very characteristic.

(4) *Orientation*.—Unlike the pyramidal cells they are never definitely orientated.

In my previous communications I have given instances in the human brain which seem to demonstrate a direct connection between the protoplasmic processes of the intercalary cells and the beaded network around the pyramidal. In dealing, however, with such extremely minute structures, dogmatic statements must be received with caution; but, apart from such seemingly direct evidence, there is much of an indirect nature which also favours this assumption. I would especially call attention to the long, beaded fibrils which pass off from the dendrites of the intercalary cells of the striate body, and which have identically the same appearance as those forming the pericellular network. The objection that from this point of view the so-called law of dynamic polarisation is contravened, has with our present knowledge of the intimate structure of nerve-cells no longer much, if any, weight. The presence of neurofibrillæ, and the way in which they are arranged in the cells, shows that, as applied to the pyramidal cells, this doctrine is untenable. It is now a well-established fact, which anyone can verify for himself, that neurofibrillæ pass directly from one dendrite to another, and therefore, in whichever direction the nervous impulse traverses such a fibril, it must pass in opposite directions in the two dendrites in which a particular fibril lies. I am assuming that there are such phenomena as nervous impulses, and that they pass along neurofibrillæ. For the experiments of Bethe seem to show that the fibrils are the only material substance along which they can pass, for, according to him, they are the only structures which run continuously through the nodes of Ranvier.

(B) HEAT-FIXED REACTION.

If the staining mixture is added to the brain and cord and the whole subjected to heat in a covered glass capsule for some time, and then left in the oven at 25° to 30° C. for three or four days, one obtains occasionally, especially in young rats, a quite different reaction. I am unable to give any hard and fast rules, as my experience is not sufficiently extended,

but I have obtained successful results in several cases, more especially when the brain in its staining fluid was slowly raised to 60° C. on the water bath and then allowed to cool to 50° C. and again slowly raised to 70° C. and kept at this last temperature for some time, according to the size of the material to be stained (half to one or more hours). It is then placed in the warm oven (26° to 30° C.) for four days, and the subsequent treatment is as before described. In one case, which showed the lateral projections on the processes of the pyramidal cells, the brain had been subjected to a temperature of 100° C. for several minutes; in another, which showed Golgi nets around the Purkinje cells, the temperature was only raised to 50° C. and kept at this point for two hours. Under these circumstances, with successful preparations, one gets appearances quite distinct from the pseudo-vital reaction. No darkly-stained, intercalary cells, nor beaded network, nor other fine detail of an undoubted nervous nature is shown, but numerous glia cells are shown, sparsely scattered about in the cortex white matter, and in the central nuclei and stem; and besides these well-developed cells with many branches, a number of round or oval bodies are seen, which vary in colour from a dull pink to almost black. They are about 9 μ in diameter and generally have a pale, round area within them occupying the greater part of their mass. These bodies are especially numerous in the white matter and amongst the cells of the nuclei of the stem. A variety of transitional forms between these just described and fully developed black glia cells occur; at one end of the scale there is an oval mass without processes; one passes from this to intermediate forms which show several short unbranched processes; at the other end of the scale the processes have become long, numerous, and freely branched, and the body less round (see fig 4). I have observed all forms of these cells in large numbers in the human cerebrum and cerebellum, treated in the manner first described for the rat's brain (*i.e.*, raised to 70° C.). In the latter region, cells which appear quite similar to the immature-looking forms in the rat's brain make up a large bulk of the entire granular layer (see

FIG. 4 ($\times 780$).

In the upper part are two well-developed multi-branched glia cells lying in between the bodies of the long pyramids of the cornu ammonis. They are much smaller than the dark nerve-cells which are met with in this region (*vide* fig. 2). In the right-hand figure the end-branchings are shown (somewhat diagrammatically), breaking up into a lattice-work covering the pyramidal cells.

In the middle of the page are three glia cells from the white matter, showing more or less immature-looking forms; the upper has no processes visible, the left-hand one has three stunted, unbranched processes, and the right-hand one has many. Note the pale area within two of these bodies.

In the lower part, to the left, are three well-developed glia cells from the molecular layer of human cerebellum. To the right are four small glia cells from the granular layer, with stunted branches. These are often clustered together into clumps of three or four. At X is a somewhat longer (8 by 5 μ) and more developed cell, which lies in the lowermost part of the molecular layer, and has the appearance of being a more developed form of the granular glia cell. (The pale centre, although represented as granular for facilities of reproduction is really quite homogeneous).

right hand side of fig. 4, lower half). At the junction between this and the molecular layer and in the innermost part of the latter the cells become larger and more branched, whilst in the outer parts of the molecular layer well-developed forms occur (left hand fig. 4, lower half). In nearly all cases they show a pale central part.

My sections show very clearly that there is a very intimate relation between the branches of these cells and the trabeculæ of the Golgi nets; in fact, I believe they demonstrate a direct passage over of their branches into the net structure.

Golgi nets.—These are shown very distinctly, closely investing the bodies and dendrites of the cells of the dentate nucleus of the cerebellum, and most of the cells of the different nuclei within the pons, especially those of Deiters' nucleus, and the fore horn cells of the cord; they appear less distinctly shown around the pyramidal cells of the cerebrum, the cells of the corpora quadrigemina and the Purkinje cells.

They consist essentially of a close-fitting trellis structure, something similar in appearance to a piece of perforated zinc; the apertures are usually polygonal, and often contain a dark dot in the centre; the trabeculæ are slender. Bethe states that cells of different classes have nets of different patterns, so that from inspection of only a small piece of the net one is able to tell whether it belongs to a cell from the cortex, dentate nucleus, or trapezoid body, &c. The meshes over the dendrites are usually more elongated than those over the body. This authority also states that the net invests the axon for a short distance, ceasing either before or at the place where the myeline sheath begins.

It is very evident to the most casual inspection that this investment and the beaded pericellular network revealed by the pseudo-vital method are entirely distinct.

I have not been able to make out any extension of the trabeculæ of the Golgi nets to the matrix between the cells such as Bethe describes, but where two cells lie in apposition it passes continuously from one to the other, as he also notes. My preparations appear to show very conclusively that they

are derived from the terminal ramifications of the branches of glia cells, and although my personal acquaintance with these nets is confined to sections from several rats' and human brains, yet I have already met with, in the former, many instances which appear to establish this connection without doubt (see fig. 5).

FIG. 5 ($\times 780$).

Golgi nets surrounding, in the upper part of the figure, two cells from the dentate nucleus of the rat's cerebellum. In the lower part a large cell from one of the nuclei of the stem. In each case the darkly-stained glia cell from which the net appears to arise is shown. Note the pentagonal form of the mesh-work, and the frequent presence of a black spot in the centre. In the two upper figures the nerve-cell is merely indicated by its covering, but in the lower one the nucleus is shown.

Thorns.—In these heat-fixed preparations very often the apices and main dendrites of the pyramidal cells of the cortex stain somewhat darkly. The apices, under these circumstances, can be seen throughout almost the entire depth of the cortex, like a forest of pine trunks, lying closely together and ending apparently in the inner part of the first layer,

where they incline slightly to one side or the other ; sometimes, especially in the case of the second layer cells, they bifurcate. The bundles formed by these apices are reinforced as they pass up by apices from shorter cells, so that they are more densely packed towards the surface than lower down. The cell body itself does not stain deeply, but the dark colour begins somewhat abruptly not far from it. The axons of these cells also stain deeply, but dwindle and disappear after a short course. In the more distal parts of the apical process, very fine, closely set, short twigs, ending in a knob, are seen passing away at right angles from its sides, and in cross sections through the dark apices these projections can be seen passing off all round like the spokes of a wheel (fig. 6). They appear quite similar to the lateral twigs already referred to, which show when the pyramidal apices stain darkly by the pseudo-vital method. Very often, also, in this view, the cut-through process is only dark on the surface and has an almost colourless centre. When examined under a high power it is seen that the dark colour is not uniform, but consists of darker granules on a somewhat lighter background. The beads can often be distinctly seen joined to the dark apex by delicate pedicles, but very often, in the immediate neighbourhood, others are seen scattered fairly thickly about and showing no connecting fibrils.

REMARKS.

Golgi nets were originally described by the distinguished Italian histologist of that name in 1882. He ascribes to them an isolating function, and believes that they are a neurokeratin structure, but opposed to this idea is the fact, which Bethe points out, that this substance is not digested by pepsin, whilst the network is. Ramon y Cajal, Donaggio, Apáthy, and, recently, Held, regard them as glial in nature, and the two first as representing merely a peripheral extension continuous with the endocellular network of Golgi. Bethe and Semi Meyer believe that they are nervous structures, and Bethe represents them as extensions of axis

FIG. 6 ($\times 780$).

On the right side are four pyramidal cells with darkly-stained apices. At some distance from the cell body fine dots are visible at the side of these processes, and generally a fibril connects the dot to the edge of the process. But, in places, collections of these dots occur which do not seem to have any connection to the pyramidal cells, as shown on the right side of the longest cell.

The left-hand figure gives a cross section of the pyramidal cells.

The apices now appear as a black spot or a black ring with colourless centre, and the little lateral projections come off all round like the spokes of a wheel. The long processes with thorns are dendrites. The two large, pale nuclei, with clear rims, represent two pyramidal cells cut through their bodies. Although these figures were outlined by aid of Zeiss's Camera Lucida, the lateral projections are so minute that it is not pretended that they have been individually traced, but only give the general appearance produced to the eye.

cylinder processes which, coming from other parts, join to the network and are thus brought into relation with the enclosed nerve-cell. The neurofibrillæ contained in the axis cylinders pass along in the trabeculæ of the nets, and leave them to enter the substance of the ganglion cells within. He believes that these nets, and a diffuse network which in places he sees in connection with them lying in the general ground substance, are identical with Nissl's hypothetical "gray" (but on this particular point Nissl does not agree with him).

My observations point strongly to the glial origin of these structures. The fact that I have only succeeded in showing them in preparations in which the stain only selected elements which appeared to be of a glial nature favours this view, but apart from such indirect evidence I have, as already mentioned, frequently seen what has every appearance of being a direct continuity between the branches of the glia cells and the network.

It is a pity that there are not greater facilities whereby different workers could compare one another's sections; if this were feasible probably many discrepancies and seemingly antagonistic views would be brought into harmony. This is the more to be desired as modern methods of staining nervous structures tend to become more special and limited in their range, so that taking any one method, whilst some details are shown, others which, perhaps, may be very closely related, are not revealed at all; and although one worker may not, and usually does not, limit himself to a single method, he will probably not reach an equal degree of perfection at all; thus, whilst he may obtain results which, after a careful examination of very many sections, appear to him convincing, he has not the time, nor perhaps the opportunities, to examine with equal thoroughness large numbers of preparations stained by different methods. Taking the case of these Golgi nets, I feel very strongly that my sections do show that they result from the splitting up of the glia cell branches, but I am unable to speak of their relation to the endocellular network, because the method I use does not show the slightest trace of these

structures. If, as Cajal and Donaggio think, they are both parts of one and the same thing, and in view of Holmgren's observations on the intracellular networks and "sap" canals in posterior spinal ganglia and gland cells, then they are probably a mechanism concerned with the metabolism of the cell.

On the other hand, the junction of axons with Golgi nets is strongly insisted on by Semi Meyer, and Nissl, after an inspection of Bethe's specimens, was also convinced of it. Nevertheless, in spite of the weight which opinions from such eminent observers carry, the minuteness of the structures under discussion must make it extremely difficult for anyone to definitely decide between actual continuity or mere proximity. It may be that the axons surround the ganglion cells, enter at the small pentagonal openings of the net, and break up within into fibrillæ. But, even should Bethe's observation prove correct, I should still be inclined to regard the network as not essentially a nervous structure, but as affording a covering for the fibrillæ, which Bethe states he has seen *within* its trabeculæ, without being itself concerned directly with the functional nervous activity of the ganglion cell.

On the assumption that the Golgi net is an extension of the axis cylinders rests the main fabric of Bethe's argument for the continuity throughout the central nervous system of individual neurofibrillæ as expounded by him in his recent publication, "Allgemeine Anatomie und Physiologie des Nervensystems," and this, to my way of thinking, is a weak link in his chain of reasoning. I myself believe in the continuity of nerve-cells with each other by their processes, but I think it occurs in a different way to that which he describes.

Thorns.—The thickly-clustered, delicate, lateral offshoots from the darkly-stained apices and dendrites of the pyramidal cells are to be seen, as already mentioned, not only in heat-fixed preparations, which do not, apparently, lend themselves to the staining of fine nerve details, but occasionally on the pyramidal apices which have, contrary to their usual custom, stained darkly in preparations which

give the pseudo-vital reaction. Although they show a great resemblance to the projections on the dendrites of some of the darkly-stained cells in the striate body, I am inclined to think there is a distinction between them and those on the pyramidal processes, and that the latter are not integral parts of the cells to which they adhere. They appear to me to be finer, more closely clustered and shorter than those on the striate body cells. Without accepting the interpretation which Bethe gives to the function of the Golgi nets, perhaps, as he suggests, these lateral projections represent the stained trabeculae of Golgi nets passing from the cellular to the diffuse portions of these structures. An objection to this view is that whilst we know that the Golgi net envelops not only the processes but the cell body, this latter part does not show the lateral projections, either with the pseudo-vital or heat-fixed methods. Speaking now of the appearances seen in heat-fixed preparations, if the Golgi net over the apices causes them to stain deeply, we may reasonably ask why the cell body is not also similarly affected. This may possibly be due to a difference in the size of the meshes over the cell and processes; if those over the dendrites are smaller they might hold the stain so as to cause the whole surface to appear dark, whilst the bigger meshes over the cell body would fail to do so.

However this may be, I do not feel qualified at present to express any decided opinion as to their precise nature, except to reiterate what I have before stated, that they do not appear to me (especially in the heat-fixed preparations) to be integral parts of the processes themselves.

A few words in conclusion concerning the differentiation by the heat-fixed method of the granules of the human cerebellum. There is still much uncertainty about the nature of these bodies; true it is that Golgi's method shows here and there in the granular layer a small cell with ascending axon and claw-like dendrites, but these only occur in comparatively few numbers, and I have always felt that their presence was no sufficient justification for looking on all the granules as of the same nature. My preparations show that a very large number stain differently from the rest. These

stain deeply, show a few stunted processes and a pale central portion. They resemble very closely the immature-looking glia bodies in the rat's brain, and show grades differing only slightly one from another between the most immature form and the well-developed glia cell, and to my mind can with very little doubt be classified as glia elements. But as I have, up to the present, only seen these bodies in more or less pathological brains, I merely wish to draw attention to the fact without drawing any conclusions therefrom.

REFERENCES.

- BETHE, A. "Allgemeine Anatomie und Physiologie des Nervensystems." G. Thieme, Leipzig, 1903. He gives a very full bibliography, and many of my references concerning Golgi nets were taken from this work.
- FORD, ROBERTSON. "Pathology of Mental Diseases." W. R. Clay, Edinburgh, 1900. He gives a large number of references in relation to Golgi nets up to that year.
- HOLMGREN, E. "Weitere über das 'Trophospongium' der Nervenzellen, &c.," *Archiv. für Mikroskopische Anatomie*, July, 1902.
- TURNER, J. "A Method of Examining Fresh Nerve-Cells, &c.," *BRAIN*, vol. xx., 1897. "Notes on the Minute Structure of the Human Caudate Nucleus, &c.," *BRAIN*, vol. xxvi., 1903.

All the figures were drawn by the aid of Zeiss's Camera Lucida.