A CONTRIBUTION TO THE STUDY OF HUMAN NEUROGLIA.

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Plates XLVI–XLIX.

The neuroglia has been a peculiarly elusive tissue. What knowledge we have had has been gained laboriously and by means of inadequate methods. Certain of the more recent means of staining, while throwing an added light on various histological details, have tended rather to obscure than elucidate the real problem at issue. This is unquestionably true of the numerous silver precipitations which go under the general name of the Golgi method. With all the wealth of knowledge it has given us, there can also be no doubt that from its essential crudeness this method has tended to perpetuate error. It has failed, in the hands even of its most skilful advocates, to demonstrate the intimate histological peculiarities which we need to know and understand. It should, however, at once be said that the function of the method lies in a different sphere, for which it is admirably adapted. But there appears to be a certain danger that it may be misused in the attempted solving of questions for which it is inadequate. One of these deals with the histological structure of neuroglia. A method which colors by precipitation is *a priori* incapable of giving us the information we require; it must, of necessity, be confusing in its pictures of structural detail. Nevertheless, in connection with the neuroglia, conclusions of moment have been drawn from its use* which, in the light of more appropriate methods, must be regarded as misleading, if not actually wrong.

* von Lenhossék, Der feinere Bau des Nervensystems. Berlin, 1895.

With the appearance of Weigert's* long-awaited monograph, and the almost simultaneous publication of Mallory's† analogous method, an absolutely new light has been thrown upon the question. Working independently, the two investigators, using practically an identical method, have reached the same general conclusion of the disassociation of neuroglia cells and neuroglia fibres in the adult human nervous system, a conclusion which has been rendered possible by the use of a differential chemical stain. There can be no doubt of the essential correctness of this histological fact, and it is not our purpose to argue in detail its claim to acceptance. Weigert's monograph, though defective in certain respects, leaves no reasonable doubt in one's mind of the essential justice of his claim, which Mallory shares. It goes without saying that many questions remain unanswered, and that we must reconstruct in great measure our conception of the neuroglia, as with the advent of the neuron we have been faithfully doing in regard to the architecture of the nervous system in general.

Stroebe has vigorously and skilfully opposed Weigert's views in the most significant monograph on the subject of "Glioma" published within the last few years,‡ and also in the discussion of the Naturforscherversammlung, held at Frankfurt in September, 1896, on the structure of newly formed neuroglia in pathological conditions.§ Stroebe bases his claims on results furnished by Mallory's phosphomolybdic-acid-hæmatoxylin stain, a method whose advantages and shortcomings in the solving of the question at issue we shall have occasion to allude to later.

Whatever may be the ultimate outcome of the controversy, it is clearly evident that the significance of the new methods is extremely great. If, as there is every reason to suppose will be the case, they fulfil their early promise, we shall have, what we have so much needed, a definite and certain means of studying normal conditions and

* Weigert, Beiträge zur Kenntnis der normalen menschlichen Neuroglia. Frankfurt a. M., 1895.

† Mallory, *Centralbl. f. allg. Pathol. u. path. Anat.*, vi (1895), 753.

‡ Stroebe, Ueber Entstehung und Bau der Gehirngliome. Ziegler's *Beiträge*, xviii (1895), 405.

§ Stroebe, *Centralbl. f. allg. Path. u. path. Anat.*, vii (1896), 864.

pathological processes, with the accompanying hope that some of the problems of neuroglial proliferation may at least be intelligently met.

It is the purpose of the present paper to bring up certain questions which this most recent work forces upon our attention, in connection with a study, chiefly anatomical, of two brain tumors of peculiar interest.

The clinical history* and gross pathological anatomy of the first and more significant case is as follows:

Case I. Anna H., 8 years, American; admitted to Massachusetts General Hospital, Nov. 29, 1895.

Family history negative. Has had whooping cough, measles, chicken pox, bronchitis. Four years ago patient began to stammer, and developed spasm of the right foot and leg. The motor disturbance apparently improved, but the speech remained hesitating. This condition continued essentially unchanged for two years. At the end of this time, paresis of the right leg and spasm of right arm were noted. Increase of symptoms took place during the following months, so that an attempt to go up stairs became difficult. In June, 1895, vomiting appeared independent of eating, and more frequent at night; in August, marked defect in vision. Appetite and sleep poor. Defective control of bladder and rectum. Increasing difficulty with speech. Some headache.

Physical Examination on entrance to hospital. Patient somewhat emaciated, dull, cries out occasionally. Right arm and leg smaller than left. Paralysis of right facial nerve. Hemiparesis, right arm and leg. Right forearm and fingers flexed. Pupils dilated. Double optic neuritis.

Sleeps much of the time; shows but slight evidence of pain. Incontinence of urine and feces. Moves the right arm at shoulder.

Operation by Dr. S. J. Mixter, December 5. Ether. Usual incision. Dura normal, bulging. Dura incised. Brain bulged through opening. Injection of vessels, otherwise normal appearances. Bone returned; dura loosely sutured. Much shock from operation. Slight improvement under stimulation. Death the following morning.

During the patient's stay in the hospital her temperature was for the most part slightly above normal, not, however, reaching higher than 100.2°F.

Autopsy, by Dr. W. F. Whitney. Skull alone opened. Calvaria normal. Convolutions somewhat flattened. Cortex normal in appearance. Lying beneath the floor of the left ventricle was a cystic cavity,

* Taken from Records, Mass. General Hospital.

containing about two ounces of serous fluid. Into this projected a new growth, apparently arising in and replacing the optic thalamus and corpus striatum. The growth was also detected in the region of the optic chiasm, close to which were several small cystic protuberances.

Further examination of the fresh specimen was deferred for fear of destroying relations in the process of manipulation. The brain was preserved entire in formalin.

After hardening, frontal sections were made, passing respectively through the tumor at its point of greatest development, viz., two centimetres anterior to the pons (see Plate XLVI); through the pulvinaria of the optic thalami, and through the cerebellum and posterior horns of the lateral ventricles.

For the preservation of topographical detail the method of sectioning after formalin hardening is altogether preferable to the immediate study of the fresh specimen. Cross section No. 1 (Plate XLVI), taken through the tips of the temporal lobes and the optic chiasm, 2 cm. anterior to the pons, shows the gross appearances and position of the new growth. At this level it lies as a solid discrete mass about the size of a walnut, chiefly in the left hemisphere, from which it undoubtedly originated, extending, however, considerably over the median line, leading to the distortion of the third ventricle, as shown in the plate. The tumor itself is dense and granular in appearance, suggesting fibrous tissue. Surrounding the main tumor mass on every side are larger and smaller cysts, which were in part filled with a serous fluid and in part with a gelatinous substance. These cysts originally had definite walls, as shown in the partially reconstructed plate, and were entirely unconnected with the ventricular cavities. The cyst of largest size was situated in the left lateral ventricle, filling a large portion of its cavity, and contained a fluid not unlike cerebro-spinal fluid in appearance and consistence. The tumor might be described as extending into this large cyst-cavity, from which it is, however, sharply separated. A similar but much smaller cyst lies at the base of the brain, pressing upon and, no doubt, in large measure destroying the optic nerves in the neighborhood of the chiasm. Smaller cysts of similar character are to be seen, differing from the larger ones chiefly in the fact that their contents are gelatinous.

A section (No. 2) made 2 cm. posterior to that already described, and passing through the posterior portions of the optic thalami, shows the tumor to have reached its limit in this direction. Occupying the pulvinar of the left thalamus is a cyst filled, as were many of the others, with a jelly-like mass. Anteriorly, the growth reaches only slightly beyond the anterior extremity of the nucleus caudatus.

Both lateral ventricles are moderately dilated, the left somewhat more than the right, particularly in its descending horn. The centrum semiovale is symmetrically reduced in size; the corpus callosum is flattened and the fornix difficult to follow in its relation to the corpus callosum. The basal ganglia of the right side have suffered somewhat through pressure. It is altogether probable that the tumor had its origin in the optic thalamus or corpus striatum of the left hemisphere. These ganglia are almost completely replaced by the new growth. Nothing corresponding to the caudate nucleus is to be made out; the putamen of the lenticular nucleus only is visible with the claustrum and external capsule. The optic thalamus is wholly converted into tumor. On the right side the ganglia have suffered only through pressure.

To summarize the macroscopic appearances: We have a dense discrete tumor replacing the basal ganglia of the left hemisphere, associated with extensive cyst formation and consequent distortion both of the cavities and the parenchyma of the brain.*

Methods. After hardening in formalin sections were taken from several portions of the growth which forms the basis of this study, and prepared in various ways for special staining methods. For purposes of comparison two other brain tumors were particularly studied, one a rapidly growing glioma with proliferation of connective tissue—already alluded to—and the other a sarcoma, together with tumors already in my collection. A variety of staining methods has been used, of which the differential neuroglia stain of Mallory† is by far the most satisfactory. Slight modifications of the method as originally published have been made by its author,‡ which justify the following detailed description:

Small pieces of the tissue to be examined, from 2 to 5 mm. in thickness, are hardened in

1. Formol, 10% aqueous solution, 4 or more days.
2. Picric acid (saturated aqueous sol.), 4-5 days.
3. Directly into bichromate of ammonium (5% aq. sol.), 4-5 days.
4. 80% alcohol.
5. 95% alcohol.
6. Imbed in celloidin.
7. Cut, thin sections.

* For a tumor very similar in gross appearance see Oppenheim, Die Geschwülste des Gehirns, in Spec. Pathol. u. Ther., herausg. von H. Nothnagel, ix, I, 3, p. 5. Wien, 1896.

† Loc. cit.

‡ Mallory, *The Journal of Experimental Medicine*, ii, 532.

Staining. 8. Fasten section to slide with ether vapor out of 95% alcohol, and harden in 80% alcohol.

9. Stain with solution of aniline oil-gentian violet, 15-20 minutes.

10. Wash in salt solution.

11. I, K I, ½-1 minute.

12. Wash with water.

13. Dry with filter paper.

14. Aniline oil and xylol, equal parts. Several changes, blotting after each washing, until clearly differentiated.

15. Xylol, wash thoroughly 3-4 times.

16. Canada balsam.

It will be seen that this method is essentially the same in principle as that published by Weigert, with the advantage that it is somewhat less complex and therefore easier of execution. The difference in the two methods lies in great measure in the preliminary hardening and fixing, Weigert using a fixative, followed by a mordant and afterwards by a reducing agent, whereas Mallory employs a mordant after the reducing agent.* The procedure of staining in both cases is a slight modification of Weigert's well known fibrin method. In the Mallory method, however, no intensifier is used, for which Weigert employs chromogen, the danger being that with the use of an intensifying agent structures other than neuroglia may stain, *e. g.*, neuraxon processes.†

In this investigation Mallory's method has been used to the exclusion of Weigert's largely as a matter of convenience, since the two are identical in their results. The value of the method, over others hitherto employed in the study of neuroglia, lies in its delicacy, in its absolutely selective action, and in its property of staining nuclei and neuroglia fibres to the exclusion of the protoplasm of the neuroglia cells. Such a combination of attributes in a single method has not hitherto been attained; its significance to pathological histology can hardly be overestimated. What it has already done for normal histology Weigert's exhaustive monograph has shown.

Other methods used are those already familiar in neuropathological work, with the addition of one recently published by Mallory.

1. Mallory's phospho-molybdic-acid-hæmatoxylin.
2. Van Gieson's hæmatoxylin-picric-acid-fuchsin.

* For details, see the respective publications of Weigert and Mallory, to which the references have already been given.

† Weigert has himself recently stated that in addition to the glia fibres, fibrin and degenerated neuraxon processes may stain. He does not, however, admit that normal neuraxon processes may do so also, which is possible when an intensifying agent is used.

3. Weigert's myeline-sheath-copper-hæmatoxylin method, with the use of the quick hardening solution following formalin (10%).

4. Alum-hæmatoxylin-eosin.

5. Mallory's phospho-tungstic-acid-hæmatoxylin.*

Each of these methods has given information of value, and has likewise demonstrated its shortcomings as applied to the investigation of a tissue to which its use is not essentially adapted.

Histological Examination. Examination with low power shows the mass of the tumor to be made up essentially of finer and coarser neuroglia fibres, irregularly associated in bundles of varying size, giving the general appearance of a dense fibrous tissue (Plate XLVII, Fig. 1). The fibre bundles are in many places seen to be cut transversely or obliquely, and in general do not lie predominantly in any one plane. Throughout the entire tumor spaces are visible between the various fibre bundles, varying in size and everywhere more or less completely filled with a homogeneous material and desquamated cells of somewhat uncertain character and origin. They are probably endothelial and large and small mononuclear lymphoid cells. It is probable that the spaces referred to are lymph channels, whose relation to the vessels is inconstant. Occasionally a capillary may be seen in the middle of such a space, but more often the spaces lie in the substance of the tumor quite free from and independent of contained vessels.

Vessels with thin walls, containing a small amount of blood, are numerous. They apparently bear no distinct histological relation to the surrounding proliferated neuroglia. In the gliomatous tissue proper, comparatively few nuclei are to be made out, when studied with a high power, and such as are present for the most part cannot be shown to bear any clear relationship to the greatly developed fibres (Plate XLVII, Fig. 2). The cellular element is absolutely in the background, the essential and striking feature of the tumor being the presence of fibres, whose number is out of all proportion to that of the cells. Careful study of individual fibres shows them to be coarsely wavy in outline, extremely like those forming a dense, coarse, fibrous tissue, and rarely showing sharp bends or angles in their course. In calibre there is much variation. There are many fine fibres, but nowhere so delicate as seen under normal conditions

* A valuable method recently published by Mallory in *The Journal of Experimental Medicine*, ii (1897), 531. The dye has a special affinity for neuroglia fibres, and stains also nuclei and to a slight extent protoplasm of neuroglia cells. Its results are more satisfactory in our hands than those gained by the phospho-molybdic-acid-hæmatoxylin method. Other uses than that for neuroglia do not at present concern us.

by the same method of staining. Very striking and characteristic are fibres of wide calibre, which are plentifully seen throughout the section and to which the epithet "hypertrophied" may properly be applied. Such coarse fibres are frequently seen to be undergoing retrograde changes suggesting hyaline degeneration, characterized by local swellings and slight alteration in staining reaction. The nuclei (neuroglia cells) of the tumor proper are, as already stated, insignificant in number; in shape they show a considerable degree of variation, an elongated form is frequent. Certain cells, not frequently met with, are of special interest. Studied by the phospho-molybdic-acid-hæmatoxylin and phospho-tungstic-acid methods, they show usually at one pole of an elongated cell body a process first of wide calibre and gradually narrowing toward its extremity, finally becoming a fibre similar in appearance to the differentiated form. So far as one may determine from this staining method such processes stand in direct relation to the cell protoplasm, and are therefore to be regarded as undifferentiated fibres. In many cases the difficulty of determining the relations of cells and fibres is extremely great. Typical "Astrocytes," as described by Stroebe, are not discoverable. In spite of the large size of the fibres and the ease with which each may be followed, no evidence whatever of branching is to be found. The tumor, excepting at its point of transition to normal brain tissue, contains no myeline fibres (Plate XLVIII, Fig. 3). Ganglion cells do not occur.

Microscopically, as well as macroscopically, the tumor is sharply circumscribed, though not encysted. Its character remains unchanged until within a short distance of its transition to normal tissue, excepting for the finer calibre of the neuroglia fibres. Indication of transition is given by the appearance of an occasional partially degenerated myeline fibre (Plate XLVIII, Fig. 3). A slight infiltration of the white matter occurs, but it is much less in degree than is ordinarily the case. Very striking, however, is the altered character of the neuroglia at this slightly infiltrated point. The fibres are of fine calibre and it is quite impossible to determine absolutely whether or not they stand in direct relation to the cell protoplasm. The appearances in some instances are exceedingly deceptive, and a most conscientious examination leaves one undecided as to the actual condition. Evidently the proliferation of the neuroglia is here actively going on, and, although we venture no dogmatic opinion, the appearances on the whole justify us in the statement that in many instances we probably have attenuated protoplasmic processes which are as yet undifferentiated in Weigert's sense. An exceedingly instructive picture is afforded by a thin section stained with phospho-tungstic-acid-hæmatoxylin, though we do not pretend to say that it gives conclusive

information in support of one or the other of the opposing views regarding the structure of the neuroglia (Plate XLVIII, Fig. 4).

In brief, we have microscopically a circumscribed tumor composed largely of newly formed differentiated neuroglia fibres without marked cellular proliferation, to which the diagnosis Glioma durum may be properly applied.

There are evidently certain points of special interest in this case, to which we here make brief allusion in view of a complete discussion to follow. 1. Its macroscopic appearance, circumscribed and associated with extensive cyst formation. 2. Its microscopic structure, dense, consisting chiefly of differentiated fibres, rather than of cells. 3. The presence in it of certain cells, possibly belonging to a transitional form. To the second and third of these points we shall devote particular attention.

Upon the general subject of glioma, the researches of the past few years have thrown much light, and that from various directions, so that a statement of our present position on certain disputed points is desirable. Embryological research and new staining methods have together done much toward overthrowing old conceptions and bringing a degree of scientific exactness into our knowledge of normal and pathological neuroglia.

The recognition through the studies of W. His, Cajal, von Lenhossék and others of the ectodermal origin of the neuroglia has of necessity led to a fundamental change in our conception of that tissue, both in its normal and pathological conditions. In speaking of those pathological processes peculiar to the nervous system, *e. g.*, the so-called scleroses, the term connective tissue should be absolutely dropped as unnecessary and misleading in its significance, in that it suggests a tissue of mesoblastic origin. The small part which mesoblastic connective tissue plays in the reparative or compensatory processes occurring in the central nervous system is by degrees being recognized, and the use of the term in describing pathological changes is less often, though still frequently, met with. We must clearly understand that neuroglia, from the histogenetic point of view, has no affinity whatever with connective tissue, and also the second fact, upon which Weigert insists, that its function for the nervous system is precisely that which belongs to the connective tissue

in the case of other organs. With these two points in mind, much of the vague phraseology everywhere met with might easily be avoided. Until the neuroglia is recognized as an epiblastic tissue, possessing, however, the functional and anatomical significance of mesoblastic connective tissue, we shall hardly reach that accuracy in speaking of the pathological changes in the nervous system which is so clearly desirable.

Our purpose in bringing up this point is a definite one, with particular reference to a matter which is ripe for discussion, viz., the relation of the gliomata to the sarcomata, or, put more broadly, the relation of epiblastic new growths to mesoblastic new growths. It is clearly evident that there is much confusion both of a theoretical and practical sort regarding these two classes of tumors. The confusion has arisen from two chief sources; first, the failure to recognize clearly the epiblastic origin of neuroglial tissue, and, secondly, the inadequacy of staining methods largely in vogue, *e. g.*, hæmatoxylin-eosin, to determine histological distinctions of a fundamental sort between neuroglia and connective tissue, or between the cells of gliomatous and sarcomatous new growths. So long as the development of the nervous system remained unstudied it was natural that the neuroglia should be regarded as an ordinary connective tissue of mesoblastic type, and as a consequence that pathologists should include the gliomatous tumors among those of the connective tissue class. Hence the inevitable confusion which has prevailed and still exists to a certain degree regarding a proper classification. Particularly noticeable is this fact in the continued use of the term "glio-sarcoma" as descriptive of a mixed growth, in which both gliomatous and sarcomatous elements are conceived as growing side by side. Such a nomenclature was clearly natural when neuroglia was regarded merely as a connective mesoblastic tissue of peculiar character. With the growth of our knowledge concerning its epiblastic origin the anomaly of a mixed mesoblastic and epiblastic tumor, bearing the name glio-sarcoma, becomes apparent, and has already been the subject of considerable discussion; nevertheless the term glio-sarcoma still maintains its place in most of the text-books as a definite form of new growth.

It will be of interest to pass quickly in review some of the expressed conceptions of gliomatous and so-called glio-sarcomatous tumors by pathologists of recognized authority.

Orth,* after speaking of gliomata, glio-sarcomata and sarcomata as distinct forms of tumor of the brain, goes on to say: "Ist nur das Faserwerk vorhanden, so liegt ein reines Gliom vor, sind viele freie Zellen dabei, so wird man ein Gliosarcom diagnosticiren."

* Orth, Pathologisch-anatomische Diagnostik, pp. 116, 117. Berlin, 1894.

Thoma* lays stress upon the epiblastic origin of the gliomatous tumor, but admits the possibility of such tumors going over into quickly growing glio-sarcomata: "in rasch wachsende Gliosarkome übergehen."

Birch-Hirschfeld† allows the term glio-sarcoma and distinguishes sarcoma from glioma, "durch rascheres Wachsthum, reichliche Entwicklung der zelligen Elemente und bedeutendere Grösse derselben"—evidently not differences of a fundamental sort.

Ziegler‡ faces the difficulty and after criticising the term glio-sarcoma as ordinarily used, says: "Ein wahres Gliosarkom kann dadurch entstehen, dass im Gliom eine perivasculäre, adventitielle Zellwucherung auftritt deren Product einen integrirenden Bestandtheil der Geschwulst bildet."

Stroebe§ admits the possibility of mixed forms, but would limit the term glio-sarcoma, as Ziegler does, to gliomatous tumors with sarcomatous growth starting from the blood-vessels. Such a distinction as that made, for example, by Birch-Hirschfeld, he would discard.

Without discussing the matter at length, Oppenheim|| admits the occurrence of mixed tumors in which sarcomatous tissue and gliomatous tissue alike appear. Starr¶ likewise uses the term. Strümpell, on the other hand, in the last edition of his "Specielle Pathologie und Therapie," makes no mention of glio-sarcoma as a distinct form of tumor.

Evidently from the few examples above cited the term is one deeply rooted in the literature and regarding which much confusion exists. Few writers are willing to give it up entirely, though in certain cases modifying it to a degree which amounts to its complete renunciation (Ziegler, Stroebe). As popularly employed and used by writers on classification of tumors the word is manifestly unfortunate and misleading. Lenhossék** states clearly the absurdity involved when he says that from the histogenetic point of view—and that is the only one we may properly use in such a matter—it is impossible that a sarcoma should at the same time be a glioma, since their cells are differently derived, in the one from mesoblast and in the other from epiblast. It is conceivable that a glioma and a sarcoma might grow side by side, but we have no reason to suppose that this ever actually occurs, nor do we have any analogy to such a process in other forms of new growth.

Considering the inadequacy of previous methods to determine the true

* Thoma, Lehrb. d. path. Anatomie, Th. I, pp. 661-663. Stuttgart, 1894.
† Birch-Hirschfeld, Lehrb. d. path. Anatomie, Bd. ii, pp. 332-334.
‡ Ziegler, Lehrb. d. path. Anatomie, Bd. ii, p. 363.
§ Stroebe, op. cit.
|| Oppenheim, op. cit., p. 10.
¶ Starr, Brain Surgery. New York, 1893.
** Lenhossék, op. cit., p. 245.

character of the component cells of such growths, and also the pathological anomaly involved in the term, we are justified in excluding the class of so-called glio-sarcomata from the brain tumors. In Ziegler's and Stroebe's sense, the term may be admitted, viz., glioma with sarcomatous elements derived from proliferation of perivascular connective tissue, but with the old confusion still in our minds it would unquestionably be better to drop the term entirely, as Strümpell apparently has done. Unquestionably the essential character of a new growth in the brain, as elsewhere, is either epiblastic or mesoblastic, and it is certainly conducive to clearness, in the present state of our knowledge, to speak of it only from the point of view of its characteristic histological element.*

Reference has already been made to Stroebe's detailed paper on glioma cerebri. Its interest and importance lie chiefly in the use of a special method of staining, Mallory's phospho-molybdic-acid-hæmatoxylin, and the somewhat far-reaching conclusions drawn therefrom. The method as originally given out by Mallory† was designed chiefly as a neuroglia stain, but in no sense as a selective one, the protoplasm, nucleus, and processes of neuroglia cells staining sharply as well as other elements, *e. g.*, axis-cylinder processes. By its use a chemical differentiation of neuroglia fibres, as attained by Weigert's and Mallory's modified fibrin stain, was clearly impossible. As a result of the sole use of an inadequate method, Stroebe finds the neuroglia fibres to be, in every case, processes of neuroglia cells and places himself in direct opposition to Weigert, who maintains the definite disassociation of fibres and cells in developed neuroglial tissue. At a discussion in the pathological section of the Naturforscherversammlung,‡ held at Frankfurt in September, 1896, Stroebe and Weigert have reiterated their distinctive views and are still opposed on this most fundamental histological point.

As is well known, previous to Weigert's monograph the general conception of the neuroglia was one which the Golgi methods had done much to strengthen, namely, that neuroglia fibres were direct outgrowths of cells, and in no way to be regarded as an intercellular substance. The various diffuse staining methods had tended to make such an idea unassailable. With the appearance, however, of Mallory's and Weigert's publication of a differential stain for the neuroglia an entirely new light was thrown upon the matter, leading to Weigert's dogmatic position of the disassociation of fibres and cells in adult neuroglia. He bases his atti-

* A similar position has been taken by Eurich. *Brain*, spring and summer, 1897, 114.

† Mallory, *Anat. Anzeiger*, vi (1891), 375.

‡ *Centralbl. f. allg. Path. u. path. Anat.*, vii (1896), 864.

tude chiefly on the fact that since the protoplasm of neuroglia cells does not stain and the neuroglia fibres do, a chemical difference of a fundamental sort must exist between cells and fibres, and therefore that fibres reacting in a definite way to a chemical stain cannot stand in the relation of processes to protoplasm which reacts in an entirely different way. Stroebe cleverly answers this argument by saying that because we have Nissl granules, for example, or neuraxon processes staining differently chemically, we do not therefore maintain that they are not integral parts of the cells in which they lie or from which they proceed. In such a case there is no thought of an intercellular substance. Unquestionably this point is well taken, and Stroebe's further suggestion is also pertinent, that the question of spatial (*räumlich*) relation of cells and fibres is of importance, a relation upon which Weigert's method throws no light, since one element, viz., the protoplasm, necessary for the determination of such spatial relations, remains unstained. This relation the phosphomolybdic-acid-hæmatoxylin method of Mallory supplies, Stroebe maintains, and that by it the existence of fibres as processes of cells may be established. It is quite beyond the scope of this paper to enter into the various arguments and facts which Weigert advances in support of his view, nor can we discuss Stroebe's position at length. We must content ourselves with as brief a statement as possible of facts which are accepted, and also of those which remain a subject of dispute.

We may regard as established that: 1. There is a definite chemical difference between neuroglia fibres and the protoplasm of the neuroglia cells. Such a conclusion the Weigert-Mallory method does not permit us longer to doubt. 2. There is an enormous development of fibres in adult neuroglia, which bear no constant relation to the cellular elements. 3. Whatever the relation of cells and fibres may be, the neuroglia acts within normal and pathological conditions as a connective tissue, in spite of its epiblastic origin. To Weigert chiefly we owe the definite establishment of these facts.

Points still in dispute are: first and most important, the relation of fibres to cells; and, secondly, if fibres are completely differentiated (Weigert), how and at what stage of their development do they become so; and as a corollary, do we find cells which may be regarded as transitional forms? It is not necessary to reiterate what a study of published articles on the subject forces upon us as established. Our purpose is rather to take up in certain detail the points recog-

nized as still doubtful in the light of the somewhat unique tumor of which we have already given a description, and also of another growth which we must likewise include in the class of gliomata. Allusion has already been made to features of particular interest, in the case whose description has been given. Of chief significance for our present discussion is the enormous development of fibres, as compared with cells. In this fact alone unquestionably lies a strong argument in favor of Weigert's view. It is difficult to conceive that fibres of such numbers and density of arrangement and calibre could, from a mere mechanical point of view, be processes of the cells found in their midst. Nor would Stroebe's argument hold here, that fibres apparently passing by cells may have had their origin from distant cells, since in this tumor we have a circumscribed growth, in no part of which cells preponderate sufficiently to give such a view a reasonable foundation. One's unavoidable impression in studying the growth histologically is that the fibres are essentially distinct from the cells, and that thus far we have a substantiation of Weigert's dogmatic view.

But just here a further question arises, which, so far as it is possible for us to learn, has received much less attention than its importance warrants. Admitting with Weigert that the essential feature of the neuroglia here developed is fibres, does it necessarily follow that all the fibres are differentiated, and represent therefore an intercellular substance? Does not Weigert take a step beyond the knowledge furnished by his method when he makes so sweeping an assertion? May there not, even in developed neuroglia, be a certain number of neuroglia elements, whose fibres are still in a relation of physical continuity with cells? If such a possibility be admitted, as we believe it must be, is not Stroebe entirely right in maintaining that Weigert's method is entirely incapable of giving us that information? Must we not appeal to a more diffuse method, in which protoplasm is also stained, in order that a physical continuity of fibre and cell protoplasm may be established, if such exist?

Stroebe, using chiefly Mallory's phospho-molybdic-acid-hæmatoxylin, while admitting as a possibility the complete differentiation of fibres, is sceptical that it exists to any considerable degree, and is tenacious of the old idea of cellular processes. Why is not a middle position between the two extremes as represented by Weigert and Stroebe reasonable and

probable? For the establishment of such a conservative position the demonstration of transitional forms of cells is required. About such cells peculiarly little has, up to this time, appeared in the literature. There certainly is no *a priori* argument against the possibility of the occurrence in the same growth or in different growths of differentiated fibres and fibres still in relation to the cells as direct processes from these cells. That Weigert's method does not demonstrate such transitional forms does not argue against their existence, since the method confessedly could in no case prove their existence. In short the proper investigation of the question demands the use of more than one method, in the same sense that a study of nerve fibres demands the employment of a neuraxon stain combined with a myeline stain. In justice to Weigert it is to be stated that his work heretofore has dealt largely with normal adult neuroglia, in which he regards the disassociation of cells and fibres as complete. Any departure from this rule applies only to embryonic periods of growth. At some time in the development of neuroglia Weigert therefore tacitly admits the association of cells and fibres as cell processes, but passes over any discussion of the manner of their differentiation. In pathological conditions just here lies a point of much practical and theoretical importance. In a growing tumor we should certainly expect to find cells with associated fibres in conjunction with fibres absolutely differentiated. Such transitional forms for Stroebe have little significance, since he does not admit an ultimate differentiation; by Weigert they are passed over without detailed discussion as embryonic cells which have no place in adult neuroglia. On page 106 of his monograph, for example, he says, speaking of the opinion of previous writers, "dass die Neuroglia nur aus Zellen und deren Fortsätzen besteht so trifft dies beim Menschen nur für die Embryonalzeit zu."

Taking a position between these two extreme views, it seems altogether reasonable to suppose that in a slowly growing tumor we should find both differentiated and undifferentiated fibres. For the investigation of this question the tumor above described is peculiarly adapted, since in just such a growth, with an enormous preponderance of differentiated fibres, we should be least likely to discover cells of a transitional type. If found, therefore, we should be justified in attaching a distinct significance to their presence.

A painstaking study of sections with the end in view of discovering such cells yields, in our opinion, positive results, though, from this

growth alone, not so conclusive as might be desired. Weigert's recent method, as Lenhossék and Stroebe have shown, is valueless in this investigation, since its function is to stain differentiated fibres. Mallory's phospho-molybdic-acid-hæmatoxylin and the most recent phospho-tungstic-acid method, also of Mallory,* are, on the other hand, suitable, since they color protoplasm in addition to nuclei and fibres. Careful search with high powers shows in those parts of the tumor where the development of fibres is less dense an occasional elongated cell, somewhat pear-shaped (see general description of microscopic appearances), from one pole of which comes off a process, gradually tapering in calibre and staining with phospho-molybdic acid, in a way similar to the protoplasm of the cell body. The conclusion is justified that such fibres are direct outgrowths of the cells. It is possible and probable that such processes would not stain by the Weigert or Mallory fibrin method, owing to certain chemical peculiarities. It is certain that their relations to the cells could, in any case, not be demonstrated by its use. Typical astrocytes, as described by Stroebe and admitted by Weigert to exist in rapidly growing gliomata, we are unable to detect. These cells, which we think it reasonable to speak of as transitional forms, are rather unipolar or bipolar in character.

In such a tumor as the one hitherto discussed, evidently slow growing and made up chiefly of differentiated fibres, these transitional forms, if such they be, are infrequent, as we should expect them to be. It is therefore of much interest to turn to the study of a tumor (Case II) belonging to the same general class, but of a distinctly different type, viz., a rapidly growing glioma with undifferentiated fibres and proliferation of perivascular connective tissue.

A nuclear stain—hæmatoxylin-eosin—shows the tumor to be made up of cells closely packed and varying to a considerable degree in size. The larger cells are easily seen to be associated with the vessel walls; they are evidently proliferated, and karyokinetic figures are frequent. The mass of the growth, on the other hand, is made up of smaller, densely crowded cells, not distinctive in character by this method of staining. The use of Mallory's modified fibrin method affords absolutely no infor-

* *The Journal of Experimental Medicine*, ii (1897), 531-2.

mation regarding the presence of neuroglia fibres, nor does Mallory's phospho-molybdic-acid-hæmatoxylin in an unteased section. On teasing a small bit of the tumor, however, and then staining with phospho-molybdic acid, cells of a strikingly interesting sort are revealed (Plate XLIX, Figs. 5 and 6). They are small, provided with a fair amount of protoplasm, from which usually, at opposite poles of the elongated cell body, come out processes tapering to fine fibres, which form a close-meshed network without discoverable anastomosis. There can be no question that these fibres are direct outgrowths from the cell body, nor is it open to reasonable doubt that they represent undifferentiated neuroglia fibres. None of the cells, however, are of the Deiters' "spider" variety.

This second tumor, therefore, is a rapidly growing, cellular glioma, with undifferentiated fibres; or, to use a convenient, if not a scientifically exact term, a glioma made up of cells of a transitional type. Differentiated fibres in Weigert's sense, as intercellular substance, are not discoverable and undoubtedly do not exist. That the fibres described as cell processes might later have become differentiated and then stained by the Weigert method is probable. This tumor,* therefore, is to be regarded as belonging to the text-book variety usually described; it derives its interest for the present discussion from the study of its individual cells in teased preparations. It will be seen that the cells of which this growth is primarily composed resemble closely the few cells already described as occurring in Case I. This is a matter of significance and justifies the conservative position which we have already indicated as, in our opinion, the correct one. In short, it may be thus stated: in glioma, neuroglia consists of cells with undifferentiated fibres and of cells together with differentiated fibres, the predominance of differentiated fibres depending chiefly on the age of the growth.

In other words it is not possible to dogmatize as to the relation of fibres and cells in any given case, since a range of variation is clearly possible between the extremes represented by Stroebe on the one hand and Weigert on the other. Case I is an almost conclusive argument in favor

* This is, in general, a variety of tumor to which the term glio-sarcoma has frequently been applied.

of Weigert's view. Case II, just alluded to, is equally suggestive of the correctness of Stroebe's conception.

It is hard to see why it is so difficult to conceive of the evolution of neuroglia from cells without processes to cells with processes, and then to cells whose processes have been completely differentiated into fibres. Could this be accepted as the natural history of the process, the controversy would seem to be at an end; that it is *a priori* a reasonable explanation is evident; that it is supported by a certain amount, at least, of anatomical evidence, the study of the foregoing varieties of gliomatous tumors indicates.

Admitting the correctness of this view, another point of interest at once arises, viz., the possibility of diagnosticating differentially the sarcomata and gliomata at all stages of their growth. The term glio-sarcoma has been sufficiently abused, as we have attempted to show. In the future, glioma or sarcoma or "unable to determine" should take the place of the straddling glio-sarcoma. In what cases, then, is it possible that a differential diagnosis may be difficult or impossible? Evidently in those, and only in those, in which the component cells have not attained a growth sufficient to designate them absolutely as glia cells, or, in other words, to differentiate them from connective-tissue or sarcoma cells. In the present state of our knowledge, and if the ideas already expressed be correct, we must admit that transitional forms may occur which might lead to confusion as regards the processes of the cells. We have already shown, following Stroebe, that Weigert's fibrin method is inadequate in this question of diagnosis, for determining the character of the cellular elements at certain stages of their growth, viz., while the fibres are still processes of the cells. The phospho-molybdic-acid, or better, the phospho-tungstic-acid methods render here, for reasons already given, more definite information. With a conscientious study of any growth by the various means now at our disposal an error is not likely to occur. Essential for a diagnosis of glioma is the demonstration of cells and fibres—the fibres being either still associated with the cell protoplasm or differentiated from it, as in older forms of growth. In the latter case Weigert's and Mallory's recent methods can leave no doubt as to diagnosis, from their inevitable staining of characteristic fibres. In the former case those methods evidently do not prove the tumor to be other than glioma from their failure to stain fibres, since these fibres at this stage of their growth are only stainable by other methods. In a case where there is still doubt after the staining of cut sections, teasing is desirable and may, as in our second case, give the desired information. We are of the opinion that macroscopic appearances, although in general characteristic,

are nevertheless an unsafe guide, and that in doubtful cases recourse should always be had to the microscope. A complete and interesting discussion of this question from all points of view is to be found in Stroebe's already frequently quoted paper. In general, he has undoubtedly brought out the points of chief importance in the differential diagnosis. We must, however, take direct issue with him in one of the general conclusions to which his study has led him. After speaking somewhat in detail of the possibility of confusing with glia cells certain forms of sarcoma cell, and furthermore the possible confusion arising from the staining of fibrin threads, he concludes: " Immerhin ergiebt sich aus den letzt erwähnten Befunden die Forderung, dass zur Diagnose eines Glioms nicht nur die Anwesenheit eines Fasernetzes zwischen den Zellen beobachtet sein muss, sondern dass auch die Verbindungen der characteristischen Fasern mit den Zellen in Gestalt der eigenartigen, vielstrahligen Gliomzellen festgestellt sein muss." * In the light of our first case there can be no doubt that the above statement is incorrect. Characteristic in this tumor is the very fact which would lead Stroebe to exclude it from the class of the gliomata, viz., the presence of fibres which cannot be shown to stand in direct relation to the cells. But that the tumor is a glioma there can be absolutely no question; it is equally evident that the fibres do not stand to the cells in the relation which Stroebe holds as essential. In other words, we have a glioma with a preponderance of free fibres. Stroebe's error here, as we think elsewhere in his discussion, lies in the use of a method from which he draws too far-reaching deductions.

To a certain degree Weigert apparently agrees with Stroebe in his conception of the essential character of a gliomatous tumor. After speaking of " the fable of softened central gliosis," met with in the teaching of syringomyelia, and the general confusion arising from our previous ignorance of the marked collection of glia about the central canal, Weigert goes on to lay down the following broad distinction: " Die Verwirrung wurde noch dadurch vergrössert, dass man ' Gliose ' d. h. krankhafte Vermehrung der Neurogliafasern mit ' Gliom ' verwechselte. Bei den Gliomen sind die Gliafasern nicht vermehrt, sondern die Gliazellen. Ja nicht nur das, sondern diese letzteren verlieren zum grossen Teile die Fähigkeit, abgesetzte Fasern zu erzeugen, und *bleiben in ihrem ursprünglichen protoplasmatischen Zustande. Man darf sich daher nicht wundern, wenn man gerade in Gliomen e c h t e Deiterssche Zellen findet, wie im Embryo.* Das Verhältnis der Gliome zur Gliose ist also, wie das des

* Op. cit., p. 459.

Sarkoms zur entzündlichen Bindegewebswucherung oder wie zum Fibrom." *

Weigert is said to have previously expressed an opinion somewhat at variance with the above statement, in that he admitted that numerous glia fibres might be found in true gliomatous growths, an implication, at least, of an increase of fibres.† In his later statement on this point, already quoted, there is no room for doubt that he makes an essential distinction between glioma and gliosis, holding that the former shows an increase of glia cells and not of glia fibres, whereas in the latter condition there is a pathological increase of neuroglia fibres.

In the light of the histological study of the tumor which forms the basis of this paper, this position of Weigert's is completely untenable for the same reason that we believe Stroebe's view untenable. We are dealing in our first case with a tumor—a glioma—and not a gliosis, and yet unquestionably we have a "krankhafte Vermehrung der Neurogliafasern," which, according to Weigert, is characteristic only of gliosis. On the other hand the glia cells are not increased, which again, according to Weigert, should invariably occur in glioma. Hence in this tumor we have what Weigert would term a gliosis, evidently an absurdity, since it is inconceivable that a glioma should at the same time be a gliosis, if any distinctive meaning at all is to attach to either term. The only possible position for us to take is one quite opposed to Weigert's, in that we fail utterly to recognize his distinction as fundamental. The teaching of the tumor under discussion unquestionably is that a pathological increase of neuroglia fibres is not inconsistent with the term "glioma." It is to be mentioned that at the discussion between Weigert and Stroebe at Frankfurt‡ the former is reported to have made the following statement: "Es sei zuzugeben, dass in Gliomen ausser den Zellen auch die Fasern vermehrt sein können," with the implication that such fibres do not readily stain owing to the imperfection of the method. In any case here is a direct contradiction of the previous statement quoted that the glia fibres are not increased in glioma. We are therefore at a loss to know what Weigert's real position in the matter is, since he has apparently modified the unequivocal and dogmatic statement contained in his monograph. Admitting the incorrectness of the first statement, a further detailed criticism of the assertions following is hardly necessary. There can be no doubt, judging from our tumor, that in glioma the glia cells

* Loc. cit., p. 156-157. The italics are Weigert's.

† Quoted from Lubarsch-Ostertag, Ergeb. d. allg. Path. u. path. Anat., Abth. ii, 1895, p. 339.

‡ Loc. cit.

are capable of producing differentiated (*abgesetzte*) fibres, and that they distinctly do not in all cases remain in their original protoplasmic condition. The presence of true Deiters' cells is, according to our view, possible, but that the tumor should be formed wholly of such cells as Weigert implies does not follow, and in our case is not true.

Finally Weigert's analogy that the relation of glioma to gliosis is similar to that of sarcoma to inflammatory connective-tissue growth or to fibroma is inexact. The glioma under discussion, for example, has a much closer resemblance to a fibroma than to a sarcoma. In short Weigert's too dogmatic distinction does not hold. Though in general true, it is incapable of serving as a fundamental means of distinguishing glioma from gliosis. Whatever other points are doubtful, of this the study of the tumor before us can leave absolutely no question. Weigert has but added to the prevailing confusion by asserting the existence of a distinction, of which he himself has later become doubtful, and which we must certainly regard as totally unessential to the real question at issue.

It is not our purpose to speculate as to fundamental pathological distinctions in the present imperfect state of our knowledge regarding the neuroglia, but the suggestion is unavoidable that here as elsewhere in the nervous system we may find that the whole matter is much simpler than we now suppose, and that the various conditions which we conveniently designate by separate names may in ultimate analysis be the same process manifesting itself diversely, as excited by causes which lie quite beyond the present field of inquiry. In short, it is much more profitable to study what glioma and gliosis, for example, have in common, than to seek for distinctions which analysis is likely to show to be not fundamental. Clinically, glioma, as a new growth, may be advantageously distinguished from gliosis as evidently giving rise to an entirely different symptom-complex, but to maintain such distinctions pathologically is altogether detrimental to progress, unless histological evidence bears them out. As yet we have no adequate evidence which permits us to assert that gliosis and glioma are sharply differentiated processes; they are evidently in many respects closely analogous and, so far as our knowledge at present reaches, their differences lie rather in their methods of growth than in any fundamental histological peculiarities.

Whatever of value the foregoing discussion may have lies in two chief factors: first, in the peculiar histological structure of the tumors studied; and secondly, in the varied methods used in their investigation. As the first case goes far to substantiate Weigert's general

view, so the second case, less completely described, is equally strong evidence in favor of Stroebe's conception. Conversely, however, the first case is subversive of Stroebe's idea, as is the second, partially at least, of Weigert's. The two tumors, therefore, are of particular interest from the fact that they show neuroglia proliferation at two distinct phases of growth hitherto, we believe, undescribed in tumor formation. It is a perfectly logical deduction, if not an absolutely demonstrable fact, that the differentiation of neuroglia fibres occurs in the course of growth, if, as in these cases, we can show with a fair degree of certainty that at one stage, presumably an early one, the fibres are still associated with the cell protoplasm as processes, while at another and later stage they are completely differentiated and become neuroglia fibres in Weigert's sense. Any other hypothesis of the differentiated fibres would imply, as Dr. Mallory has suggested to me, that at some period of growth the elongated protoplasmic processes cease to exist as such and disappear, to give place to other and separate fibres of unknown origin. Unquestionably the idea of a gradual differentiation is more consistent with nature's economy, as well as with certain facts of observation which we have attempted to bring forward. It is evident that the transitional forms are of the utmost interest and importance in the establishment of such a view; and just here we find, as before suggested, the gap in our knowledge which the work of the future should fill; it is hoped that this investigation may have at least the effect of indicating a profitable line of investigation.

The views of Stroebe and Weigert evidently represent the horns of a real dilemma, whose solution must depend upon a careful weighing of the evidence by all the means at our disposal. Even when that is done, it is probable that our ultimate conclusions must in a measure rest upon other data than those of direct observation. For the rigid adherent of either Weigert's or Stroebe's views there will always be the possibility of escape, in that our present means of investigation do not permit of absolute dogmatism on either side. Nevertheless, in view of all the facts, we feel altogether justified in maintaining the conservative and natural attitude that the dilemma

probably, in last analysis, does not exist, since both its extremes are true in part but not completely. Such, at least, is to us the teaching of the pathological glia which we have described in the two tumors chiefly under consideration.

CONCLUSIONS.

The results and conclusions of the above investigation are:

1. The term glio-sarcoma should be dropped, as unscientific and misleading in its significance.

2. The problems regarding neuroglia demand varied methods for their adequate study.

3. With all the means at our command, the absolute determination of the relation of cells and fibres in individual cases remains difficult and at times impossible.

4. No criterion has yet been offered to determine a fundamental distinction between glioma and sarcoma (Stroebe); and secondly, between glioma and so-called gliosis (Weigert).

5. The development of neuroglia in all probability is from cells with protoplasmic processes to cells with differentiated and independent fibres.

6. Herein lies a possible reconciliation of the conflicting views concerning the ultimate structure of human neuroglia.

DESCRIPTION OF PLATES XLVI-XLIX.

Plate XLVI.

Frontal section—actual size—through tumor at its point of greatest development. Ventricular cavities are seen to be distinct from the cysts surrounding the tumor proper. Chief destruction has taken place in the left hemisphere.

Plate XLVII.

Fig. 1.—Section through tumor from Case I. Mallory's modified fibrin stain. Leitz oc. 3; obj. 3. Showing fibre structure; probable lymph spaces containing desquamated cells; preponderance of fibres over cells.

Fig. 2.—Section through same. Mallory's modified fibrin stain. Zeiss oc. 4; obj. $\frac{1}{12}$. Showing fibres varying in size and occasional nuclei of neuroglia and desquamated cells.

Plate XLVIII.

Fig. 3.—Section at edge of tumor from Case I. Weigert's rapid copper-hæmatoxylin method. Leitz oc. 3; obj. 5. Showing partially degenerated

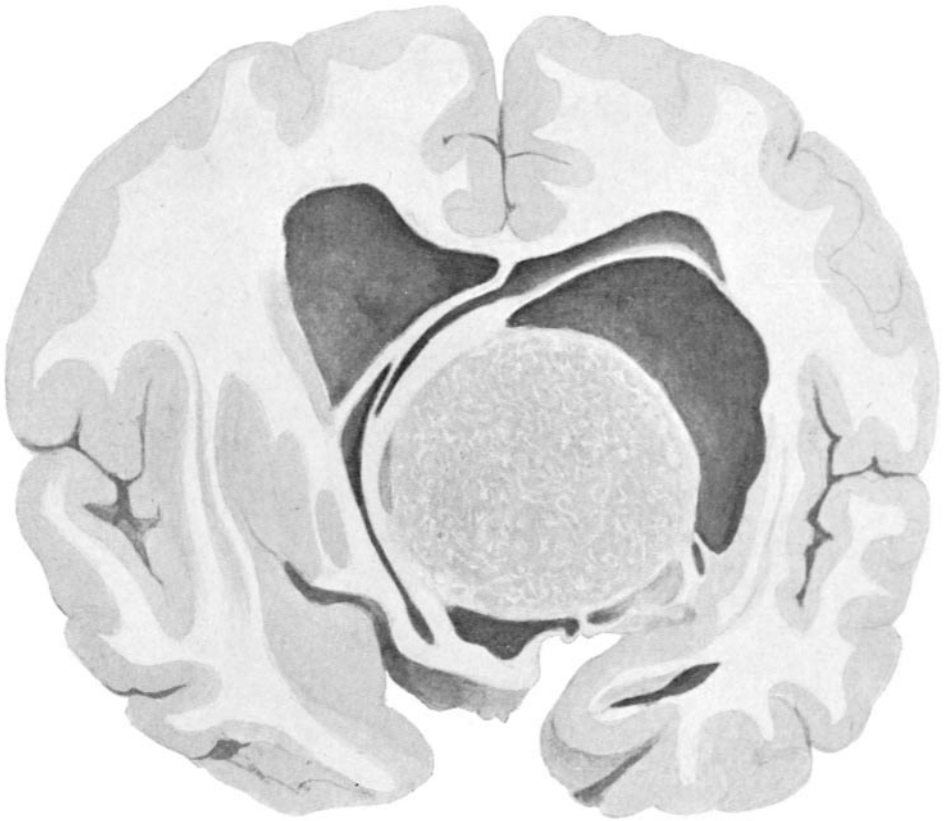
myeline fibres. The background represents the infiltrating gliomatous tissue.

Fig. 4.—Section at edge of tumor from Case I. Mallory's phospho-tungstic-acid-hæmatoxylin method. Zeiss oc. 4; obj. $\frac{1}{12}$. Showing neuroglia cells and fibres. In this section is shown the difficulty of stating dogmatically whether or not certain fibres stand in direct relation with cells. The fibres here are finer and the cells more numerous than in sections taken from the central portions of the growth representing an older phase.

Plate XLIX.

Fig. 5.—Teased preparation from Case II. Mallory's phospho-molybdic-acid-hæmatoxylin method. Leitz oc. 3; obj. 5. Showing cells with long processes and no differentiated fibres. The cells are for the most part unipolar or bipolar in character, and no doubt are young neuroglia cells.

Fig. 6.—Same. Zeiss oc. 4; obj. $\frac{1}{12}$. Selected cells, showing character of processes. It is to be noted that the nuclei are large proportionately to the amount of protoplasm.



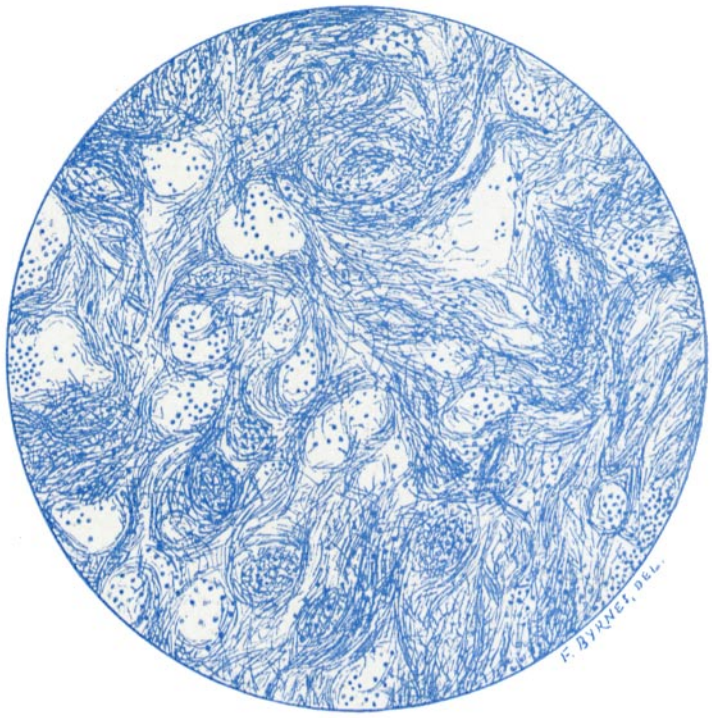


FIG. 1.

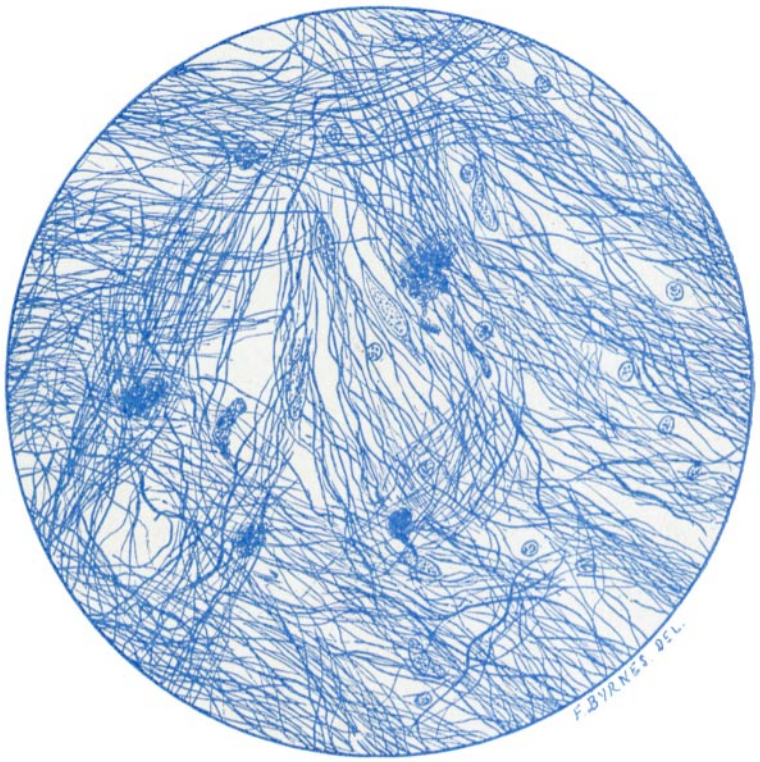


FIG. 2.

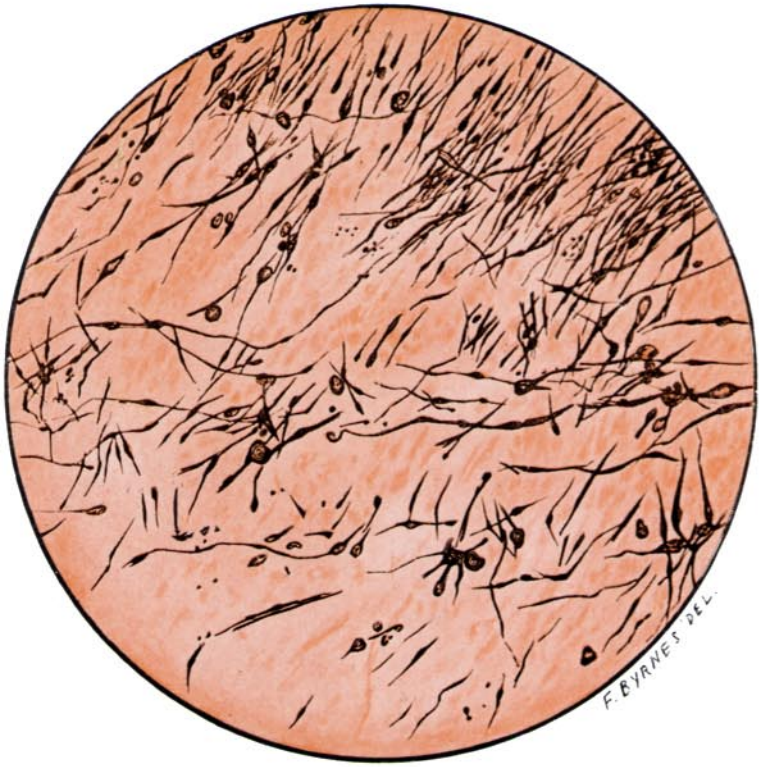


FIG. 3.

FIG. 4.

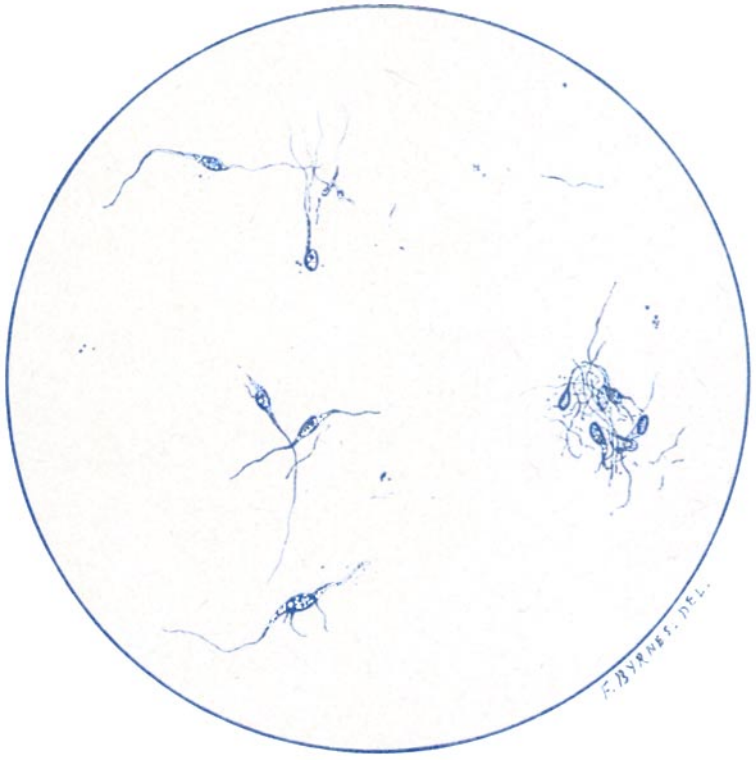


FIG. 5.

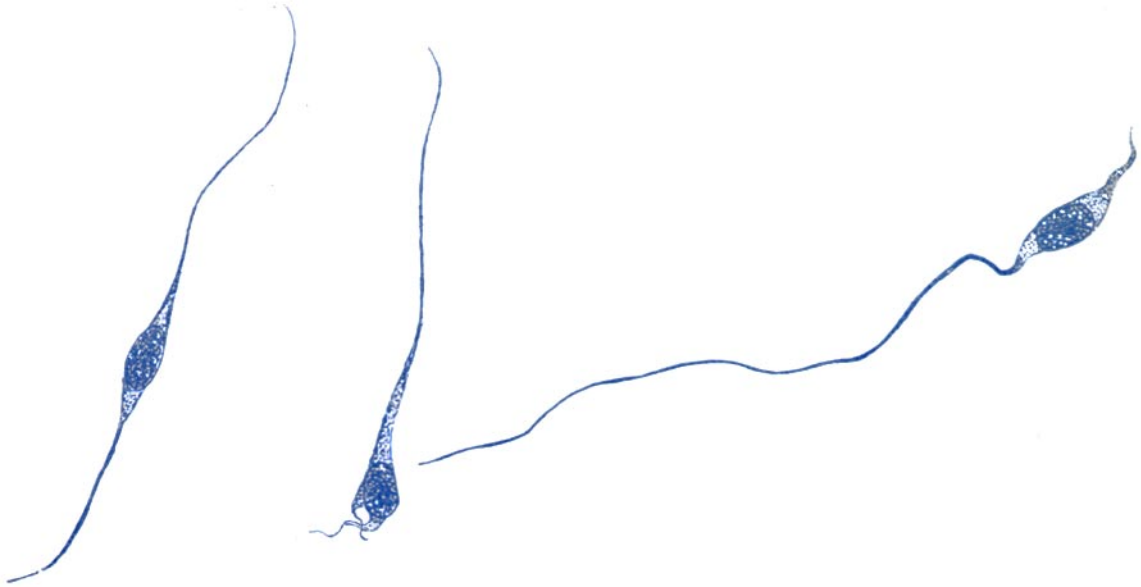


FIG. 6.