DIAGNOSIS OF INTRACRANIAL TUMORS BY SUPRAVITAL TECHNIQUE *

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With increasing satisfaction and confidence we have come to rely upon the supravital technique, devised by Sabin for the examination of the blood, as our most favored method for the making of pathological diagnoses of intracranial tumors. In this procedure the tissue is neither fixed nor frozen, but is simply prepared as a fresh smear of the living cells, and with experience immediate diagnoses can be secured which are often more dependable than those based on the study of stained tissue sections.

Our attention was first drawn to the possibilities of this method by Dr. Lawrence Kubie, who three years ago spent several weeks in the neurosurgical laboratory studying the cytological elements, more particularly the clasmatocytes, in the surgical specimens that happened to be supplied at that time. We have since then come to familiarize ourselves with the appearance of the tumors studied in this way and find that specimens, which have been prepared by the time-consuming methods in common use, fail in many instances to give the information we desire for purposes of classification. The method is of particular value in the differentiation of the gliomas into their various types, and believing that others will be interested in the subject, we propose to give a brief account of our experience with it.

Various methods for rapid microscopic diagnosis, particularly of malignant tumors elsewhere in the body, have been reported. Most of these, however, describe some process of fixation and the preparation of frozen sections. Hellwig [1] in 1926 advocated Wilson's method [2] of fixation in dextrin solution and staining of the sections with Unna's polychrome methylene blue, a procedure requiring about three minutes. They discovered that with the dextrin solution there was less shrinking and tearing of tissues than when formalin is used, and stated that individual cells were thereby more distinctly shown.

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Hoffheinz,[3] however, a year later expressed the opinion that the best results were obtained by the old method of quick fixation in hot formalin. He found that the dextrin solution caused sticky preparations which tended to form folds, and preferred the hematoxylin and eosin stain because it gave better color contrasts. Moreover, there was better preservation of the relationship of the various tissue structures. He concluded that the old method was a more dependable means of diagnosis, especially in obscure cases, justifying the five to ten minutes necessary for preparation.

Dudgeon and Patrick,[4] also in 1927, reported what they describe as a "wet-film method." The freshly-cut surface of the tissue is scraped and the juice thus obtained is spread on a slide and placed in Schaudinn's fluid for fixation. The films are then stained with haemalum and eosin. They allow eight to ten minutes for the complete process. In a series of 200 specimens they report successful results as compared with the control examination of sections prepared in paraffin by the usual technique.

MacCarty for years has used frozen sections of fresh unfixed tissue, stained with Terry's modification of Unna's polychrome methylene blue. In 1928 [5] he emphasized the importance of the cytological study of fresh unfixed material, and in a recent paper [6] states that the diagnosis of a malignant condition may be made from a single cell.

Taft and Ludlum [7] in a preliminary report have lately described a method for staining unfixed brain tissue with silver. The tissue is placed in argyrol, later washed in distilled water, and a film finally prepared.

In any method of quick diagnosis, the rapidity depends upon familiarity with a given procedure, the experience of the observer and the nature of the tissue. The morphological diagnosis of a tumor is occasionally very difficult whatever the method of preparation, and long and exhaustive study may be necessary before the final classification of the growth is possible.

Technique: The method, as stated, is that described in detail by Sabin [8] in her studies of living human blood cells. Its application to the more solid tissue of intracranial tumors is quite simple.

As soon as the tumor is exposed at operation, a fragment of it is routinely submitted for immediate examination. A minute portion is separated with dissecting instruments and placed on a glass slide.

The slide may be previously prepared with an even film of neutral red dye, or a mixture of neutral red and Janus green, the exact directions for which are given by Sabin. We have found that satisfactory results are obtained when a clean slide is used and a drop of aqueous solution of neutral red dye (1:10,000 or more) added directly to the tissue.

A cover glass is immediately placed over the tissue, which is carefully spread by gentle pressure on the cover glass, the rubber end of a pencil being convenient for the purpose. One learns by experience how much pressure to apply in making the smear. Some tumors are so soft that they spread almost like fluid, and anything more than the lightest touch damages the cells. Other tissues are very firm, fibrous or even gritty with calcium, requiring greater effort in flattening them out for a satisfactory film.

The preparation is sealed at once with a mixture of vaseline and paraffin around the edge of the cover glass, and is then examined microscopically in a warm box.

The actual preparation of the smear takes less than a minute.

There are one or two precautions which may be particularly commented upon at this point. If too strong a solution of the dye is used, the nuclei will be stained diffusely — a sign that the cells have been injured. The edges of the preparations are usually overstained in this manner. While overstaining does not necessarily so obscure a microscopic picture as to make a diagnosis uncertain, it must be avoided if one is interested in studying the cytological elements of these tumors.

Furthermore, the importance of making the preparations as soon as possible after the tissue has been removed should be emphasized whether or not an immediate microscopic report is imperative. A diagnosis may be made in most cases even though the smears are prepared an hour or several hours later, provided that the tissue has not been allowed to dry. However, there is no question but that the cells undergo changes on long standing, especially if the tumor is of a soft consistency. They become swollen and vacuolated, the cytoplasm markedly granulated, and the nuclei show degenerative changes. When extreme vacuolization occurs, one vacuole may fill the whole cell. In some areas cytoplasmic boundaries become unrecognizable, so that there is only disintegrated material to be seen.

As Kubie has pointed out in a study of the perivascular tissues of

the central nervous system by this technique,[9] preparations of solid tissue tend to be uneven. Some areas are too dense, and others too thin. But there are many fields in which the relations of the different structures are well preserved, and in the thinner portions individual cells which have become separated may be studied in minute detail.

In every case for comparison a portion of the same tissue is fixed to be subsequently sectioned and stained by the usual methods.

Advantages: Possibly the greatest value of the method herein presented lies in the opportunity it affords for the study of cells which have not undergone changes due to fixation or cutting, but which are seen under the microscope in their entirety while they are still living. The usual shrinkage and distortion of the tissues is thus avoided. Under high magnification (oil immersion) single cells, such as astrocytes and oligodendroglia, may be beautifully defined with all their delicate processes which are rarely to be seen intact in fixed histological sections. After fixation the cytoplasm is oftentimes destroyed, or in other instances it is demonstrated only by the use of special stains. An illustration of this is given in the photomicrographs of a recent oligodendroglioma (*cf.* Figs. 9 and 10) in which it may be seen how distinctly the cytoplasm of each cell is outlined in the fresh smear, while in the fixed preparations it has become changed beyond recognition.

Mitotic figures, too, may be identified in various phases in the supravital preparations of some of these tumors.

Not only are individual cells clearly shown, but, as has been pointed out, the relationship of the various structures in the tumor as a whole is well preserved, such as the formation of whorls and masses of cells in a meningioma, or the radiation of the cells of an ependymoma about a vessel and its branches, or the palisade arrangement of cells so characteristic of an acoustic neurinoma.

In invasive types of brain tumors myelin sheaths are often identified as curious irregular strands with bulbous enlargements and terminations sharply outlined by what appear as two closely parallel lines which are extremely refractive (*cf.* Fig. 6).

Collagen, too, has a distinctive appearance because of the refractive quality of its bands of wavy fibers.

Of particular interest are the histiocytes, which are frequently associated with such tumors as the rapidly growing gliomas, men-

ingiomas, and adenomas, and which are believed by Carrel [10] to influence growing cells by aiding in their nutrition. In these supravital preparations they may be observed during their activity to phagocytize the neutral red dye and store it in the digestive vacuoles within their cytoplasm. These vacuoles appear as round, highly refractive globules, which may increase greatly in size as they become filled with the inclusions of the dye. Clasmatocytes may be present in extraordinary numbers in a rapidly growing tumor like a medulloblastoma, showing evidence of great activity, or they may be scattered here and there, as in a pituitary adenoma, moving rather lazily among the tumor cells.

In some of the tumors there are other cells which contain highly refractive vacuoles in their cytoplasm, but which are non-motile and do not ingest the dye. These vacuoles are an evidence of cell degeneration. Such vacuolated cells are commonly found in acoustic neurinomas which are undergoing fatty degeneration, or occasionally in a meningioma they are conspicuous among masses of the tumor cells or within the whorls.

Diagnosis: In many cases the precise nature of the lesion is quite apparent to the operator from its gross appearance and location, as is usually true for example of such growths as the meningiomas. Under these circumstances the microscopic diagnosis is merely corroborative. On the other hand, there may be some uncertainty in the surgeon's mind as to whether, for example, an atypical soft vascular tumor exposed in the region of the sella turcica is a suprasellar meningioma or a pituitary adenoma, or whether a suspicious nodule apart from the main mass of tumor represents an implantation or metastasis, or whether a necrotic area consists possibly of degenerated brain in the neighborhood of the growth or actually is tumor.

Possibly the most important immediate differentiation that is needed is between the various types of gliomas, and although increased familiarity on the surgeon's part enables him to distinguish many of these tumors by their gross characteristics, this is only possible when he has become thoroughly familiar with its histological appearance. If the surgeon must wait for a week or two, or even for a few days before he can get a clear idea of the microscopic structure of the tumor he has seen at operation, his recollection of the gross appearance has necessarily become obscured. The immediate

knowledge of the cytological characteristics of a tumor is therefore of great value in teaching him to associate the histological picture with the gross appearance of the lesion. Furthermore it gives him an immediate indication of the prognosis so that he may modify his measures accordingly and determine what particular operative procedure to pursue and how radical or otherwise an extirpation of the lesion is desirable. Moreover if the growth is of a type benefited by roentgen-ray therapy, the latter may be instituted without delay.

This of course is less important with the tumors whose gross characteristics are better known, such as the acoustic neurinomas and the meningiomas, but even here operative difficulties of diagnosis may be encountered, a tumor, which was adherent to the dura proving histologically to be a carcinoma, whereas it was assumed to be a meningioma during the course of the operation.

Description of Tumors: A certain familiarity with the appearance of the different intracranial tumors in supravital preparations as contrasted with fixed preparations must be acquired before the various types of lesions can be distinguished with accuracy. A description of the microscopic appearance of the more common tumors when examined by supravital technique will therefore be given.

Gliomas: The gliomas represent over forty per cent of all intracranial tumors. The immediate differential diagnosis of the various types, as has been said, is important in indicating the prognosis and in determining the method of operative procedure. The three largest groups in the classified series are the astrocytomas, the glioblastomas, and the medulloblastomas, and they may accordingly be considered in the order of their frequency as follows.

Astrocytoma Fibrillare: When the tissue is quite tough and unyielding, and indeed it may contain calcium, the usual picture under the microscope is of a very dense network of neuroglial fibrillae which are rarely stained but appear as closely crisscrossing refractive processes, among which there are scattered nuclei. It is usually in the less dense areas along the margins of the preparation that the cytoplasm of the cells is most distinctly seen, and here one may find unmistakable astrocytes with their long processes extending in starlike fashion from the cell body, one of which not infrequently may be traced to a neighboring vessel.

Occasionally these tumors are soft in composition, the tissue spreading very easily. We have noticed especially in cases of such

fibrillary astrocytomas removed from the cerebellum in young children that one is likely to find instead of a dense feltwork of fibrillae an abundance of well preserved astrocytes throughout the preparation. The cells may be quite small with a few processes forming a delicate fibrillary meshwork. In Fig. 1 an astrocyte of this type is shown and particular attention may be called to the distinctness of the nucleus and chromatin particles, and the granules in the cytoplasm.

Astrocytoma Protoplasmaticum: The protoplasmic astrocytomas are usually soft. The most pronounced difference from the fibrillary type when examined microscopically is the general absence of neuroglial fibrillae. Furthermore they tend to undergo degenerative changes and become invasive, and it is not unusual to see among the fairly large oval nuclei of the tumor numerous degenerated cells packed with vacuoles, and clasmatocytes which have taken up the neutral red dye. Nerve fibers identified by their refractive myelin sheaths may also be found here and there. In this respect they are similar to the glioblastomas.

If the specimen is not too degenerated, however, the cytoplasm as well as the nucleus may be discerned, and in Fig. 2 the soft branching processes of a typical astrocyte may be seen extending out in various directions for a considerable distance. The nucleus appears darker in the photomicrograph than it should because of the attempt to reproduce satisfactorily the delicately outlined processes which were only slightly stained.

How much more informing the supravital preparation may be when contrasted with the Zenker-fixed specimen from the same tumor stained with phosphotungstic acid hematoxylin is evident from Figs. 3 and 4, which have been taken at a low magnification to show the general architecture rather than the cell type of the tumor.

Glioblastoma Multiforme: These rapidly growing tumors are composed of cells of various size and shape, including spongioblasts of all forms, astrocytes, round and spindle-shaped cells, which in a favorable piece of tissue are easily identified in the fresh smear. Multinucleated cells are present and mitotic figures may be demonstrated. However, many of these lesions are degenerated, and if the tissue examined is from a necrotic area such cells are not well shown, but instead the fields are occupied by great numbers of vacuolated cells which may be huge in size, filled with characteristic greenish

refractive globules which are often large. Active clasmatocytes ingesting the neutral red dye are also present (Fig. 5). Among them the nuclei of the tumor cells may be seen, and usually one finds scattered myelinated nerve fibers indicating the invasive nature of the growth.

It may be pointed out that occasionally a bit of tissue is submitted for diagnosis from the brain in the neighborhood of a tumor. Under these circumstances the myelinated nerve fibers (Fig. 6) present an unmistakable picture. At other times the tissue may come from the margin of the growth, and examination disclose neuroglial fibrillae and possibly astrocytes, which, however, represent a gliosis rather than the real tumor, so that a more representative piece of tissue is necessary in order not to confuse the diagnosis with that of a fibrillary astrocytoma.

Medulloblastoma: In Figs. 7 and 8 are shown for comparison the preparation by supravital technique and the preparation of the same tumor which had been fixed, cut and stained by the usual method, the nuclei being greatly shrunken and the cytoplasm practically indiscernible. These tumors are generally soft and should be spread gently. Examination by supravital technique shows a most distinctive picture of a rapidly growing exceedingly cellular tumor, composed of masses of small round cells with round or oval nuclei containing a fair amount of chromatin. The predominating type of cell found in the fresh preparations as seen under oil immersion (Fig. 7) is round rather than carrot-shaped. The cytoplasm is pale in contrast to the nucleus, which sometimes may be a little eccentric, but the cytoplasmic boundary is very well defined, as is evident in the photomicrograph. Mitoses in various phases are easily recognized and may be numerous with distinct chromosomes, as was true of this particular tumor from the roof of the fourth ventricle in a child. Occasionally spongioblasts and neuroblasts may be identified in these tumors, but in this case they were not present.

Hordes of clasmatocytes are often present in medulloblastomas and may be seen actively taking up the dye so that they stand out conspicuously among the tumor cells. We have already referred to Carrel's conclusions regarding their influence on the nutrition of growing cells, and in this particular tumor, which is obviously a very rapidly growing one, they were present in extraordinary numbers none happening to show in the photomicrograph.

Oligodendroglioma: Here again, in Figs. 9 and 10 the supravital preparation is shown in contrast with the fixed preparation of the same tumor stained by ordinary methods. These tumors are rare, representing only one per cent of the gliomas, so that only a few have been available for study by the supravital method of examination. When seen in the fresh smears they present a most interesting picture. Whereas in the fixed preparation (Fig. 10) the round though shrunken nuclei are seen, in no field of any section was the cytoplasm recognizable, though it may be noted that in some fixed preparations of similar lesions the nuclei may be surrounded by a halo. This may be contrasted with the fresh smear, which shows a typical field of the same growth at the same magnification (Fig. 9). The nuclei are spherical and unshrunken, containing quite abundant chromatin, and are surrounded by a pale cytoplasm which is very distinctly outlined. One may see in the photomicrograph how cellular the tumor is, but unlike the medulloblastomas, no mitoses are present. They are slowly growing tumors and almost invariably tend to undergo partial calcification.

The oligodendroglia are thought to develop from indifferent cells of ectodermal origin which may also differentiate into fibrillary astrocytomas. In a recent case of fibrillary astrocytoma of the cerebellum in a child of ten years, typical oligodendroglial cells were found in the supravital preparations of the tumor. In Fig. 11 two of these cells are shown with the slender processes characterized by swellings along their course and at their terminations. In fixed preparations the processes of these cells are not stained by usual methods, though they may be brought out by special silver stains as recently reported by Bailey and Bucy.[11]

Other Types of Gliomas: Of the remaining types of gliomas which form a smaller percentage of the classified group, one or two may be briefly referred to. There have been a few examples of ependymoma studied by this technique. They are highly cellular lesions and in the fresh smears the radiation of the cells around the vessels has been observed to particular advantage under low power, while under oil immersion extensions of the cells may be traced to the vessel walls. Blepharoplasten have not been distinguished in the tumors so far available for supravital study.

There have been two recent examples of spongioblastoma unipolare et bipolare. In Fig. 12 some of the individual cells are shown as they

appeared in the fresh preparation. They lie more or less parallel to one another, this arrangement having been quite consistent throughout all fields.

Pituitary Adenomas: The differential diagnosis between the chromophobe and chromophile types of pituitary adenomas is possible on examination of the supravital preparations. The chromophobe lesions are composed of masses or cords of epithelial cells which are round or polygonal in form with round or oval nuclei containing a variable amount of chromatin and usually well marked nucleoli (Fig. 13). The cytoplasm is very finely granular. Clasmatocytes are commonly found though they are generally not numerous and may be comparatively inactive. It may be noted that in the case of the youngest patient in the series with chromophobe adenoma, a girl who was first operated upon at the age of ten years, mitoses were observed in the fresh preparation of the tumor at a second operation five years later.

In the chromophile type the alpha granules which in fixed preparations are brought out best by special stains, such as Bailey's ethyl violet-orange G, are readily seen in the fresh smears as rather coarse granules most marked at the periphery of the cytoplasm. Multinucleated cells may be numerous and crescent-shaped cells are also found. There is considerable variation in the size of the cells. A rather striking picture was observed in a recent case of "fugitive acromegaly" upon examination of the fresh smear. Everywhere among other comparatively pale, delicately granular chromophobe cells there were great numbers of enormous cells which were especially conspicuous in appearance because of the very granular cytoplasm which stained deeply with the neutral red dye (Fig. 14). The nuclei were round with a prominent nucleolus, and some of the cells contained two nuclei.

Meningiomas: These tumors as a rule are unmistakable on examination by supravital technique. They vary in consistency from very soft to very firm lesions. One sees masses of cells with large nicely outlined oval, round or elongated nuclei which have a singularly typical appearance. Nucleoli are usually prominent. Whorl formation is well preserved in the fresh smears as may be seen in the photomicrograph which is taken at the same magnification as that of the fixed preparation of the same tumor (Figs. 15 and 16).

Even when calcium is present a smear satisfactory for diagnosis

may be obtained. The psammoma bodies under low power are conspicuous round refractive bodies, and when examined under oil immersion nuclei within some of these concentric concretions may be identified.

Occasionally there is evidence of degeneration, and in one of our recent cases the cytoplasm of the tumor cells within and outside of the whorls was filled with very fine greenish globules.

Clasmatocytes are associated with some of the meningiomas, as has been pointed out, and they may be observed ingesting the neutral red dye.

Although in most cases the gross appearance of a meningioma leaves no doubt in the surgeon's mind as to its histological nature, in rare instances the gross features may be misleading. On a recent occasion, for example, a tumor which was adherent to the dura and was regarded as a meningioma proved by supravital preparation to be a carcinoma.

Acoustic Neurinomas: The acoustic neurinomas are usually firm in composition. If the tissue is well preserved and cellular, the fresh smear shows oval or elongated nuclei running along in fibrillary strands in the palisade and occasionally whorl-like arrangement which is typical of these growths. The cytoplasm of the cells in such fields is ordinarily not well defined, but the general architecture of the tumor is unmistakable.

Frequently the tissue submitted has a yellowish fatty appearance, and when examined by supravital technique it shows the characteristic arrangement mentioned above, but the cytoplasm is more distinct because it is packed with fine greenish refractive vacuoles. The cells are commonly elongated and spindle-shaped, with fibrillary extensions at their extremities. When the tumor is extremely degenerated, however, many of the cells show only the nuclei, with globules of fat trailing along the fibrillary material present.

Conclusions

The supravital technique has been adopted as the most favored routine method of diagnosing and classifying tumors of the central nervous system, it being of particular value in the cytological differentiation of the various types of gliomata.

Not only can an immediate diagnosis be given to the surgeon so that he may learn to associate the microscopic type of the lesion

with its gross appearance at the operating table, but a permanent photographic record of the fresh preparations can be made for comparison with the permanent section of the fixed tissue.

The supravital method makes it possible for the examiner to see the cells with their cytoplasm and processes intact and gives pictures which are wholly unfamiliar to those who have only studied these cells in fixed sections.

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DESCRIPTION OF PLATES

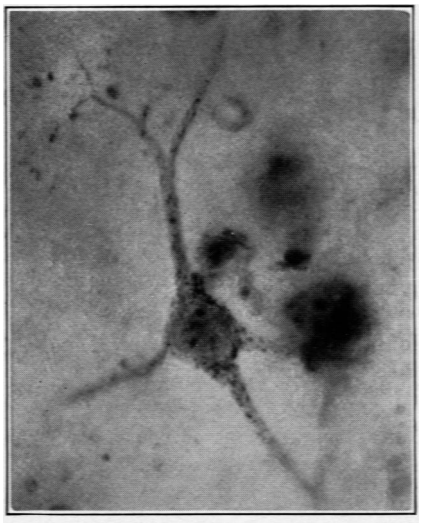
PLATE 114

FIG. 1. Supravital preparation showing a single fibrillary astrocyte. Note the distinctness of the granules in the cytoplasm. × 850.

FIG. 2. Supravital preparation showing a single protoplasmic astrocyte. Note the numerous soft branching processes. × 850.

FIG. 3. Supravital preparation of an astrocytoma. For comparison with Fig. 4. × 300.

FIG. 4. Zenker-fixed preparation of same tumor as in Fig. 3 (phosphotungstic acid hematoxylin stain). × 300.



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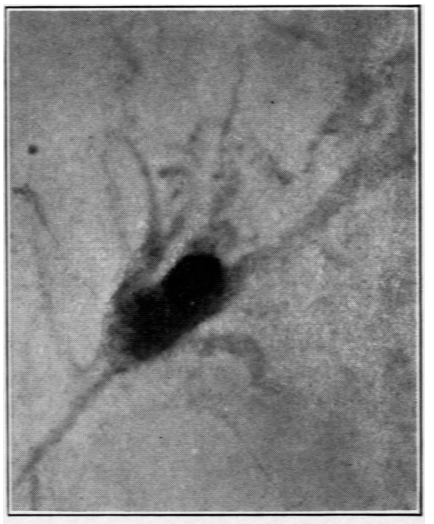

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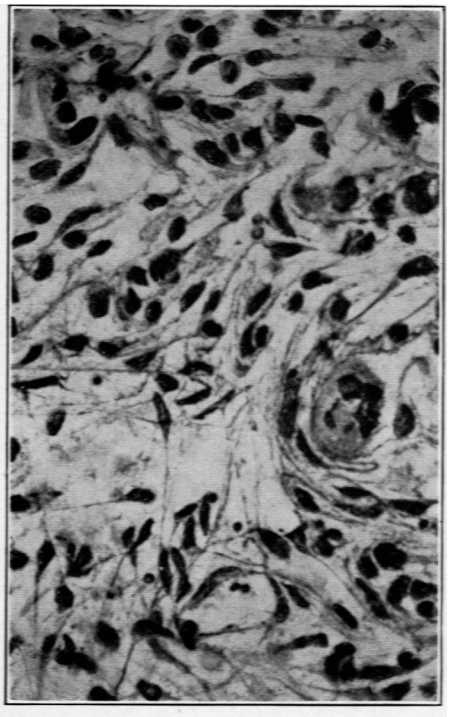

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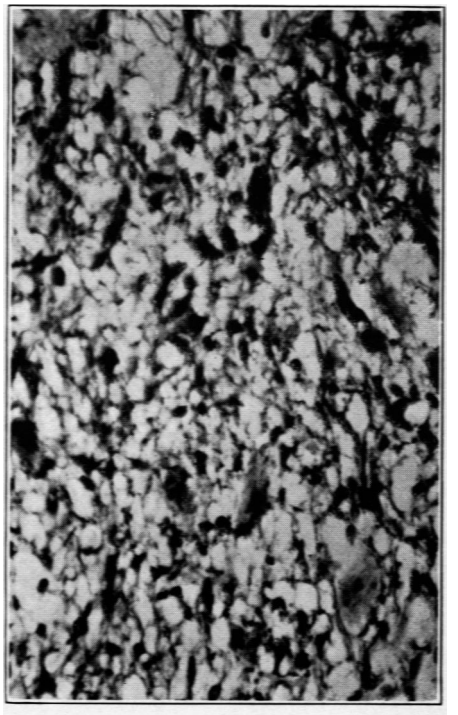

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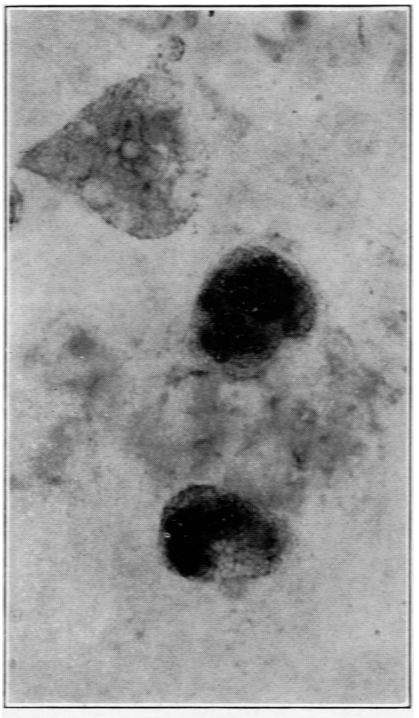
PLATE 115

FIG. 5. Supravital preparation showing vacuolated cells in a rapidly growing glioma. The upper cell is inactive, while two histiocytes below have ingested particles of neutral red dye. × 600.

FIG. 6. Supravita. preparation of fragment of brain to illustrate the typical appearance of myelinated nerve fibers. They are highly refractive and are often found among the cells of an invasive tumor. × 600.

FIG. 7. Supravital preparation of a medulloblastoma showing clearly defined cytoplasm (arrows) and uniformly round shape of cells. Two mitotic figures are included in the field. × 850.

FIG. 8. Formalin-fixed preparation of same tumor as in Fig. 7 (hematoxylin-eosin stain). The cytoplasm has been almost wholly lost and the nuclei are greatly shrunken. × 850.



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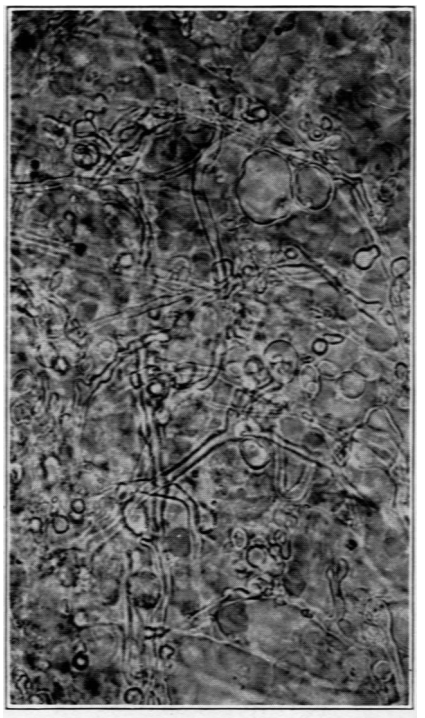

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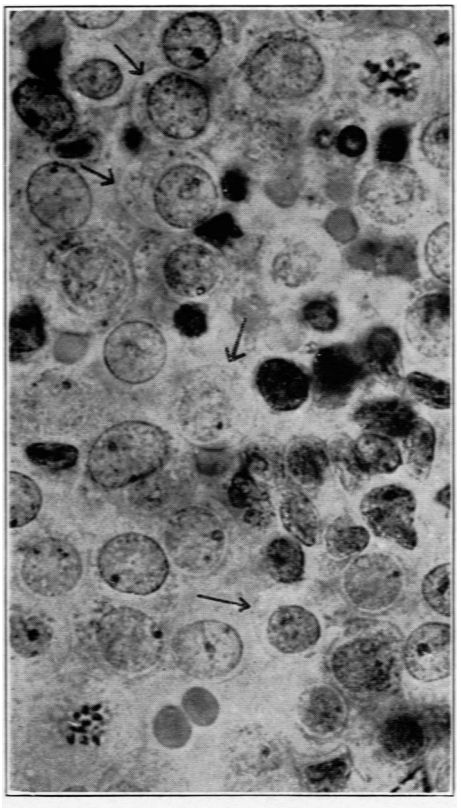

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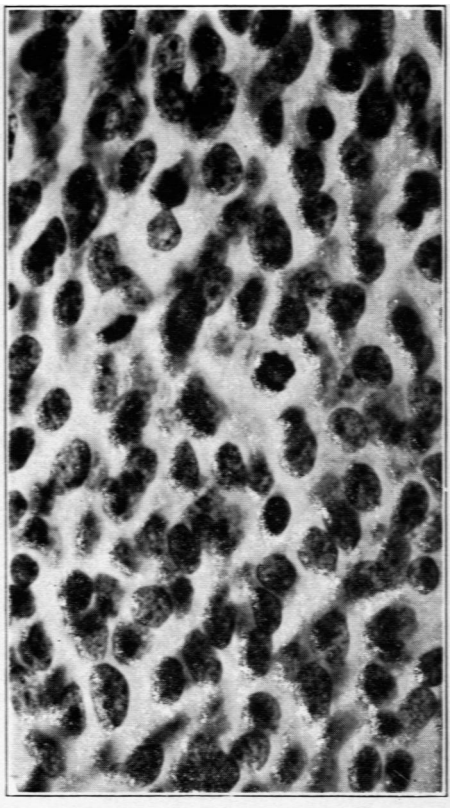

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PLATE 116

FIG. 9. Supravital preparation of an oligodendroglioma to show the sharply outlined cytoplasm of the cells and their spherical nuclei. For comparison with Fig. 10. × 850.

FIG. 10. Zenker-fixed preparation of same tumor as in Fig. 9 (eosin-methylene blue). The cytoplasm was not recognizable in any field of several sections studied. The nuclei are much shrunken. × 850.

FIG. 11. Supravital preparation showing two isolated oligodendroglial cells from a midline cerebellar fibrillary astrocytoma, with typical swellings along their processes. × 850.

FIG. 12. Supravital preparation of a spongioblastoma unipolare et bipolare, showing several of the individual cells in characteristic parallel arrangement. × 850.

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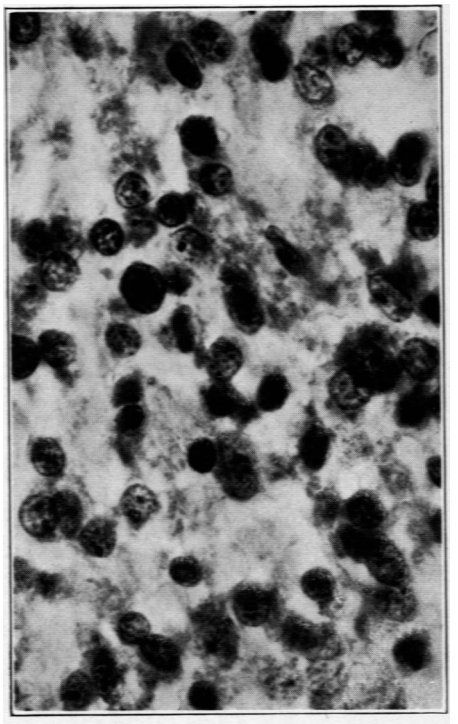

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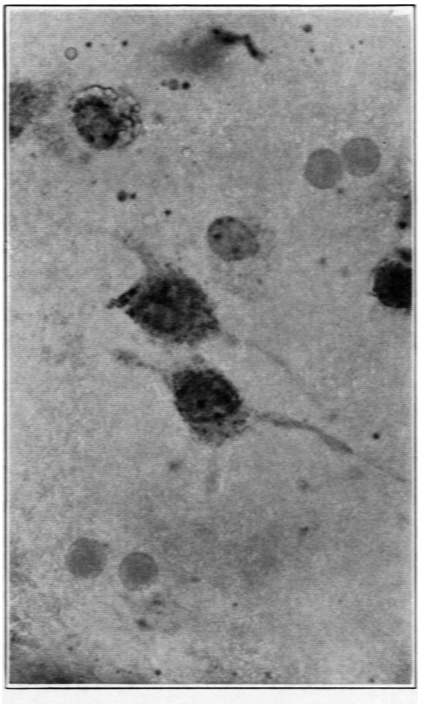

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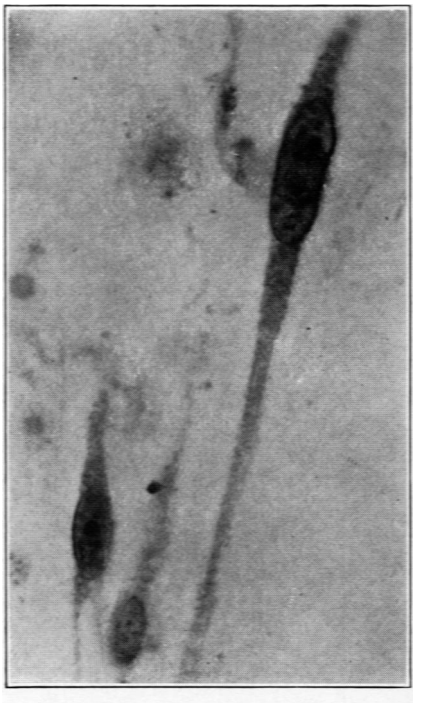

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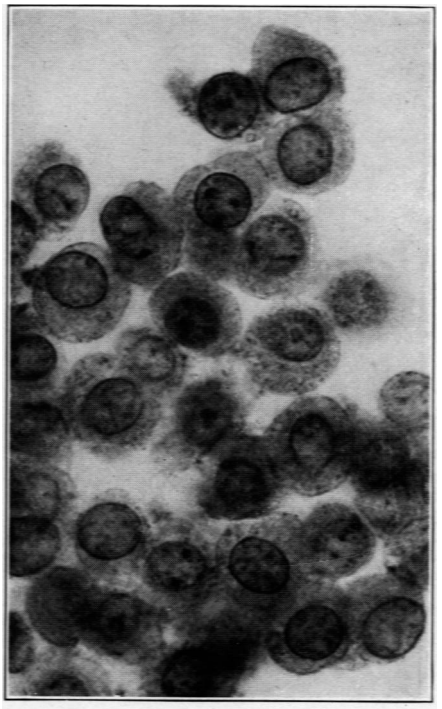
PLATE 117

FIG. 13. Supravital preparation of a chromophobe pituitary adenoma showing a group of cells of about the same size with very finely granular cytoplasm. × 850.

FIG. 14. Supravital preparation of a pituitary adenoma of mixed type. Note the size of the cells as compared with those in Fig. 13, and the granular cytoplasm stained by neutral red. One cell with two nuclei is shown. × 850.

FIG. 15. Supravital preparation of a meningioma to show how well whorl formation is preserved. × 600.

FIG. 16. Zenker-fixed preparation of same tumor as in Fig. 15 (eosin-methylene blue stain). × 600.



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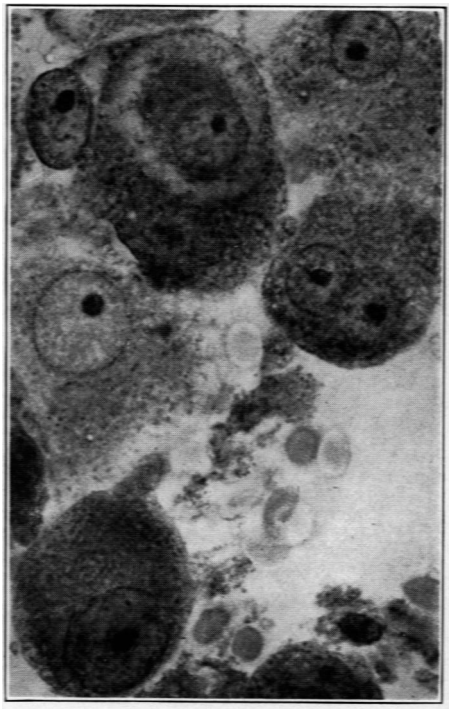

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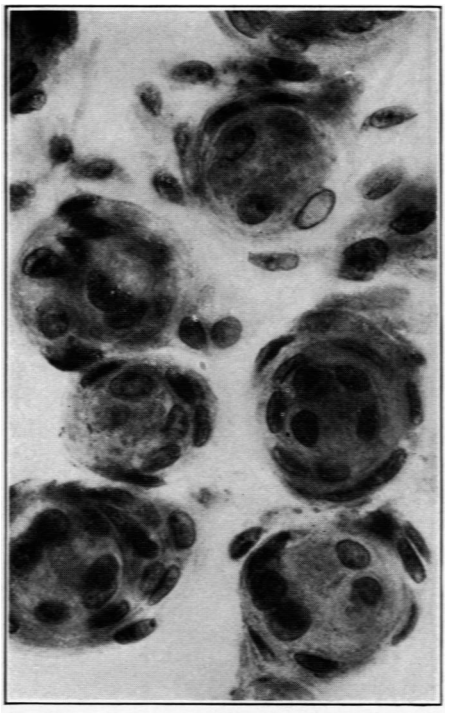
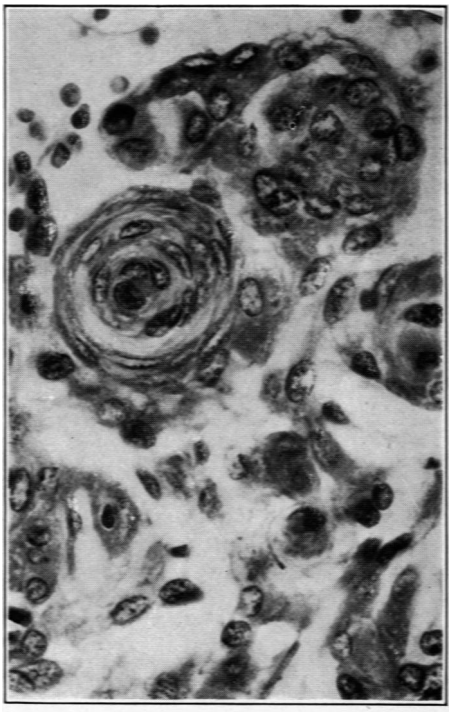


15



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