DEFINITIVE treatment of cerebral tumors has been standardized by accurate quick histological diagnoses. Though a majority of cerebral tumors can be diagnosed by their gross appearance, it is of considerable importance to the surgeon to know the tumor type prior to removal of the neoplasm. The pathologist is forever trying to improve upon the accuracy of the diagnosis and at the same time enable methods to remain within a practical reach and understanding of the surgeon. In order that a method may fulfill the latter purpose it must be rapid, provide good staining differential qualities, be adaptable to handling tiny bits of tissue, and stain all of the constituents of the tissue. It is of course desirable that any rapid method be comparable to the standard preparations commonly used.

It is the purpose of this paper to describe a simple method of rapid diagnosis of cerebral tumors which can be used in the operating room. The tissue can be ready for the surgeon to examine within 30-40 seconds following his own preparation of the smears made at the operating table between two sterile glass slides.

The technique of using smears of bits of tissue for the diagnosis of various lesions is not new. It has been described for the diagnosis of brain tumors by Badt,1 Russell,4 Deery,2 and Dudgeon and Patrick.3 It has probably been used in every pathological laboratory. The stain described here is superior to any one I have ever used for quick histological diagnosis, and was developed by Dr. William Reid, and used at the Montreal Neurological Institute.

TECHNIQUE

As soon as the tissue is removed, a tiny fragment is placed at one end of an ordinary glass slide, and a drop of normal saline solution is added. A second glass slide with its long axis perpendicular to the first slide is then inverted over the fragment. Gentle pressure on both slides is then applied with the thumb and index finger of the same hand, mashing the tissue between the two slides. After little experience the proper pressure necessary to obtain a satisfactory preparation will be acquired. With a firm hold on the uppermost slide, this slide is quickly drawn down the long axis of the lowermost slide, thus making a smear of the fragment, just as one would in preparing a smear of gastric juice, exudate, or blood. It is recommended that several slides be prepared from the same fragment and from different fragments of the same specimen. The slides are then allowed to dry in air or over a hot plate.
The staining method is as follows:

1. Place in eosin solution for 5 to 10 seconds. This solution is prepared in the following manner:
   - Eosin (water soluable) .................................... 1 gm.
   - Potassium bichromate .................................... 1 gm.
   - Distilled water .......................................... 100 cc.
2. Wash carefully in tap water.
3. Dehydrate with acetone-alcohol from dropper bottle.
   Use:
   - Absolute alcohol ...................................... 1 part
   - Acetone .............................................. 5 parts
4. Wash carefully in tap water.
5. Stain in methylene blue solution for 10 to 30 seconds. Tumor tissue requires a shorter time, usually about 10 seconds. The preparation of this solution is often difficult, and the success of having a good concentrated stain depends on carrying out the directions carefully:
   - Prepare a solution of
     - Methylene blue ........................................ 1 gm.
     - Potassium carbonate ................................... 1 gm.
     - Distilled water ........................................ 300 cc.
   - Boil the solution for 10–15 minutes. Add 3 cc. of glacial acetic acid, drop by drop with a pipette, shaking the solution rather vigorously. Continue to shake until the precipitate dissolves. Finally boil the solution until a concentrated solution of 100 cc. remains.
   - Allow to cool slowly. This stain is stable for at least a year, if not longer.
6. Wash carefully in tap water.
7. Dehydrate with acetone-alcohol solution from dropper bottle, using 1 part absolute alcohol and 5 parts acetone.
8. Dehydrate with chloroform from dropper bottle.
10. Mount in Canada Balsam.
11. Cover with large cover glass.

The preparation is then ready to be studied under the microscope. The actual preparation of the smear can be made in less than 1 minute in a large number of instances.

The results of the staining reveal blue nuclei, slight grey to pink cytoplasm, brilliant pink collagen, pink glial fibers, blue intracellular phagocytosed particles. In one instance a metastatic brain tumor from a melanoma of the eye revealed blue intracytoplasmic granules. The stain gives an excellent differentiation between the various structures listed. On several occasions I have been able to observe the intracytoplasmic fibrils in a piloid astrocyte. In another case, “gitter” cells could be easily differentiated from the tumor cells of the same size, because of the mesh-like cytoplasm in the “gitter” cells. These results were checked with fat stains on homologous smears. The smears may also be used for the routine frozen and chromatic techniques. Mucicarmine and glycogen stains have been particularly successful.

Certain alterations in the staining times will result in distinctive preparations. For example, if perineurial fibroblastomas are left longer (25 to 30 seconds) in the eosin solution, and if meningeal fibroblastomas are left exactly 8 seconds in the eosin solution and 12 seconds in the methylene blue
solution, the results will be far more gratifying. With experience and after examination of the first stained preparation, one will be able to judge the staining times with considerable advantage.

The fastness of the stain varies. The preparations are good for at least 3 years. If the smears are re-studied the differentiation often will be clearer than the original more heavily stained preparation. For example, smears of normal brain will fade more quickly than smears of tumors. When the normal brain smears fade, the neuroglial fibers become more prominent, and occasionally one will observe astrocytes fully impregnated, a demonstration not unlike Cajal's gold chloride sublimate method for demonstrating astrocytes in frozen sections.

The importance of making preparations as soon as possible is not as demanding as supravital preparations of unfixed tissues. The tissue when kept in an icebox can be accurately stained and interpreted as long as 3 days later. After the unstained slides are prepared, they may be kept and stained at any time, even years later.

The tissue when prepared by this method tends to be uneven. This, however, does not detract from the usefulness of the smear, but rather adds to it, for on a single slide it is common to have large areas of solid tissue, which are often equivalent to the cut frozen section, and small areas of isolated cells. Thus the relationship of the tumor proper can be deduced, and the isolated cytology offers an academic study as well as establishing its true form. For example in the ependymoblastoma the isolated cells appear as sperm-like objects (Fig. 1) with elongated tails, and in thicker areas as rosette-like clumps of cells, some arranged around the blood vessels.

**DISCUSSION**

*Advantages of the Technique and Staining Method.* The method is simple, requires very little time, and provides results comparable with the ordinary hematoxylin and eosin stain. Little specialized training is required for interpretation of the tumor types.

One can usually work accurately with the smallest fragment of the tissue. The following case illustrates the importance of accurate histological diagnosis on a tiny fragment of tissue, which was too small to allow a frozen section to be attempted:

A 46-year-old woman who had Jacksonian seizures for 2 years, was admitted for investigation. There were no neurological signs, except a questionable papilledema of the left eye. The CSF pressure was 220 mm. A ventriculogram revealed an expanding lesion of the left frontoparietal region. Plain X-rays of the skull were normal.

A left frontoparietal osteoplastic craniotomy was made and the rolandic fissure exposed. The bone flap had its superior margin placed within 8 cm. of the midline. A single widened gyrus and moderate increase in the intracranial pressure were the only positive findings. In view of the 2-year history of convulsions, the lack of plain X-ray changes, minimal neurological signs, and the finding of a single widened gyrus, which on stimulation was proven to be the motor area, the presumptive gross diagnosis of astrocytoma diffusum was made. If this
diagnosis was verified, no further surgery was necessary, since removal of this region would result in hemiplegia. A small (#18) biopsy needle was introduced for a distance of 4 cm. into the widened gyrus, where an increased resistance (moderate) was encountered. A tiny fragment was removed, one that was too small to risk cutting a frozen section. A quick smear was made and the diagnosis of "meningeal fibroblastoma" returned. The surgeon, reasoning that such a tumor in this region was commonly attached to the falx, enlarged the bone flap mesially towards the falx, and removed the meningeal fibroblastoma attached to the falx.

Fig. 1. Photomicrograph showing smear preparation of an ependymoblastoma of the cerebral hemispheres. The numerous elongated unipolar cells resemble spermatozoa.

Fig. 2. Medulloblastoma of the cerebellum. Note the small hyperchromatic cells and the large rosette.

from behind the motor area. The patient recovered from a temporary postoperative right hemiplegia within 12 days.

Entire discrete cells with their processes often can be studied (Figs. 1, 4, 6 and 8). In the piloid astrocytoma the numerous piloid fibers can be seen in large numbers (Fig. 6). The cells of the ependyblastoma resemble sperm cells with elongated tails (Fig. 1). This detailed cytological character of the tissue also serves to gratify the academic interest in the tissue.

The estimation of dedifferentiation of the tissue is better since in a cut section so many cells are out of plane of the section (Figs. 1, 3, and 7).

Proliferative blood vessels in malignant gliomas are often seen in great
lengths (Fig. 9). When parts of the proliferative vessels break off they appear as solid balls of cells (Fig. 9, A).

The method is of great aid in the selection of tissues for special study, such as tissue cultures and tissue transplants into laboratory animals.

A more accurate estimation of the number of mitoses is gained since the tissue is examined before many mitoses are completed, whereas even with rapid fixation of tissues a large number of cells in mitotic division are eliminated in the final paraffin or frozen section.

The unit of structure is sometimes revealed. The rosette (Fig. 2) of the medulloblastoma presents itself as an isolated structure, and the whorls of a meningeal fibroblastoma will occasionally be entirely isolated from the tissue proper.

It is beyond the scope of this manuscript to state all the advantages of the rapid methods used in the diagnosis of brain tumors since they are well known.

Disadvantages of the Technique and Staining Method. Russell states that certain tumors, such as the acoustic neurofibromas and some of the meningi-
Fig. 5. Smear preparation of a meningeal fibroblastoma (meningioma). Note the elongated fibroblasts with fine even fibrous fibers forming numerous sweeping whorls. The direction of the smearing process is not detectable.

Fig. 6. Piloid astrocytoma of cerebral hemisphere. Note heavily impregnated piloid fibers and small round nuclei.

Fig. 7. Photomicrograph of a glioblastoma multiforme of the cerebral hemisphere. Compare cell size to that of a red blood cell (A). Note pleomorphism of cells and presence of giant cells (B). Many of the nuclei reveal their chromatin granules.
Fig. 8. Smear preparation from margins of a hemorrhagic cyst. Note large astrocyte with its processes fully stained (A), and numerous clear cystic areas (B). The general mottled appearance suggests widespread degeneration.

Fig. 9. Photomicrograph showing endothelial proliferation in a glioblastoma multiforme. One vessel is shown in cross section (A) and is completely closed by endothelial proliferation. Another vessel, shown in the longitudinal plane, demonstrates the marked thickness of vessels in the adjacent neoplastic tissue.

Fig. 10. Smear preparation of normal vessels of the brain for comparison with Fig. 9.
omas, are too tough to spread upon a slide. In my experience this difficulty has not been encountered. Some of the best preparations are obtained from these tumor groups (Fig. 5).

It is true that some of the tumors present more difficulties in smearing than others, and it is in this group that the most distortion results. The diagnosis, however, can usually be made from a few fringes of the tissue near the margins of the slide.

This method is not designed to replace the diagnosis of cerebral tumors by the well established differential staining methods, but is designed to provide a presumptive diagnosis during the operation.

Difficulties in the diagnosis will obviously arise depending upon the experience and training of the interpreter. In general the diagnostic difficulties which may have arisen have been no greater than those ordinarily encountered with any rapid method of diagnosis.

SUMMARY AND CONCLUSIONS

This report is based upon a personal experience with 116 tumors of the central nervous system. This new staining method, which utilizes smears or wet film preparations of central nervous system tumors, is simple, rapid, provides results comparable with the ordinary hematoxylin and eosin stain, requires little specialized experience for the interpretation of the tumor type, enables one to work accurately with the smallest fragment of tissue, often reveals entire discrete cells with their processes intact, provides a better differentiation of the tissue, and may reveal isolated unit structures for study. The diagnosis based upon study of smears is considered only a presumptive diagnosis, and should not replace the well established differential staining methods commonly used in the diagnosis of the tumors of the central nervous system.

REFERENCES