HISTOCHEMICAL STUDY OF LIPIDS IN INTRACRANAL TUMORS*

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In recent years pathologists have become increasingly aware of the necessity for using newer methods for the investigation of morphological problems. With the introduction of such methods as phase contrast microscopy, dark field, electron microscopy, and histochemistry, it is reasonable to hope that additional clarifying information can be obtained in the study of obscure pathological problems. The importance of histochemistry in pathology has been emphasized repeatedly.1,12,14 However, a histochemical approach to pathological problems has been little used, and by far the greater portion of the work in this field has been outside the realm of neuropathology.

It was felt that application of histochemical techniques might shed some light on questions arising from the study of intracranial tumors. Preliminary work in this laboratory showed that glial tumors collected over the years were not suitable for certain histochemical techniques, e.g., the study of nucleic acids. However, old formalin-fixed material gave satisfactory staining results in demonstrating lipids.

Review of the literature disclosed a paucity of information about the presence of lipids in gliomas. A review of the older literature dealing with this topic was given by Henschen.10 Recent investigations by biochemical methods brought to light marked differences of opinion regarding the importance of lipids in glial tumors. Cumings7 found only traces of phospholipids in gliomas and concluded that their presence was ascribable either to necrosis or to contamination with normal brain tissue. Selverstone and Moulton20 on the other hand found that the values for phospholipids in gliomas, while lower than the values of normal brain tissue, were still high enough to have a basic significance and suggested a metabolic as well as a structural function for lipids in gliomas.

The purpose of this paper is to study by histochemical techniques the lipids in intracranial tumors in regard to their location, and to their eventual formative influence upon characteristic histological features of these neoplasms. Further, it was felt that an approach to the problem of lipids in gliomas with the help of histochemical techniques might resolve the above-mentioned discrepancy of results obtained by biochemical methods.

MATERIALS AND METHODS

Thirteen autopsied cases of intracranial tumors were incorporated into this study. With the exception of Case 3 (FSH 2075), the autopsy numbers refer to postmortem examinations performed at the Grace-New Haven Community Hospital. Case 3 was autopsied at the Fairfield State Hospital (Table 1). None of these patients had received roentgen-ray therapy. Only those regions of the tumors showing changes pertinent to this study will be described in detail. All specimens were fixed in formalin, some for as long as 10 years, while others had been obtained only days before they were incorporated into this study. There were no detectable differences in the staining qualities of the tumors using histochemical techniques for demonstration of lipids, such as Nile blue sulfate and Sudan black.

Carbowax 1540, a water-soluble hydrocarbon, was used for embedding. Pieces of tumor were processed by washing briefly in water and then

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* None of the patients had received roentgen-ray therapy.

immersing them in Carbowax 1540 in dilutions of water of 70 per cent, 90 per cent, and 100 per cent for \(\frac{3}{4}\) hour, \(\frac{1}{2}\) hour, and 1 hour respectively, at a temperature of 50°C. The sections were then placed in 100 per cent Carbowax 1540 and refrigerated for 1 hour after which they were ready for sectioning with the paraffin microtome. Sections of tissue known to contain fatty material, e.g., fatty liver, were prepared in carbowax and compared with sections of the same tissue cut with the freezing microtome. No differences were observed in the staining of lipids between carbowax and frozen sections.

Carbowax 1540 was found to give excellent results, when consecutive sections were needed. When sectioning on the paraffin microtome, ribbons of tissue could be obtained. Hematoxylin and eosin stain was used to determine the fine cellular details of the tumors. Consecutive sections were examined for lipids using the Nile blue sulfate\(^{25}\) and the Sudan black\(^{23}\) techniques. The techniques for the demonstration of lipids were as follows:

Nile blue sulfate—Sections were washed briefly in water to remove the Carbowax 1540 from the tissue and then placed in Nile blue sulfate (saturated solution in water) for 15 minutes. They were then washed in water and differentiated for approximately 5 minutes in 1 per cent aqueous acetic acid. After another washing in water, the
sections were mounted in glycerine jelly.

Sudan black—After a brief washing in water to remove the Carbowax 1540, the sections were placed in 100 per cent propylene glycol for 5 minutes and then directly into Sudan black (0.7 per cent solution in propylene glycol) for 15 minutes. Sections were then differentiated in 90 per cent aqueous propylene glycol for approximately 5 minutes and mounted in glycerine jelly.

Menschik has recently reviewed the literature and carried out his own investigations of Nile blue sulfate technique as a stain for lipids. It has been demonstrated that this stain acts in two ways. “Neutral fats” absorb the red oxazone of this dye and stain red. These fats consist of saturated and unsaturated triglycerides, hydrocarbons, higher alcohols, esters, and waxes. “Acid” lipids react with the blue oxazine of this dye and stain blue. These lipids are free fatty acids, phospholipids, galactolipids, and chroomolipids. Nile blue sulfate, being a basic stain, also stains some protein blue. When doubts occurred concerning the lipid or possible protein nature of the blue-staining material, sections were controlled with fat solvents, e.g., acetone, alcohol. To check the sensitivity of Nile blue sulfate for phospholipids, smears of .001 per cent solution of commercially obtained lecithin were prepared. Even for such low concentrations of lecithin the Nile blue sulfate technique was found to be sufficiently sensitive.

Sudan black B was used during this investigation, since it has been demonstrated by Lillie to be one of the more sensitive stains for lipids. Therefore it was felt that Nile blue sulfate and Sudan black were ideally suited to this investigation: Nile blue sulfate for demonstrating staining differences in types of lipids and Sudan black for insuring that all lipids would be demonstrated and to control the results of the Nile blue sulfate staining.

All tumors were examined using a triple microscopic setup. That is, since sections were stained with the various histochemical dyes and hematoxylin and eosin in a consecutive fashion, it was always possible to locate identical fields in Nile blue sulfate, Sudan black, and hematoxylin and eosin preparations. In many cases the same cell could be seen in two of the three differently stained slides. Thus it was feasible to determine the exact location of lipids, both intra- and extracellularly, in the tumor sections and
Fig. 2. Case 1. Glioblastoma multiforme. Adventitial proliferation is present in one side of a vessel. At right side of the figure necrosis and pseudopalisading of tumor cells are shown. Hematoxylin and cosin, X440.

Fig. 3. Case 1. Glioblastoma multiforme. Consecutive section of slide shown in Fig. 1. There is concentration of fat in region of pseudopalisading. Sudan black, X154. (Sudan black stain was utilized for photomicrography for technical reasons.)
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Fig. 4. Case 1. Glioblastoma multiforme. Consecutive section of preparation shown in Fig. 2. Within the region of adventitial proliferation and in the vicinity of pseudopalisading lipids are demonstrated. Sudan black, ×440.

to identify the cellular elements in a precise manner in the consecutive section stained with hematoxylin and eosin.

RESULTS

Case 1. Glioblastoma multiforme (A10375). The tumor had the typical cytoarchitecture of a glioblastoma multiforme; necrosis and pseudopalisading were seen frequently (Fig. 1). The tumor cells showing pseudopalisading revealed many pyknotic regressive forms. Many of the blood vessels in the tumor showed marked adventitial proliferation which was most marked in the neighborhood of necrotic foci. Often the adventitial thickening was not symmetrical and only in one part of the vessel was marked thickening of the adventitia seen (Fig. 2). In some other instances marked adventitial thickening was seen in tumor areas which appeared slightly acellular and edematous, probably representing regressive alterations of the tumor.

The Nile blue sulfate stain revealed neither "neutral" fat nor "acid" lipids in the solid parts of the tumor. No lipids were found in tumor giant cells. There was, however, an abundance of fat in necrotic foci, especially in their edges. In the Nile blue sulfate stained sections there was a diffuse pink discoloration of varying shade in the necrotic foci which was not identical in location to the varying shades of eosinophilia often seen in hematoxylin and eosin preparations of corresponding foci. It was of interest to observe pink-stained lipids in a zone immediately adjacent to and within the zone of the tumor cells composing the edges of pseudopalisading (Fig. 3). In many instances the following interesting observations could be made by comparing the adventitial proliferation of the vessels with the corresponding section stained with Nile blue sulfate: it could be seen that there was a relationship of the adventitial proliferation to the presence of neutral fat in the proliferating parts of a given vessel (Fig. 4).

No such correlation between the presence of neutral fat and endothelial proliferation could be made.

Case 2. Glioblastoma multiforme (A12750). Histologically the tumor was as typical as that in Case 1. Some foci of complete necrosis stained blue with hematoxylin dyes, while others revealed a pink discoloration. In some necrotic foci round-cell infiltrates were seen.

Fat generally was seen closely associated with necrosis and with regions of pseudopalisading. No fat was seen within the partial adventitial proliferation of vessels. However, "neutral" fat was present in their immediate neighborhood. In areas where round-cell infiltrates were seen, pink-staining lipids were present. In those less cellular
Fig. 5. *Case 3.* Glioblastoma multiforme. Gemistocytic astrocytes are present in region revealing mild regressive changes. Hematoxylin and eosin, X1760.

Fig. 6. *Case 3.* Glioblastoma multiforme. Dark-stained fat is seen between gemistocytic astrocytes and in areas of cellular degeneration. Similar field as shown in Fig. 5. Sudan black, X1760.
foci of the tumor where some large pale astrocyte-like cells were present, neutral fat was seen. No lipids were present in the solid parts of the tumor or in regions of endothelial proliferation.

**Case 3.** Glioblastoma multiforme (FSH 2075). This tumor was composed of pleomorphic cells and large numbers of gemistocytic astrocytes. Often the gemistocytic astrocytes were observed in foci revealing mild regressive changes (Fig. 5).

Fat was present only in necrotic foci. Around some vessels showing adventitial proliferation pink deposits were present, while around others no fat was observed. No lipid material was seen within the proliferating adventitial cells. In regions in which gemistocytic astrocytes were present pink-stained material was seen in Nile blue sulfate preparations. This material stained darkly in a consecutive section stained with Sudan black (Fig. 6). Even with oil immersion it was difficult to demonstrate clearly the lipids within the cytoplasm of the gemistocytic astrocytes, partly because these cells do not reveal a well outlined cell body. It was our impression that the lipids were present between and not within the gemistocytic astrocytes.

**Case 4.** Astrocytoma (A12523). The tumor was predominantly a fibrillary astrocytoma. In addition, some gemistocytic astrocytes could be detected. Occasionally there were small foci of acidophilic discoloration with pyknotic cells. Many small circumscribed foci showed marked reduction of tumor cells to the point of being almost acellular. In some instances a few well preserved tumor cells, mostly gemistocytic astrocytes, were scattered haphazardly. As a rule, these less cellular or acellular foci showed in their center a fibrillary appearance and many tiny, cyst-like empty spaces (Fig. 7). In addition, nuclear debris and regressive tumor cells were present revealing pyknosis and varying stages of karyolysis. Only rarely in such regions could gitter cells be seen. These foci probably represent precursors of cystic degeneration. In addition, the tumor showed many small cysts surrounded by tissue with a fibrillary appearance and mostly well preserved tumor cells (Fig. 8). No stained material could be demonstrated within the cysts.

"Neutral" fat was seen only in those areas that were designated as "precursors of cystic degeneration" (Fig. 9). In the solid parts of the tumor and in the cystic areas no lipids were seen. Also in gemistocytic astrocytes no fat was seen either in or around the cells.

**Case 5.** Astrocytoma (A4760). The tumor was predominantly a fibrillary astrocytoma. Occasionally gemistocytic astrocytes were present. No "neutral" fat or "acid" lipids were demonstrated in the tumor cells or in regions of cystic
Fig. 8. Case 4. Astrocytoma. The tumor shows several small cysts surrounded by tissue with a fibrillary appearance and well preserved tumor cells. Hematoxylin and eosin, X440.

Fig. 9. Case 4. Astrocytoma. Consecutive section of preparation shown in Fig. 7. Lipids are seen distributed in scattered fashion. Sudan black, ×1100.
Fig. 10. Case 7. Oligodendrogioma. Cyst-like degenerated area with coagulated fluid in the vicinity of a vessel (better seen in upper part of the vessel). In the periphery of the degenerated area some regressive pyknotic cells are shown with some nuclear debris. Hematoxylin and cosin, ×440.

Fig. 11. Case 7. Oligodendrogioma. Consecutive section of the preparation shown in Fig. 10. Lipids can be seen in the cyst-like region illustrated in Fig. 10. Sudan black, ×1760.
were regions representing organization and scar-
ing subsequent to the operative procedure with
activity of macro-

phages, and large amounts of collagenous tissue.

In the well preserved parts of the tumor neither
“neutral” fat nor “acid” lipids could be seen. In
small foci of cellular degeneration of the tumor
some pink material was present in Nile blue sul-

fate preparations. The region of scarring and
fibroblastic organiz-
tion showed large quantities
of “neutral” fat in Nile blue sulfate stains.

Case 7. Oligodendroglioma (A610). This
tumor showed the pattern of an oligodendro-
glioma. There were circumscribed foci with fewer
tumor cells, others with markedly pyknotic tumor
cells, and others which were acellular. Most of
these foci were perivascular. Areas revealing a
cystic degeneration with a pinkish coagulated
material could be seen and were in most instances
perivascular in location (Fig. 10).

In Nile blue sulfate preparations one could see
small circumscribed areas in which “neutral” fat
and other lipids of different colors ranging from
blue to pink could be seen (Fig. 11). In addition,
these areas demonstrated, in Nile blue sulfate,
vacuoles that did not give a positive lipid reac-
tion. Such areas were examined in hematoxylin
and eosin preparations and were found to be dis-
crete circumscribed areas empty of cells or con-
taining occasionally one or two nuclei revealing
varying stages of lysis. The size of the vacuoles in
hematoxylin and eosin preparations varied con-
siderably as well as the size of the circumscribed
areas showing cellular degeneration. The network
of these vacuolated areas revealed a coagulated-
like pink discoloration. Areas of calcification did
not reveal any fat in Nile blue sulfate stains.
Occasionally, however, in Nile blue sulfate prepa-

rations some dark blue-stained granules could be
seen in some of the regions that appear to corre-
spond to areas revealing calcification. One section
of the tumor was immersed in acetone for 15
minutes so as to remove the lipid and then the
areas as described above were reviewed after a
Nile blue sulfate stain. Another such section was
repeated with acetone and subsequently stained
with Nile blue sulfate and with the Prussian blue
technique. After acetone treatment and Nile blue
sulfate stain, the granules stained dark blue as
before. After immersion in acetone and staining
with Nile blue sulfate and Prussian blue, the
granules stained greenish-blue. It was concluded
that this material that stained dark blue in Nile
blue sulfate did not represent lipids, and further
that it was, or at least contained, iron.

Case 8. Oligodendroglioma (A4287). Micro-
scopically the tumor was similar to the one de-
scribed in Case 7. No calcifications were seen in
the microscopic slide examine I.

In the solid parts of the tumor no “neutral”
fat or “fatty” acids were present. Tumor cells
with honeycomb-like appearance were examined
in Nile blue sulfate preparations and were not
found to contain lipids. In most of the cystic
regions, whether in the vicinity of or far from
vessels, gitter cells containing “neutral” fat were
seen, while in others no fat could be detected.

Case 9. Medulloblastoma (A10216). Histo-
logically the tumor was a medulloblastoma. Occa-
sionally there were large necrotic acellular foci in
the periphery of which nuclear debris could be
seen. The center of some of these lesions had a
vacuolated appearance and a few nuclear shadows.

In the Nile blue sulfate preparations, lipids
were seen in these necrotic foci. Mostly they
stained mauve and occasionally pink. Comparison
of foci of necrosis in hematoxylin and eosin and
Nile blue sulfate preparations revealed that the
lipids corresponded to vacuolated areas seen in
hematoxylin and eosin stain. Only a few gitter
cells were seen in hematoxylin and eosin prepa-
rations and in the corresponding Nile blue sulfate
stain. In the periphery of the tumor there was an
extensive area of necrosis; many vacuoles were
present. Nile blue sulfate preparations revealed
many cloud-like lipid deposits staining from
mauve to pink. No neutral fat was seen in the
solid part of the tumor.

Case 10. Medulloblastoma (A7110). Micro-
scopically the tumor was similar to the medul-
loblastoma in Case 9. Occasionally there were small
foci of necrosis.

In Nile blue sulfate preparations such foci
revealed pink-stained “neutral” fat. None of the
well preserved tumor cells showed either “acid”
lipids or “neutral” fat.

Case 11. Ependymoma (A6005). Histological
sections showed a tumor composed of epithelial-
like cells arranged in a papillary fashion. Large
foci of degeneration of the connective-tissue
septa and areas of necrosis were seen.

There was an abundance of pink-staining ma-
terial in the zones of necrosis in Nile blue sulfate
preparations. In the solid parts of the tumor no
lipids were detected. Occasionally in the vicinity
of tumor cells revealing regressive changes, pink-
staining material was present. The same held
true for the connective-tissue septa of the tumor.

Case 12. Pinealoma (A5067). Microscopically
the tumor was composed of islands of cells sepa-

rated by connective-tissue septa. Both the extent of the tumor islands and the thickness of the connective-tissue septa varied from field to field. In the well preserved parts the tumor was composed of large epithelial-like cells having a large nucleus surrounded by varying amounts of cytoplasm. The nuclei were rich in chromatin revealing several nucleoli-like condensations of chromatin. Occasionally smaller lymphocyte-like tumor cells intermingled with the above-described large cells. Large parts of the tumor were necrotic, and in such parts macrophages with a foamy vacuolated cytoplasm were seen.

In Nile blue sulfate preparations necrotic areas revealed large amounts of neutral fat. In addition, perinucleolarly there were small granule-like cytoplasmic deposits which gave a staining reaction similar to the neutral fat observed in necrotic foci (Fig. 12). Most of these deposits revealed on examination with oil immersion a crescentic formation consisting of a lipophobe vacuole-like structure with a covering of lipid material. This material was seen in all well preserved large tumor cells.

Case 13. Chromophobe Adenoma (A12936). The histological appearance was that of a chromophobe adenoma. Occasionally very fine vacuoles were present in the cytoplasm of the tumor cells. Areas of necrosis were not seen. The connective-tissue septa occasionally appeared edematous.

No pink-stained material was seen in the Nile blue sulfate preparations. However, in the cytoplasm of the tumor cells a varying number of granules staining blue were observed in slides stained with Nile blue sulfate. A similar section of the tumor treated for half an hour in acetone and subsequently stained with Nile blue sulfate still revealed these granules. In addition to these granules in the cytoplasm of the tumor cells there were vacuoles having bluish-staining borders and centers without stainable material. These vacuoles were also observed in the section treated with acetone. Both vacuole- and granule-containing cells appeared normal and without evidence of regressive changes. It was concluded that these granules and vacuoles were proteinaceous in nature.

DISCUSSION

Using the Nile blue sulfate and the Sudan black techniques no lipids could be detected in the well preserved parts of the glioblastomas multiforme examined (Cases 1, 2 and 3). Giant cells occurring in the material examined were free of lipids. No relationship could be found between endothelial proliferation of blood vessels and necrosis. In all instances the proliferated endothelial cells
were free of lipids. Often in the material examined adventitial proliferation of the vessels was present. In some instances (Case 1) lipids staining pink in Nile blue sulfate preparations were seen in form of large droplets within the proliferated adventitial tissue. In the other cases (2 and 3) no lipids were seen within the proliferated adventitia. However, in all 3 cases of glioblastoma multiforme investigated in this study, lipids were present in the immediate vicinity of proliferated adventitia. It is therefore reasonable to assume that the presence of lipids had at least partially, if not entirely, some formative influence upon the proliferation of the adventitial cells. Furthermore, often the adventitial proliferation was present on one side of the vessel and this side was adjacent to a necrotic focus. The fact that lipids were present within the proliferated adventitia of a case of an astrocytoma examined (Case 5) speaks also in favor of the above statement.

In general there was fat only in the necrotic parts of the 3 cases of glioblastoma multiforme. The maximal amount of fat was concentrated at the edges of necrotic foci. The center revealed in some necrotic foci a pink discoloration in Nile blue sulfate preparations. In almost all instances in the immediate vicinity and within regions of palisading large quantities of pink-stained lipids were observed. Lipids were seen in tumor cells showing regressive changes leading to rhexis and lysis of the nucleus and fatty degeneration of the cytoplasm. Many of the cells forming the palisading disclosed such regressive changes. The frequent presence of palisading next to foci of necrosis and adjacent to accumulations of lipids indicated that disintegration of tumor tissue had a formative influence upon the pattern of and the cellular arrangement in palisading. A similar view concerning the influence of necrosis upon palisading has also been expressed by Henschcn.

The lipids were at the periphery of the necrotic lesions in gitter cells, around vessels, in the walls of vessels, and in the form of fat droplets apparently free in the tissue. In regions of scarring, lipids were present in the connective-tissue cells (Case 6).

While this investigation was being carried out, a paper was published on the histochemistry of tumors of the central nervous system, with special reference to the lipids in glioblastoma multiforme. In general, the authors of this paper found the same distribution of lipids in glioblastomas as in our study. However, some of their interpretations are in disagreement with the views expressed in the present discussion. They do not believe that palisading is secondary to or induced by the presence of necrosis (see discussion above), but rather that this arrangement is preexistent and corresponds to the specific tendency of glial cells to arrange themselves radially to form rosettes, pseudorosettes and gliovascular systems. This, according to the authors, shows clearly the character of the functional unity of the gliovascular system. In our opinion, in most parts of a glioblastoma no specific tendency of the tumor cells to arrange themselves radially has been seen. Furthermore, palisading was seen around necrotic lesions and was entirely absent around vessels in the well preserved parts of the tumor. A functional unity of the so-called “gliovascular” system was not seen in glioblastomas.

Degenerated tumor cells and disintegration of the preexisting structures, especially of myelin, were in our opinion the major sources for the lipids demonstrated in the tumors examined (Cases 1–13). It is well known that edema is associated with tumors of the central nervous system. Jacob has shown that edema fluid has a deleterious influence upon myelin structures, often leading to necrosis of myelin. Scholz also has demonstrated in a variety of pathological disorders that myelin, as opposed to the gray matter, is very sensitive to edema fluid and plasmatic infiltrates. The cause of demyelination is therefore edema, and demyelination in gliomas is not secondary to the “many disturbances that affect neurones” as reported. Further, in our cases it often has been seen that nerve cells were well preserved
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among infiltrating tumor cells, a fact well known from the literature.

Foci of necrosis have been described, e.g., Cases 1 and 2, disclosing in hematoxylin and eosin preparations an eosinophilic or basophilic discoloration. Consecutive sections of such foci stained with Nile blue sulfate revealed the following findings: 1) Most of these necrotic foci showed pink-stained lipids, the extent of which was not identical with the eosinophilia or basophilia seen in hematoxylin and eosin preparations. 2) The pink discoloration seen in Nile blue sulfate preparations was always less than the discoloration of the necrotic foci seen in hematoxylin and eosin stains. 3) A few of these basophilic or acidophilic foci did not reveal any lipids. The question arises as to what caused the eosinophilic or basophilic discoloration seen in loci of necrosis stained with hematoxylin and eosin. Although for reasons already stated our material did not permit accurate staining for nucleic acid, we do, however, believe that the basophilia or eosinophilia was ascribable to the presence of desoxyribonucleic acids and associated histones. Bunting found in regions of necrosis (i.e., infarcts, caseous exudate) basophilia and acidophilia which gave the Feulgen reaction and which were not affected by ribonuclease. He concluded that these staining qualities were consistent with the presence of desoxyribonucleic acid and associated histones derived from nuclei of necrotic cells. In this connection it is worth mentioning the possibility that the presence of desoxyribonucleic acids associated with necrosis may have, in addition to the lipids, a formative influence on the genesis of the palisading tumor cells.

It has been reported that gemistocytic astrocytes revealed in gliomas a mild fatty degeneration. In 3 cases in our material (Cases 3, 4 and 5) gemistocytic astrocytes have been observed. In 2 astrocytomas (Cases 4 and 5) no lipids were seen in these cells either with the Nile blue sulfate or with the Sudan black technique. In the third case, a glioblastoma (Case 3), it was very difficult to demonstrate even with oil immersion whether or not some lipids were present in the most peripheral parts of the cytoplasm of the gemistocytic astrocytes. This difficulty arose because the cellular outlines of these cells were not clearly demonstrable. In this case lipids were seen between the gemistocytic astrocytes in Nile blue sulfate as well as in Sudan black stain (see Fig. 7). However, in those areas where the lipids were present, consecutive sections stained with hematoxylin and eosin disclosed small islands of tumor cells showing regressive changes. Although our material was not large enough to draw definite conclusions about gemistocytic astrocytes, nevertheless it seems probable that lipids are not characteristic inclusions of the cells, but probably may occur under adverse cellular conditions. We agree with Henschen that pyknosis of the nucleus, reduction of its size, and hyaline-like discoloration of cytoplasm do not necessarily represent regressive changes of the gemistocytic astrocytes, since these are morphological features normally present in these cells.

Lipids were seen in astrocytomas only in some regions of degeneration. No lipids were present either in the solid parts of the astrocytomas or in those regions revealing fully developed cystic degeneration of the tumor tissue (Cases 4, 5, and 6). However, in most instances in lesions designated as “precursors of cystic degeneration” some “neutral” fat was seen in form of pink-stained droplets in Nile blue sulfate preparations. Such lesions probably represent developmental stages of the cystic degeneration and were, in hematoxylin and eosin preparations, less cellular and showed a fibrillary appearance. In addition, many tiny, cyst-like empty spaces were observed within such foci and by comparison with corresponding lipid stains not all tiny empty spaces revealed a positive staining reaction. Probably the majority of the tiny cyst-like spaces represented interstitial edema, which was low in protein and did not give a staining reaction in hematoxylin and eosin preparations. In this connection, it is worth mentioning that according to
some investigators\textsuperscript{10} edema is one of the major causes for the development of cystic degeneration in astrocytomas. Also, fully developed cysts in the astrocytomas of our material failed to reveal stainable material in hematoxylin and eosin preparations. Probably the material occupying the cysts was low in protein and did not give a positive staining reaction in hematoxylin and eosin preparations. Stern\textsuperscript{21} examined chemically the content of the cysts and found their protein content lower than that of blood.

In both oligodendrogliomas examined (Cases 7 and 8) many circumscribed areas were seen revealing few tumor cells or being acellular and showing cystic degeneration. In contrast to similar lesions seen in astrocytomas, the cyst-like spaces gave a pink-blue staining reaction in hematoxylin and eosin preparations and seemed to contain a coagulated proteinaceous material. In many instances in such foci, which were often perivascular, gitter cells were present. Some of the lesions gave a positive staining reaction with Nile blue sulfate and in 1 case of oligodendroglioma (Case 7) the lipids showed different colors ranging from blue to pink. By no means were all cystic spaces in Nile blue sulfate or in Sudan black preparations found to contain lipids, and in some cystic lesions (these were in the minority) no lipids whatsoever were seen. As in the astrocytomas, it is our opinion that edema was the most important cause for the cystic degeneration in oligodendrogliomas. Nevertheless in these tumors, as opposed to the astrocytomas, the fluid of the cysts seemed to be rich in protein and therefore appeared as coagulated material staining blue-pinkish in hematoxylin and eosin preparations.

The well preserved parts of the oligodendrogliomas did not disclose any lipids. The so-called „honeycomb”-appearing cells did not give any positive reaction either with the Nile blue sulfate or with the Sudan black techniques.

In one of our oligodendroglioma cases (Case 7) blue-staining granules were seen near foci of calcification in Nile blue sulfate preparations. Since this staining reaction persisted after treating a consecutive section with acetone and another section gave a positive stain with the Prussian blue technique, it was concluded that this material is not fat, and further that it is, or at least contains, iron.

In both cases of medulloblastoma examined (Cases 9 and 10) only in the necrotic part of the tumor were lipids seen in Nile blue sulfate preparations, in 1 case (Case 10) giving a pink discoloration and in the other (Case 9) a staining reaction ranging from mauve to pink.

In the case of the papillary type of ependymoma evaluated (Case 11) only the necrotic parts of the tumor revealed lipids in Nile blue sulfate and Sudan black preparations.

The only tumor examined in this series in which lipids were found in the well preserved parts of the tumor in addition to the areas of necrosis was the pinealoma (Case 12). In the cytoplasm of well preserved tumor cells granule-like deposits were observed, which stained pink in Nile blue sulfate preparations. The number of these deposits varied from cell to cell. Examined with oil immersion, most of these droplets are composed of a lipophobe vacuole centrally located surrounded by a pink-stained rim. According to the best of our knowledge, no observations on the occurrence of lipids in pinealomas has been reported in the literature. Lipid structures similar to those observed in our case of pinealoma were seen in the normal pineal glands of man.\textsuperscript{16} On the basis of the above-described lipids in pinealomas, it may be possible in the future to better classify the tumors occurring in the pineal gland. Russell\textsuperscript{17} expressed the view that most of the so-called pinealomas did not arise from the parenchyma of the pineal body, but were atypical teratomas. Further, she reported that the so-called pinealomas bear a great resemblance to other teratoid tumors and the testicular seminoma.\textsuperscript{17} It would be of importance to determine whether lipids occur in all pinealomas and whether it would be possible by the presence or absence of lipids to differentiate pinealomas, atypical teratomas, and testicular seminomas.
In the only case of chromphobe adenoma of the pituitary examined (Case 13) no foci of necrosis were seen and no lipids could be detected in Nile blue sulfate preparations. However, with this staining technique blue granules were detected in the tumor cells. These blue granules were still present after treating a slide for half an hour in acetone. Since the Nile blue sulfate technique stains, in addition to the lipids, some protein blue, it was concluded that these deposits observed in the cytoplasm were proteinaceous in nature. They probably are similar to the granules that can be demonstrated in adenomas of the pituitary with the Mallory technique.

Using the Nile blue sulfate and the Sudan black techniques, with the exception of a case of pinealoma, no lipids were detected in the healthy, well preserved parts of the tumors of the central nervous system examined. Only in necrotic lesions were lipids present. Our histochemical findings concerning lipids of gliomas were in agreement with the results reported by Cumings in his chemical investigation of the same problem. Selverstone and Moulton, on the other hand, found values of phospholipids in gliomas high enough to suggest a basic significance and a metabolic as well as a structural function for the lipids in gliomas. Although our histochemical methods were sensitive for demonstrating phospholipids, the different methodical approach, namely the use of biochemical techniques, by the above-mentioned authors in their study may account for the difference in the results obtained. It is also possible that their findings were influenced by the presence of necrosis. It is surprising to find foci of necrosis often, microscopically, in pieces of tumors that were judged grossly as well preserved.

SUMMARY

Thirteen intracranial tumors were examined with the Nile blue sulfate and Sudan black techniques. Lipids were found in the healthy well preserved cells of the tumor in only 1 case, that of a pinealoma. In the other cases fat was present only in necrotic foci.

The influence of necrosis, lipids and desoxyribonucleic acid on the palisading of tumor cells in glioblastomas was discussed. Lipids were thought to have a formative influence upon the adventitial proliferation of the vessels seen in glioblastomas and in 1 case of astrocytoma.

No causal relationship could be established between the presence of lipids and endothelial proliferation.

No fat was seen clearly in gemistocytic astrocytes; however, in one instance lipids were observed between these cells.

Fat was present in foci of degeneration and in lesions designated as "precursors of cystic degeneration" in astrocytomas. No lipids were seen in cystic areas. Cystic degeneration as seen in astrocytomas and oligodendrogliomas was compared and the differences observed were outlined. Oligodendrogliomas revealed lipids in regions of cystic degeneration. In 1 oligodendroglioma, blue-stained granules were present in areas of calcification. It was shown that there were no lipids in these, and that they contained iron.

In 1 chromophobe adenoma blue-stained granules were seen in the cytoplasm of tumor cells. They were found to be proteinaceous in nature.

The basophilia and acidophilia seen in hematoxylin and eosin preparations were attributed to the presence of desoxyribonucleic acids.

The lipids seen in a case of pinealoma were considered critically. Their importance for the classification of tumors occurring in the pineal body was outlined. The hope was expressed that these lipids in the pinealoma may contribute to the discrimination among morphologically related tumors such as pinealomas, atypical teratomas, and testicular seminomas.

REFERENCES

3. Cain, A. J. The use of Nile blue in the examina-