



1967

Cytochemistry of Retrograde Neuronal Degeneration in Feline Lateral Geniculate Body

John B. Oldershaw
Loyola University Chicago

Follow this and additional works at: https://ecommons.luc.edu/luc_theses

 Part of the [Medicine and Health Sciences Commons](#)

Recommended Citation

Oldershaw, John B., "Cytochemistry of Retrograde Neuronal Degeneration in Feline Lateral Geniculate Body" (1967). *Master's Theses*. 2171.

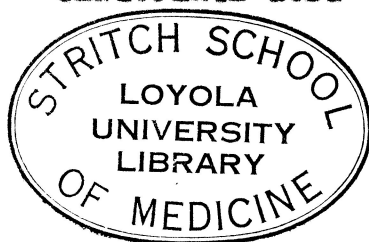
https://ecommons.luc.edu/luc_theses/2171

This Thesis is brought to you for free and open access by the Theses and Dissertations at Loyola eCommons. It has been accepted for inclusion in Master's Theses by an authorized administrator of Loyola eCommons. For more information, please contact ecommons@luc.edu.



This work is licensed under a [Creative Commons Attribution-Noncommercial-No Derivative Works 3.0 License](#).
Copyright © 1967 John B. Oldershaw

CYTOCHEMISTRY OF RETROGRADE NEURONAL
DEGENERATION IN FELINE LATERAL
GENICULATE BODY



by

John B. Oldershaw, M.D.

A Thesis Submitted to the Faculty of the Graduate School
of Loyola University in Partial Fulfillment of
the Requirements for the Degree of
Master of Science

February

1967

TABLE OF CONTENTS

	PAGE
ACKNOWLEDGMENT	111
BIOGRAPHY	1v
INTRODUCTION	1
PROCEDURE	7
RESULTS	11
DISCUSSION	21
SUMMARY AND CONCLUSIONS	28
BIBLIOGRAPHY	29
FIGURE CAPTIONS	38

ACKNOWLEDGMENT

I wish to express my indebtedness to Doctor Kevin D. Barron, Chief of Neurology, Veterans Administration Hospital, Hines, Illinois, whose efforts to teach me the rudiments of experimental neurology and neuropathology are greatly appreciated. He has graciously permitted me to use portions of our collaborative work on Retrograde Neuronal Degeneration in the preparation of this thesis and has generously given of his time in the review of it.

BIOGRAPHY

Doctor John B. Oldershaw was born in Evanston, Illinois on November 10, 1932.

He was graduated from Loyola Academy in 1950. He attended Loyola University from 1950 to 1957 obtaining a B. S. degree in 1955 and an M. D. degree from the Stritch School of Medicine in 1957.

He interned at the National Naval Medical Center at Bethesda, Maryland followed by training at the Naval Aviation Medical Center in Pensacola, Florida. He was designated a Naval Flight Surgeon in 1958 and was subsequently ordered to duty with the Fleet Marine Corps.

He began general surgery training at the United States Naval Hospital, Balboa, San Diego, California in 1961 which was followed by a neurosurgical residency with Doctor Harold C. Voris under the auspices of the University of Illinois from 1962 to 1966.

His next assignment is a tour of duty as neurosurgeon on the hospital ship U. S. S. Repose in Vietnam waters.

INTRODUCTION

Study of the effect of lesions of the visual cortex upon the lateral geniculate body was begun in 1872 when Von Monakow described atrophy in the ipsilateral lateral geniculate nucleus, pulvinar, superior colliculus and transverse peduncular tract following occipital cortex lesions. In a later report, he postulated the presence of internuncials in the lateral geniculate nucleus. In 1913, Winkowski showed a precise point-to-point degeneration in the lateral geniculate nucleus following lesions in the striate cortex. In 1920, he described the systematic termination of crossed and uncrossed optic tract fibers within distinct laminae of the lateral geniculate nucleus of the cat and monkey.

In the cat, the primary thalamic termination of optic fibers is the lateral geniculate nucleus which consists chiefly of a trilaminated pars dorsalis and a small, ventrolaterally-placed pars ventralis. Thuma (1928) has described three principal cellular laminae of the pars dorsalis which he designated A, A₁, and B, from dorsal to ventral. From transneuronal atrophy studies by Winkowski (1920), Barris (1935), and Silva (1956), from Nauta studies by Hayhow (1958), and from microelectrode electrophysiological studies by

Cohn (1956), it has been established that crossed optic tract fibers terminate exclusively in laminae A and B, whereas uncrossed optic tract fibers terminate exclusively in the intervening lamina A₁ of each lateral geniculate nucleus. Laminae A and A₁ receive predominantly optic tract fibers of large size and lamina B is supplied almost exclusively by optic tract fibers of small diameter (Bishop and Clara, 1955). Individual optic tract fibers end within a single cellular layer and in the adjacent interlaminar margin (O'Leary, 1940).

From the lateral geniculate nucleus, efferent fibers pass chiefly through the optic radiations to the cerebral cortex where they terminate within the histologically defined striate area. Projection fibers from the lateral geniculate nucleus terminate in this area in a rather precise manner; the medial part of the nucleus projecting to the medial part of the striate area, the lateral part of the nucleus to the lateral striate area, and the rostral and caudal parts of the nucleus to the rostral and caudal parts respectively of the striate area (Minkowski, 1913). Fibers from the lateral geniculate nucleus also terminate outside the striate area, as was proposed by Von Monakow (1882) and demonstrated by Minkowski (1913) and Polyak (1927).

Retrograde neuronal degeneration occurs when axons of neurons which begin and end in the central nervous system are severed. Brodal in 1940 (2) reported an extreme example of retrograde neuronal degeneration in the inferior olivary nucleus of young animals following cerebellar lesions.

The earliest changes seen in retrograde neuronal degeneration occur approximately the 4th day after axon severance. The cells show moderate, atypical tigrolysis beginning in the central part of the cytoplasm. During the following days the tigrolysis increases; at the same time the nucleus and the cell gradually decrease. The nucleus is not displaced toward the periphery of the cell. This picture, seen in nearly all cells in the affected area about the eighth day, represents the maximum retrograde reaction in these cells. Already at this time cells may appear as "cell shadows" or as very pale, shrunken nuclei, surrounded by a scanty mass of cytoplasm. Such cells, obviously disintegrating, are seen in increasing numbers during the following days. At the same time the total number of cells is gradually reduced. About sixteen days after the operation from one-third to one-half of the cells have disappeared, and disintegrating cells are relatively seldom seen. At later stages, approximately the

same degree of cell loss is noted indicating that most of the cells which disintegrate acutely do so within sixteen days after the lesion.

The cells which do not disappear undergo a change, but in another way; they grow steadily smaller; some recover some of their Nissl bodies; others remain pale. These cells thus become atrophied, and after some time are extremely reduced in size. In the later stages there is a greater concentration of the glia cells in places where some of the nerve cells have disappeared.

In contrast, neurons whose processes are entirely or partly outside the central nervous system respond to axonic interruption by a regenerative phenomenon known as central chromatolysis or axon reaction. This process is histologically characterized by an expansion in perikaryal volume, eccentricity of the nucleus, increase in nucleolar volume and fragmentation and peripheral displacement of the Nissl substance and Golgi apparatus. The eccentric nucleus regularly comes to lie opposite the axonic process. The chemical changes which occur in axonal reaction are directed toward the reconstitution of the lost axoplasm. There is a net accumulation of lipid, ribonucleic acid and protein in

the nerve cell soma.

The comparison of the responses of central and peripheral nerve cells to axon section may lead to an understanding of the phenomena that determine the capacity of a nerve cell to regenerate after damage to its axonic process. Examination by enzyme histochemical methods has already been carried out in the process of central chromatolysis (6, 10, 12). Similar investigation of the process of retrograde neuronal degeneration is to be carried out here, followed by a comparison of the enzyme histochemical findings in the two neuronal responses to axon injury.

The lateral geniculate body of the cat has been chosen as the object of study because it appears to receive little cortical innervation so that transneuronal effects are not a complicating issue.

Prior study of thalamic nuclei by standard histologic methods has been carried out. However, this study marks the first attempt to study retrograde neuronal degeneration in the lateral geniculate body by enzyme histochemical methods.

The following enzyme histochemistry is to be studied; acid phosphatase by the Gomori and Barka-Anderson methods, nucleoside diphosphatase by the Novikoff-Goldfischer method,

and 5-nucleotidase, adenosine triphosphatase, non-specific esterase and acetylcholinesterase by the standard methods.

PROCEDURE

Twenty-one cats were used. Thirteen were operated and eight were controls. Under Nembutal anesthesia, the cats to be operated were placed on a special cat head holder which maintained them in firm, immovable position. A scalp incision was made in some cases in the midline and in others in the fashion of a left parietal flap. The bone overlying the left striate cortex was removed with small trephines, rongeurs and bone punches. This was carried to the bony tentorium inferiorly, to the level of the vertex superiorly, to $\frac{1}{2}$ cm. across the midline medially and 5 cm. laterally so that adequate exposure of the sylvian fissure would be obtained. After the bone removal the dura was opened lateral to the sagittal sinus and then reflected laterally toward the sylvian fissure. The elements of the striate cortex were then identified, (Fig. A). In all animals the pia overlying the lateral gyrus was incised in its entire extent. From this point using various sized suction tips the lateral gyrus, and the postero-lateral gyrus, were removed. To a lesser extent lesions of this nature were made in the middle suprasylvian gyrus, the posterior suprasylvian gyrus and the ectosylvian gyrus. In two animals an undercutting procedure was carried out using a scimitar

scalpel blade. The blade was passed through the extent of the lateral and postero-lateral gyri on the left in each case. Closure was effected with careful attention to hemostasis. The animals were allowed to survive for varying periods of time. The survival times for the cortex removals were as follows: 3, 5, 6 (2 animals), 14, 33 (2 animals), 41, 55, 89 and 96 days. In the two undercut animals the survival times were 62 and 236 days. The animals including eight unoperated control animals were all sacrificed by intra-aortic perfusion of 1% calcium-formalin solution containing 5% sucrose at 2-4°C in an amount of 1.5-2 liters. After perfusion the brain was removed and a block 1.2 to 1.5 cm. square and 0.3 cm. thick containing the entire lateral geniculate body except for the anterior and posterior poles of the nucleus was dissected from each side of the brain in the coronal plane. The blocks were immersed in calcium-formalin 1% at 2-4°C for 18-22 hours. They were rinsed in three changes of 5% sucrose and then placed in 100-150 times their volumes of gum-sucrose (22) for 24 hours at 2-4°C. Next, frozen sections were cut at 10-15 μ on a freezing microtome. The sections were collected in ice-chilled distilled water and processed quickly thereafter for the following enzyme activities: non-specific acid phosphatase

by Gomori (20) and Barka-Anderson (33) methods; nucleoside diphosphatase (21) with thiamine pyrophosphate as substrate; "apparent" (25) adenosine triphosphatase (34); 5-nucleotidase; non-specific esterase (36) with alpha-naphthyl acetate as substrate; acetylcholinesterase with acetylthiocholine as substrate (20) but with substitution of 30% Na_2SO_4 for the saturated solution. The incubation times were as follows: 10-15 minutes for adenosine triphosphatase, 30-45 minutes for acid phosphatase, nucleoside diphosphatase and non-specific esterase, 60-90 minutes for 5-nucleotidase, 180 minutes for acetylcholinesterase. All incubations were at 37°C .

Frozen sections from every animal were stained by buffered thionin (37) and Oil Red O (38) techniques. Sudan Black B (38) and Hirano-Zimmerman (39) stains were prepared also.

The following additives were used in selected experimental material: Sodium fluoride; p-chloromercuribenzoic acid (PCMB); cupric sulfate (Cu^{++}); sodium DL-tartrate; methanol-free formaldehyde (CH_2O); diethyl-p-nitrophenyl phosphate (E600).

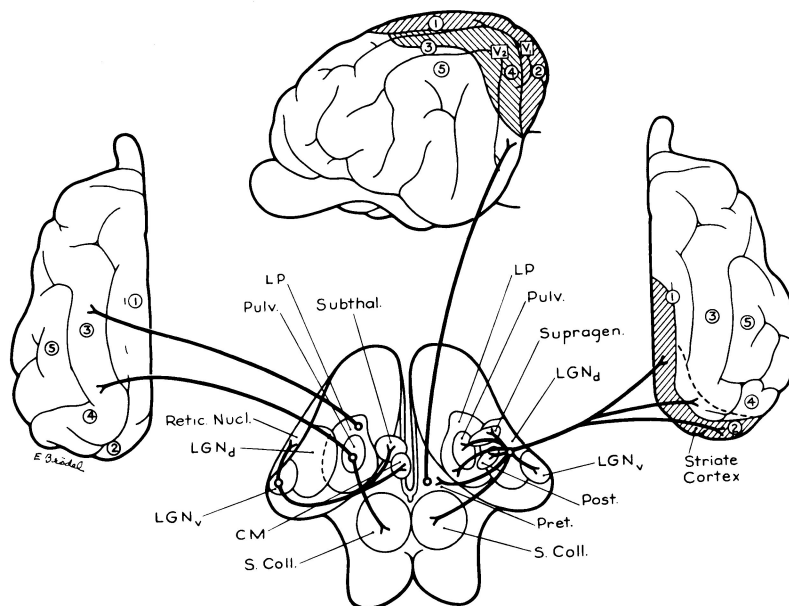


Figure A

Thalamic fiber projections related to the visual system indicated on dorsal view of the thalamus and midbrain (after Thuma, 1928) and on dorsal and lateral views of the cerebral cortex of the cat. The striate area (after O'Leary, 1941) is cross-hatched on the dorsal view of the right hemisphere, and visual areas V_1 and V_2 (after Woolsey, 1962) are cross-hatched on the lateral view of the left hemisphere.

Numerals represent: (1) lateral gyrus; (2) posterolateral gyrus; (3) middle suprasylvian gyrus; (4) posterior suprasylvian gyrus; and (5) ectosylvian gyrus.

Abbreviations: LGN_d - lateral geniculate nucleus, pars dorsalis; LGN_v - lateral geniculate nucleus, pars ventralis; S. Coll. - superior colliculus; V_1 - visual area 1; V_2 - visual area 2.

RESULTS

Unless otherwise stated, the results described below refer solely to the pars dorsalis of lateral geniculate body. They do not necessarily apply to other regions of cat central nervous system. The lateral geniculate body contralateral to cortical extirpation was not different from that of unoperated animals. Separate reference is made at the conclusion of the section to findings attendant upon undercutting of the visual cortex.

Observations on Tissue Sections

Gomori Acid Phosphatase: In normal lateral geniculate body the cytoplasm of neurons of large (30-40u), small (8-15u) and intermediate (20-25u) dimension contains numerous black sulfide droplets. These droplets are assumed to be lysosomes (22,23). They range in size from 0.2 to 1.0u, the majority being 0.4-0.8u in diameter. They are most densely packed in the larger cells, (Fig. 1). Droplets and lamellae are seen in the cytoplasm of perineuronal satellites (Fig. 2). Droplets are scattered also in inter-neuronal parenchyma or neuropil.

Three days post-operatively, when loss of basophilia was already apparent in thionin preparations, retrograde

degeneration was accompanied by a minimal, though definite, loss of acid phosphatase activity from affected neurons. By 5-6 days post-operatively, a definite loss of droplets from both soma and processes was apparent. Occasional acid phosphatase-positive granules in pericytes appeared enlarged (Fig. 3). By 14 days the cytoplasm of atrophic left-sided neurons contained a relatively sparse population of lead sulfide droplets (Figs. 4, 5) which appeared to be of reduced size as compared to normal neurons. Many nerve cells were virtually devoid of granules. The neuropil, however, contained numerous swollen droplets of 2-3 μ diameter.

Preparations studied 33-35 days post-operatively were similar to 14-day material except for marked nuclear staining of the pericytes and glia of the atrophic nucleus. At 89-96 days the swollen droplets of pericytes and neuropil were no longer present. Many atrophic neurons remained in the shrunken lateral geniculate body. They contained only a few droplets of acid phosphatase activity or entirely lacked demonstrable enzyme. At no point in the process was swelling of neuronal lysosomes apparent.

Barka-Anderson Acid Phosphatase Method: The advantages of the Barka-Anderson technique include the rapid coupling of

the hexazonium salt at acid pH and the insolubility of the azo dye reaction product. In normal tissue there is a diffuse reaction of neuronal cytoplasm, the perikaryon being stained varying shades of red, the large neurons most deeply (Figs. 6a, 7a).

At three days post-operatively, a minimal diminution of cytoplasmic staining was apparent on the operated side when careful comparisons were made with the contralateral lateral geniculate body. In 5-6 day survivals, loss of enzyme activity was striking in atrophic neurons. By the 14th post-operative day neurons of the affected nucleus were difficult to discern at low magnifications (Fig. 6b). In later survivals striking cytoplasmic pallor of atrophic neurons was a consistent finding (Fig. 7b). These findings corroborate the results in the Gomori Acid Phosphatase preparations.

5-Nucleotidase (adenosine 5-monophosphatase):

At high magnifications the outlines of glial processes are sometimes discernible against a dense neuropilar stain in normal tissue. In addition to the diffuse reaction observed in neuropil, numerous droplets of reaction product are found in neuronal cytoplasm (Figs. 8, 9). In number, size and distribution these resemble the granules developed in Gomori

preparations. Thin lamellar threads are occasionally seen. Substitution of 5-cytidylic acid for 5-adenylic acid at the same concentration ($10^{-3}M$) results in similar localizations of reaction product though neuropilar staining is less intense.

To determine the nature of the droplet activity against 5-adenylic acid and 5-cytidylic acid, these compounds were dissolved at $10^{-3}M$ and $5 \times 10^{-3}M$ concentrations in substrate media containing 0.1M acetate or trismaleate buffers at pH 5.0 and 6.5 respectively. Manganous ions were alternately omitted from or added to the media. At pH 5.0 the 5-mononucleotides produced histochemical preparations identical to those described under the Gomori method whether or not manganous salts were included in the substrate. At pH 6.5, in the absence of manganous ions, neuropilar activity was minimal though neurons continued to exhibit droplet activity. The results, in so far as droplets were concerned, revealed severe or complete inhibition by fluoride, tartrate and p-chloromercuribenzoic acid (PCMB) (Fig. 10) and moderate inhibition by Cu^{++} . Pre-incubation in buffer at pH 4.0 abolished neuropilar enzyme (Fig. 11) while appearing to activate the droplets. The droplet enzyme has characteristics of a non-specific acid phosphatase identical to that localized by the Gomori procedure. The neuropilar enzyme of

5-nucleotidase preparations is distinctly different since it is inactive at pH 5.0, has a requirement for manganous salts and is relatively resistant to fluoride and p-chloromercuribenzoic acid (PCMB) inhibition while remarkably sensitive to acid pre-incubation. Further it was noted that the neuropilar enzyme appeared to be activated by Cu^{++} and tartrate.

Retrograde atrophy was associated with loss of neuronal lysosomes as described for Gomori preparations. No definite alteration of neuropilar staining was encountered.

Nucleoside diphosphatase: Use of thiamine pyrophosphate as substrate is associated with staining of an intraneuronal reticulum composed of delicate anastomosing threads (Figs. 12-15). The reticulum fills most of the cytoplasm but often is bounded peripherally, especially in the larger cells, by a narrow rim of cytoplasm (Fig. 12) which can be shown to be basophilic by thionin counterstains. At the light microscopic level the nucleoside diphosphatase-positive reticulum is equatable with the Golgi organelle (21). Diffuse staining of capillary loops and glial processes is encountered (Figs. 12-14).

Magnesium salts at 10^{-2}M concentration resulted in preparations having crisply-defined, intensely-stained Golgi organelles in neurons, especially when the substrate concentra-

tion was increased to twice the usual, while reactive capillaries were abundant and glial and neuropilar activity was virtually absent (Fig. 14). Glial and neuropilar activities were severely suppressed by 0.5% CH_2O and abolished by 0.75% CH_2O while capillary activity was moderately suppressed by the higher concentration (Fig. 15).

Retrograde neuronal atrophy was accompanied in the 5-6 day animals by a loss of staining intensity and an apparent attenuation of the constituent lamellae of the Golgi network. By 14 days the Golgi system of some neurons was pale and difficult to photograph. At this and subsequent survival periods, a minority of nerve cells showed a concentration of nucleoside diphosphatase-positive lamellae about the nucleus, the peripheral cytoplasmic halo noted in normal neurons seeming accentuated (Fig. 16). Yet, even in the 55-96 day survivals, some neurons contained a well-developed Golgi network in the midst of obviously atrophic tissue. At no point was a peripheral dispersion ("retispersion") of the Golgi reticulum noted. The dominant neuronal alteration in the atrophied nucleus was the pallor and diminished bulk of the nucleoside diphosphatase-positive Golgi net. Associated with the neuronal changes was an enhancement of glial, vascular and neuropilar activities

which was so prominent as not to be attributable to the condensation of tissue which accompanied atrophy of the nucleus (Fig. 17).

Adenosine Triphosphatase: This enzyme activity is localized to walls of blood vessels, especially those of capillary and pre-capillary dimension, as well as to glia and neuropil (Fig. 18). Neuropilar activity visualized by this substrate is distinguished by the presence of patchy foci of concentration (Fig. 18).

Retrograde atrophy was associated with increased staining of glial and neuropilar elements, especially the latter (Fig. 19).

Non-specific esterase: After 30 minutes' incubation with alpha-naphthyl acetate, neuronal cytoplasm is stained a deep red, azo dye being largely restricted to the perikaryon. The intensity of the neuronal reaction varies greatly but generally the larger neurons contain a greater concentration of azo dye than small nerve cells (Fig. 20). In some neurons, red droplets may be discerned against the diffuse staining of the cytoplasm (Fig. 20). These droplets resemble the smaller granules of the Gomori method in size and distribution and are assumed to be lysosomes, though they are always far fewer in

number, even in large neurons where they are seen most readily, than the bodies visualized by the Gomori technique. Reaction product in droplet form is present also in cells associated with capillaries. At least a proportion of these cells possess long branching processes (Fig. 21) and are probably pericytes. Many of the droplets, especially those in the cytoplasmic extensions of pericytes, are approximately 1.2μ in diameter and are quite regular in size and form, but other droplets measure 3μ or more in diameter and are clearly vacuolated (Fig. 22). These large droplets tend to be concentrated in perinuclear cytoplasm. Droplets of similar morphologic appearance are stained in cytoplasm of pericytes by Sudan dyes (Fig. 23).

By the fifth post-operative day a loss of the E600-sensitive esteratic activity of neuronal cytoplasm was suggested in the lateral geniculate body ipsilateral to cortical extirpation and was readily apparent in 14-day survivals. In 55-96 day animals the cytoplasm of shrunken neurocytes stained a pale pink which contrasted with the deep red of neurons from the contralateral lateral geniculate body. In survivals of 14-55 days there was prominent organophosphate-resistant activity in pericytes and glia. This was often in the form of large droplets lying in the inter-neuronal parenchyma and resembling the

enlarged droplets described for Gomori preparations (Fig. 24).

Acetylcholinesterase: In normal lateral geniculate body there is a dense brown coloration of neuropil. Neuronal cytoplasm stains varying degrees of yellow-brown, often being virtually colorless and rarely exhibiting strong reactivity.

It may be concluded that in normal lateral geniculate body, under the conditions employed in this work, acetylthiocholine hydrolysis is accomplished solely by acetylcholinesterase.

Retrograde atrophy was not accompanied by clearly defined alterations of acetylcholinesterase content. However, in 55-96 day survivals the collapsed lateral geniculate body of the operated side appeared to be stained more intensely than the contralateral nucleus.

Nissl and Other Stains: Neurons of the normal lateral geniculate body frequently display eccentric nuclei and in some there is an appearance of peripheral margination of Nissl substance (Fig. 25). In others Nissl material is concentrated about the nucleus.

Three days post-operatively the outstanding abnormality on the operated side was a loss of neuronal basophilia. At 5-6 days pallor of neuronal cytoplasm was even more conspicuous (Fig. 26).

The loss of basophilia was accompanied by apparent decomposition of the coarse Nissl bodies, the basophilic substance of the cell becoming finely granular. Glial nuclei were increased in number. In a few areas one or more neurons appeared to have fallen out. However, even at 96 days many shrunken neurons remained. They were crowded together within the collapsed lateral geniculate body. At no point in these events did nerve cells present the classic picture of central chromatolysis, viz. peripheral dispersion of Nissl substance etc.

Effects of Undercutting of Visual Cortex: Alterations in the lateral geniculate body homolateral to the side of operation were entirely similar to those described for extirpation. The rapidity of progression and the degree of degenerative change was somewhat less. Noteworthy was the persistence of numerous, pale, shrunken neurons in the atrophic lateral geniculate body 236 days after undercutting of the visual cortex.

DISCUSSION

That central chromatolysis is accompanied by an increase in the histochemical reaction for acid phosphatase was first shown by Bodian and Mellors (6) who examined spinal motor nerve cells in acetone-fixed, paraffin-embedded tissue by the original Gomori method (44). Accordingly, in 1961, Barron and Sklar (10) re-investigated the acid phosphatase histochemistry of central chromatolysis by application of the improved Gomori method (45) to frozen sections of tissue perfuse-fixed in 1% calcium-formalin and subsequently impregnated in gum-sucrose (22). Their studies confirmed the heightened acid phosphatase activity of chromatolytic neurons described by Bodian and Mellors (6). Further, they demonstrated a localization of the enzyme to granules or droplets (lysosomes) which increased in number and size in the cytoplasm of chromatolyzed cells. The results detailed in the present study of lateral geniculate body neurons show that, in contrast to the situation which obtains in central chromatolysis, retrograde neuronal degeneration is accompanied by a dramatic loss of acid phosphatase activity, this alteration being apparent within five days of the operative insult whether the Gomori method or the Barka-Anderson technique is used. Since the function of non-specific acid phosphatase is unknown,

it is not possible to establish the significance of the differing behavior of the enzyme in the two neuronal responses under consideration. However, it is tempting to speculate, in the case of central chromatolysis, that increased acid phosphomonoesterase activity may be concerned with the mobilization of phosphate for synthesis of compounds, such as nucleotides and phospholipids (7, 13, 49), which are constituted at an increased rate during axon regrowth. Conversely, the reduction in enzyme associated with retrograde neuronal degeneration may be an adaptation to or reflection of the diminished metabolism of the nerve cell undergoing atrophy. Enzymes, in common with other proteins, undergo turnover (50, 51), and the loss of acid phosphatase from damaged lateral geniculate body neurons may be a consequence of a general failure of protein synthesis and a resultant unbalanced catabolism of enzyme protein within that structure.

The assumption that the acid phosphatase-positive droplets or granules seen in Gomori preparations by light microscopy are lysosomes requires some amplification. Electron micrographs of neural tissue reacted for acid phosphatase have demonstrated the presence of enzyme in Golgi apparatus (46) in addition to dense bodies (lysosomes) and endoplasmic reticulum (52). The

amount of reaction product demonstrable in the last-named structure is apparently minor quantitatively and may not contribute to light microscopic pictures. The lamellar loci of reaction product described here for lateral geniculate body neurons probably represent the disposition of enzyme within the limiting membranes of the Golgi apparatus. Similar lamellar distributions may be seen under the light microscope in other types of nerve cell (42). Nevertheless, the bulk of the reaction product of Gomori preparations of lateral geniculate body neurons is localized to discrete cytoplasmic bodies which, by virtue of their possession of acid phosphatase activity, may be considered to be lysosomes, at least tentatively (52,53,56). If acid phosphatase as demonstrated by the Gomori technique is assumed to be a morphologic "marker" for lysosomes, the rapid loss of Gomori-positive bodies from injured lateral geniculate body neurons could indicate that lysosomes are in a state of active turnover and disappear quickly from cells undergoing atrophy whose synthetic capacities are impaired. It has been suggested that rat liver lysosomes have a life span of between 15 and 30 days (55). By 14 days after cortical resection, most atrophic lateral geniculate body nerve cells contain a much-reduced population of acid phosphatase-positive droplets, a

fact which might support the accuracy of the lower range of the life-span suggested for lysosomes by deDuve (55). However, our speculations regarding the turnover of the structure or structures included under the term lysosome must be carefully qualified, since they are based upon a single histochemical criterion - the possession of acid phosphatase activity. It is a plausible assumption that the turnover rate of the structural matrix of an organelle could differ from the enzymes it contains and that, under pathologic conditions, cytochemical methods for these enzymes, such as the Gomori or other procedures, might not be valid indicators of the presence or absence of the anatomic entity they normally delineate. Thus estimates of the life span of the organelle may only reflect the life span of the enzymatic material within the matrix and not the lysosome itself.

Retrograde neuronal degeneration is a regressive cellular response to injury which terminates eventually in cell death (1, 5). However, prominent, swollen droplets of acid phosphatase activity (cytolysomes) such as described in damaged cells in other situations (56, 57) and often interpreted as an indicator of fatal injury, were not observed in lateral geniculate body neurons undergoing retrograde atrophy. Enlarged

droplets rich in acid phosphatase were discerned only in glial cells activated by the degenerative process. It would seem that the role played by lysosomes in physiological events, wherein cellular constituents are broken down prior to subsequent re-utilization in synthetic processes, should be stressed. Thus, the increase in lysosomal number which accompanies axon regeneration by chromatolytic neurons may be an expected accompaniment of the heightened turnover of metabolites that has been demonstrated to occur in these regenerating cells (7, 13, 49). Conversely, where metabolic turnover is depressed, as, presumably, in the course of the slow dissolution of lateral geniculate body neurons following cortical resection, the gradual disappearance of lysosomes from the affected cells might not be unexpected.

No certain alterations in distribution or staining intensity of neuropilar hydrolysis of 5-mononucleotides accompanied retrograde atrophy of lateral geniculate body. In contrast, transneuronal atrophy following ocular extirpation is accompanied by a striking loss of 5-nucleotidase activity from denervated laminae (62).

The appearance of the Golgi apparatus in nucleoside diphosphatase preparations of lateral geniculate body neurons

undergoing retrograde atrophy differs markedly from the appearance of that organelle in chromatolytic cervical motoneurons (12). "Retispersion" of Golgi membranes to the periphery of the cell body, an alteration characteristic of light microscope pictures of axon reaction in bulbospinal motor neurons and posterior root ganglion nerve cells (63, 64) is not encountered in lateral geniculate body neurons after cortical extirpation. Rather there is a gradual loss of stainability of the neuronal Golgi apparatus in the enzyme preparations with, rarely, an apparent condensation of nucleoside diphosphatase-reactive lamellae about the nucleus.

Retrograde neuronal atrophy is accompanied by early loss of A- and B- esterases from nerve cell droplets and cytoplasm. Comparisons of the behavior of the A- and B- enzymes in retrograde atrophy and central chromatolysis cannot be made since these enzymes have not been studied in cells exhibiting the latter phenomenon.

The experiments described here did not provide evidence of loss of acetylcholinesterase activity from any part of the nucleus as a result of cortical extirpation.

The possibility that the histochemical and histological alterations described in lateral geniculate body neurons after

cortical resection may be partly transneuronal seems remote. It has been remarked that afferents to lateral geniculate body from visual cortex are relatively inconsequential (76). The possibility has even been considered that degenerating fibers observed in Nauta preparations of lateral geniculate body after lesions in striate cortex derive from degenerating axons of the atrophied lateral geniculate body nerve cells (78). Further, transneuronal changes in lateral geniculate body after removal of an eye occur much more slowly than retrograde atrophy after cortical resection (62).

It may be that neurons whose processes are confined to the central nervous system generally respond to axon section by cytochemical changes resembling those of lateral geniculate body neurons and differing from those described for axon reaction in peripheral motor and sensory nerve cells.

Attention has been given (82) to the possible roles of glial or connective tissue scars in the prevention of axon regeneration in central tracts. It may be, however, that the key to failure of regeneration of severed axons in the central nervous system lies in the nature of the reaction of the parent soma rather than in local conditions at the site of nerve fiber interruption.

SUMMARY AND CONCLUSIONS

Retrograde atrophy of lateral geniculate body neurons following resection of the visual cortex is associated with a loss of acid phosphatase activity. This has been demonstrated by both Gomori and Barka-Anderson preparations. Nerve cells also show a loss of nucleoside diphosphatase activity manifested by diminished stainability of the Golgi apparatus. These alterations contrast conspicuously with changes during central chromatolysis. In the latter process, acid phosphatase activity is increased following axon section. It is suggested that, in the case of central chromatolysis, increased acid phosphatase activity may be concerned with the mobilization of phosphate for synthesis of compounds such as nucleotides and phospholipids (7, 13, 49), which are produced at an increased rate during axon regrowth. Conversely, the reduction in enzyme associated with retrograde neuronal degeneration may be an adaptation to or reflection of the diminished metabolism of the nerve cell undergoing atrophy. The decrease and eventual loss of metabolic activity as demonstrated by the enzymes studied in this project suggests that the failure of regeneration of severed axons in the central nervous system is the result of the nature of the reaction of the neurons whose axons are severed.

BIBLIOGRAPHY

1. Lashley, K. S. The Mechanism of Vision. The Projection of the Retina upon the Cerebral Cortex of the Rat. *J. Comp. Neurol.*, 60:57, 1934.
2. Brodal, A. Modification of Gudden Method for the Study of Cerebral Localization. *Arch. Neurol. & Psychiat.*, 43:45, 1940.
3. Rose, J. E. and Woolsey, C. N. A Study of Thalamo-Cortical Relations in the Rabbit. *Bull. Johns Hopk. Hosp.*, 73:65, 1943.
4. Clark, W. E. L. and Powell, T. P. S. On the Thalamo-Cortical Connexions of the General Sensory Cortex of Macaca. *Proc. Roy. Soc. B.*, 141:467, 1953.
5. Torvik, A. The Ascending Fibers from the Main Trigeminal Sensory Nucleus. An Experimental Study in the Cat. *Amer. J. Anat.*, 100:1, 1957.
6. Bodian, D. and Mellors, R. C. The Regenerative Cycle of Motoneurons, with Special Reference to Phosphatase Activity. *J. Exp. Med.*, 81:469, 1945.
7. Brattgard, S. O., Edstrom, J. E. and Hyden, H. The Chemical Changes in Regenerating Neurons. *J. Neurochem.*, 1:316, 1957.
8. Schwarzscher, H. G. Der Cholinesterasegehalt Motorischer Nervenzellen Wahrend der Axonalen Reaktion. *Acta Anat.*, 32:51, 1958.
9. Friede, R. Transport of Oxidative Enzymes in Nerve Fibers; a Histochemical Investigation of the Regenerative Cycle in Neurons. *Exptl. Neurol.*, 1:441, 1959.
10. Barron, K. D. and Sklar, S. Response of Lysosomes of Bulbo-spinal Motoneurons to Axon Section. *Neurology*. 11:866, 1961.

11. Robins, E., Kossana, J. M. and Lowe, K. P. Quantitative Biochemical Studies of Chromatolysis. In: Chemical Pathology of the Nervous System. Folch-Pi, J. (Ed.). New York, Pergamon Press, 1961.
12. Barron, K. D. and Tunobay, T. O. Phosphatase Histochemistry of Feline Cervical Spinal Cord after Brachial Plexectomy. J. Neuropath. Exper. Neurol., 23:368, 1964.
13. Brattgard, S. O., Hyden, H. and Sjostrand, J. Incorporation of Orotic Acid - ^{14}C and Lysine - ^{14}C in Regenerating Single Nerve Cells. Nature, 182:801, 1958.
14. Fischer, J., Lodin, Z. and Kolonsek, J. A Histoautoradiographic Study of the Effect of Section of the Facial Nerve on the Uptake of Methionine - ^{35}S by the Cells of the Facial Nerve Nucleus. Nature, 181:341, 1958.
15. Rhodes, A., Ford, D. and Rhines, R. Comparative Uptake of DL-Lysine- H^3 by Normal and Regenerative Hypoglossal Nerve Cells in Euthyroid, Hypothyroid and Hyperthyroid Male Rats. Exptl. Neurol., 10:251, 1964.
16. Hudson, G., Lazarow, A. and Hartmann, J. F. A Quantitative Electron Microscopic Study of Mitochondria in Motor Neurons Following Axonal Section. Exp. Cell Res., 24:440, 1961.
17. Navarro, J. Elektronenmicroscopische Befunde an Spinalganglionzellen der Ratte nach Ischiadikotomie. In: Proc. 4th Internat. Congr. Neuropath., Stuttgart, verlag, G. T., 1962, p. 99-104.
18. Bodian, D. An Electron-Microscopic Study of the Monkey Spinal Cord. Bull. Johns Hopk. Hosp., 114:13, 1964.
19. Mackey, E. A., Spiro, D. and Wiener, J. A Study of Chromatolysis in Dorsal Root Ganglia at the Cellular Level. J. Neuropath. & Exper. Neurol., 23:508, 1964.
20. Gomori, G. Microscopic Histochemistry; Principles and Practice. Chicago, Univ. of Chicago Press, 1952.

21. Novikoff, A. B. and Goldfischer, S. Nucleoside Diphosphatase Activity in the Golgi Apparatus and its Usefulness for Cytological Studies. *Proc. Natl. Acad. Sci.*, 47:802, 1961.
22. Holt, S. J. Factors Governing the Validity of Staining Methods for Enzymes, and their Bearing upon the Gomori Acid Phosphatase Technique. *Exp. Cell Res.*, (Suppl. 7): 1, 1959.
23. Novikoff, A. B. Lysosomes and the Physiology and Pathology of Cells (Abstract). *Biol. Bull.*, 117:385, 1959.
24. Novikoff, A. B., Goldfischer, S. and Essner, E. The Importance of Fixation in a Cytochemical Method for the Golgi Apparatus. *J. Histochem. Cytochem.*, 9:459, 1961.
25. Novikoff, A. B., Drucker, J., Shin, W. Y. and Goldfischer, S. Further Studies of the Apparent Adenosinetriphosphatase Activity of Cell Membranes in Formol-Calcium-Fixed Tissues. *J. Histochem. & Cytochem.*, 9:434, 1961.
26. Barron, K. D. and Tuncbay, T. O. Phosphatases of Cuneate Nuclei after Brachial Plexectomy. *Arch. Neurol.*, 7:203, 1962.
27. Shute, C. C. D. and Lewis, P. R. Cholinesterase-Containing Systems of the Brain of the Rat. *Nature*, 199:1160, 1963.
28. Friede, R. L. and Knoller, M. Quantitative Tests of Histochemical Methods for Phosphomonoesterases in Brain Tissue. *J. Histochem. & Cytochem.*, 13:125, 1965.
29. Janigan, D. T. Tissue enzyme fixation studies. 1. The effects of aldehyde fixation on B-glucuronidase, B-galactosidase, N-acetyl-B-glucosaminidase, and B-glucosidase in tissue blocks. *Lab. Invest.*, 13:1038, 1964.
30. Seligman, A. M., Chauncey, H. H. and Nachlas, M. M. Effect of Formalin Fixation on the Activity of Five Enzymes of Rat Liver. *Stain Technol.*, 26:19, 1951.

31. Hunter, R. L. and Markert, C. L. Histochemical Demonstration of Enzymes Separated by Zone Electrophoresis in Starch Gels. *Science*, 125:1294, 1957.
32. Smithies, O. An Improved Procedure for Starch-Gel Electrophoresis: Further variations in the Serum Proteins of Normal Individuals. *Biochem. J.*, 71:585, 1959.
33. Barka, T. and Anderson, P. J. Histochemical Methods for Acid Phosphatase Using Hexazonium Pararosaniline as Coupler. *J. Histochem. & Cytochem.*, 10:741, 1962.
34. Wachstein, M. and Meisel, E. Histochemistry of Hepatic Phosphatases at a Physiologic pH with Special Reference to the Demonstration of Bile Canaliculi. *Am. J. Clin. Path.*, 27:13, 1957.
35. Barron, K. D. and Boshes, B. Histochemical Demonstration of 5-Nucleotidase in Central Nervous System: Effects of Manganous Ion and pH. *J. Histochem. & Cytochem.*, 9:455, 1961.
36. Lehrer, G. M. and Ornstein, L. A Diazo Coupling Method for the Electron Microscopic Localization of Cholinesterase. *J. Biophysic. Biochem. Cytol.*, 6:399, 1959.
37. Windle, W. F., Rhines, R. and Rankin, J. A Nissl Method Using Buffered Solutions of Thionin. *Stain Technol.*, 18:77, 1943.
38. Chiffelle, T. L. and Putt, F. A. Propylene and Ethylene Glycol as Solvents for Sudan IV and Sudan Black B. *Stain Technol.*, 26:51, 1951.
39. Hirano, A. and Zimmerman, H. M. Silver Impregnation of Nerve Cells and Fibers in Celloidin Sections. *Arch. Neurol.*, 6:114, 1962.
40. Barron, K. D., Bernsohn, J. and Hess, A. R. Separation and Properties of Human Brain Esterases. *J. Histochem. & Cytochem.*, 11:139, 1963.

41. Barron, K. D., Bernsohn, J. and Hess, A. R. Zymograms of Neural Acid Phosphatases. Implications for Slide Histochemistry. *J. Histochem. & Cytochem.*, 12:42, 1964.
42. Barron, K.D. Enzyme Histochemistry of Central Nervous System. In: *Neuropathology*, Minckler, J. (ed.). In Press.
43. Barron, K. D. and Oldershaw, J. B. Cytochemical Studies of Transneuronal Degeneration. In preparation.
44. Gomori, G. Distribution of Acid Phosphatase in the Tissues under Normal and under Pathologic Conditions. *Arch. Path.*, 32:189, 1941.
45. Gomori, G. An Improved Histochemical Technic for Acid Phosphatase. *Stain Technol.*, 25:81, 1950.
46. Novikoff, A. B. and Essner, E. Pathological Changes in Cytoplasmic Organelles. *Fed. Proc.*, 21:1130, 1962.
47. Kawai, H. Cytoplasmic Granules in Nerve Cells of the Spinal Cord Following Transection of Sciatic Nerve of Guinea Pig. *Exptl. Neurol.*, 7:457, 1963.
48. Coimbra, A. and Tavares, A. S. Deoxyribonuclease II, Acid Ribonuclease and Acid Phosphatase Activities in Normal and Chromatolytic Neurons. *Histochemie*, 3:509, 1964.
49. Miani, N. Metabolic and Chemical Changes in Regenerating Neurons. III. The Rate of Incorporation of Radioactive Phosphate into Individual Phospholipids of the Nerve-Cell Perikaryon of the C₈ Spinal Ganglion in vitro. *J. Neurochem.*, 9:537-541, 1962.
50. McConnell, K. P. and Dallam, R. D. Time-Distribution Examination of the in vivo Incorporation of Selenium into Cytochrome-C of the Rat and Its Turnover. *Nature*, 193:746, 1962.

51. Schapira, G., Kruk, J., Dreyfus, J. C. and Schapira, P. The Molecular Turnover of Muscle Aldolase. *J. Biol. Chem.*, 235:1738, 1960.
52. Novikoff, A. B. Lysosomes in the Physiology and Pathology of Cells: Contributions of Staining Methods. IN: Lysosomes. Giba Foundation Symposium. de Rauck, A.V.S. and Cameron, M.P. (eds.). Boston, Little, Brown and Co., 1963.
53. Novikoff, A. B., Essner, E. and Quintana, N. Golgi Apparatus and Lysosomes. *Fed. Proc.*, 23:1010, 1964.
54. Novikoff, A. B. Lysosomes and Related Particles. In: The Cell. Biochemistry, Physiology, Morphology. Vol. 2. Cells and Their Component Parts. Brachet, J. and Mirsky, E. (eds.). New York, Academic Press, Inc., 1961.
55. deDuve, C. The Lysosome Concept. In: Lysosomes. Giba Foundation Symposium. deRauck, A.V.S. and Cameron, M.P. (eds.). Boston, Little, Brown and Co., 1963.
56. Novikoff, A. B. Biochemical and Staining Reactions of Cytoplasmic Constituents. In: Developing Cell Systems and Their Control. Rudnick, D. (ed.). New York, Ronald Press, 1960.
57. Becker, N. H. and Barron, K. D. The Cytochemistry of Anoxic and Anoxic-Ischemic Encephalopathy in Rats. *Amer. J. Path.*, 38:161, 1961.
58. Barron, K. D. Enzymatic Hydrolysis of Adenosine-5-Phosphate in Nervous Tissues (Abstract). *J. Neuropath. & Exper. Neurol.*, 21:324, 1962.
59. Essner, E., Novikoff, A. B. and Quintana, N. Nucleoside Phosphatase Activities in Rat Cardiac Muscle. *J. Cell Biol.*, 25:201, 1965.
60. Lane, N. J. and Novikoff, A. B. Effects of Arginine Deprivation, Ultraviolet Radiation, and X-radiation on Cultured KB Cells. A Cytochemical and Ultrastructural Study. *J. Cell Biol.*, 27:603, 1965.

61. Scott, T. G. The Specificity of 5'-Nucleotidase in the Brain of the Mouse. *J. Histochem. & Cytochem.*, 13:657, 1965.
62. Barron, K. D. and Oldershaw, J. B. Cytochemistry of Lateral Geniculate Body: Transneuronal and Retrograde Neuronal Degeneration (Abstract). *Neurology*, 15:289, 1965.
63. Marcora, F. Sur les alterations de l'appareil reticulaire interne des cellules nervouses motrices, consecutives a des lesions des nerfs. *Arch. Ital. de Biol.*, 53:346, 1910.
64. Penfield, W. G. Alterations of the Golgi Apparatus in Nerve Cells. *Brain*, 43:290, 1920.
65. Barron, K. D., Oldershaw, J. B. and Doolin, P. P. Cytochemistry and Fine Structure of Retrograde Neuronal Degeneration in Lateral Geniculate Body (Abstract). *J. Neuropath. & Exper. Neurol.*, 25:135, 1966.
66. Torack, R. M. Adenosine Triphosphatase Activity in Rat Brain Following Differential Fixation with Formaldehyde, Glutaraldehyde and Hydroxyadipaldehyde. *J. Histochem. Cytochem.*, 13:191, 1965.
67. Aldridge, W. N. Serum Esterases. 1. Two Types of Esterase (A and B) Hydrolyzing p-Nitrophenyl Acetate, Propionate and Butyrate, and a Method for their Determination. *Biochem. J.*, 53:110, 1953.
68. Bernsohn, J., Barron, K. D. and Hedrick, M. T. Distribution of Rat Brain Esterases and their Differential Inhibition by Organophosphorus Inhibitors in Cell Particulate Fractions in vivo. *Anat. Rec.* 151:323, 1965.
69. Shnitka, T. K. and Seligman, A. M. Role of Esteratic Inhibition on Localization of Esterase and the Simultaneous Cytochemical Demonstration of Inhibitor Sensitive and Resistant Enzyme Species. *J. Histochem. Cytochem.*, 9:504, 1961.

70. Wachstein, M., Meisel, E. and Falcon, C. Histochemistry of Thiolacetic Acid Esterase: A Comparison with Non-specific Esterase with Special Regard to the Effect of Fixatives and Inhibitors on Intracellular Localization. *J. Histochem. Cytochem.*, 9:325, 1961.
71. Holt, S. J. Some Observations on the Occurrence and Nature of Esterases in Lysosomes. In: *Lysosomes*. Ciba Foundation Symposium. deReuck, A.V.S. and Cameron, M.P. (eds.). Boston, Little, Brown and Co., 1963.
72. Miller, F. and Palade, G. E. Lytic Activities in Renal Protein Absorption Droplets. An Electron Microscopical Cytochemical Study. *J. Cell Biol.*, 23:519, 1964.
73. Gordon, G. B., Miller, L. R. and Bensch, K. G. Studies on the Intracellular Digestive Process in Mammalian Tissue Culture Cells. *J. Cell Biol.*, 25:41, 1965.
74. Kanczak, N. M., Krall, J. I., Hayes, E. R. and Elliott, W.B. Lysosomal Fractions from Transitional Epithelium. *J. Cell Biol.*, 24:259, 1965.
75. Meikle, T. H. and Sprague, J. M. The Neural Organization of the Visual Pathways in the Cat. In: *International Review of Neurobiology*, vol. 6. Pfeiffer, C. C. and Smythies, J. R. (eds.). New York, Academic Press, 1964.
76. Altman, J. Some Fiber Projections to the Superior Colliculus in the Cat. *J. Comp. Neur.*, 119:77, 1962.
77. Barron, K. D., Bernsohn, J. and Hess, A. R. Proteins and Esterases in Normal and Atrophic Feline Muscle. *J. Histochem. Cytochem.* In Press.
78. Beresford, W. A. A Nauta and Galloccyanin Study of the Cortico-Lateral Geniculate Projection in the Cat and Monkey. *J. Hirnforsch.*, 5:210, 1962.
79. Gonda, V. E. and Malamud, N. Retrograde Degeneration of the Thalamus Following Self-Inflicted Prefrontal Lobotomy. Comparative Study with Cases of Surgical Lobotomy. *Tr. Am. Neur. Ass.*, 79:192, 1954.

80. Levin, P. M. and Bradford, F. K. The Exact Origin of the Cortico-spinal Tract in the Monkey. J. Comp. Neur., 68:411, 1938.
81. Albers, W. R. in discussion of Pope, A. Implication of Histochemical Studies for Metabolism of the Neuroglia. In: Biology of Neuroglia. Windle, W. F. (ed.). Springfield, C. C. Thomas, 1958.
82. Clemente, C. D. Regeneration in the Vertebrate Central Nervous System. Internat. Rev. Neurobiol. 6:257, 1964.

PLATE I

- Figure 1. Normal lateral geniculate body. Droplets (lysosomes) in large neurons are more densely packed than in neurons of small (short arrows) or intermediate (long arrows) size (480X).
- Figure 2. Normal lateral geniculate body. Lightly-stained, lamellar configurations of reaction product (arrow) contrast with darker droplets. Note lysosomes in glia (G) (1536X).
- Figure 3. Left lateral geniculate body five days post-operatively. Note large droplets (tending to coalesce) associated with vascular cell (V). Vascular activity was always more prominent on the side of operation (856X).
- Figure 4. Left lateral geniculate body at 14 days. Note that few neurons are visible though outlines of two atrophic nerve cells containing a relatively sparse population of lysosomes are discernible at arrows. Large extra-neuronal droplets of acid phosphatase activity are scattered through the tissue. Vessel above V (1230X).

Figures 1 to 4 are of Gomori preparations.

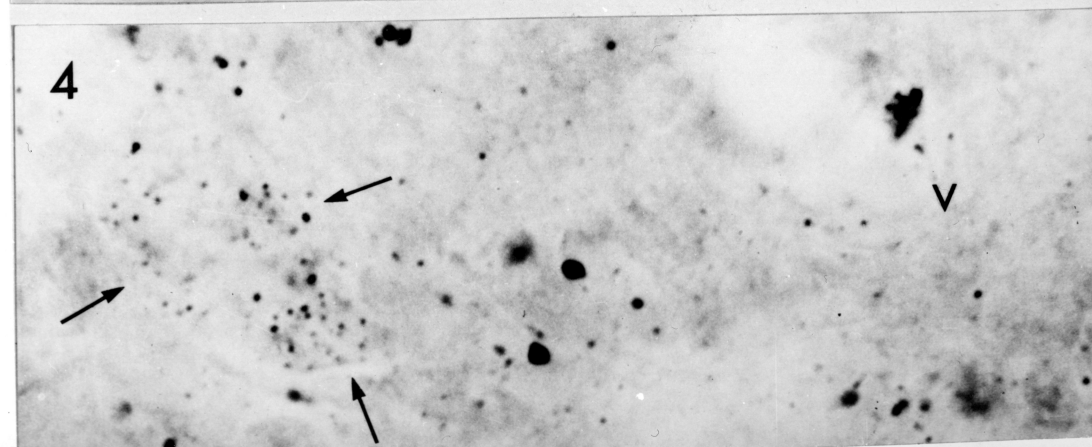
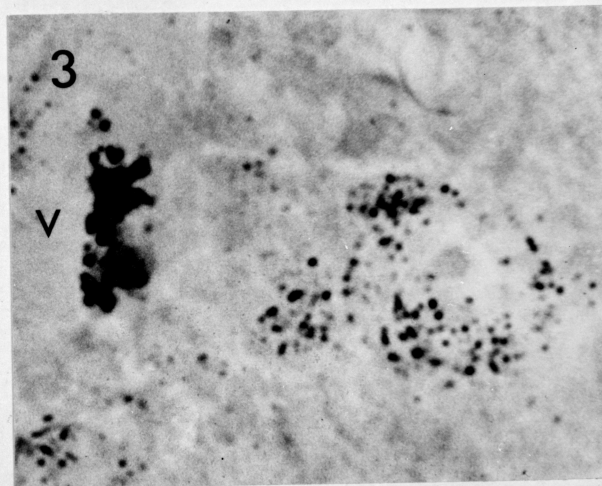
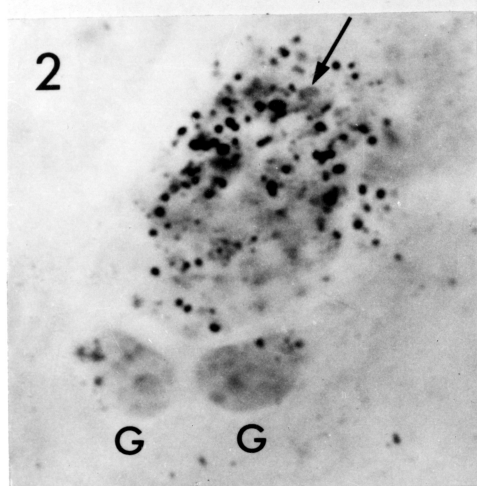
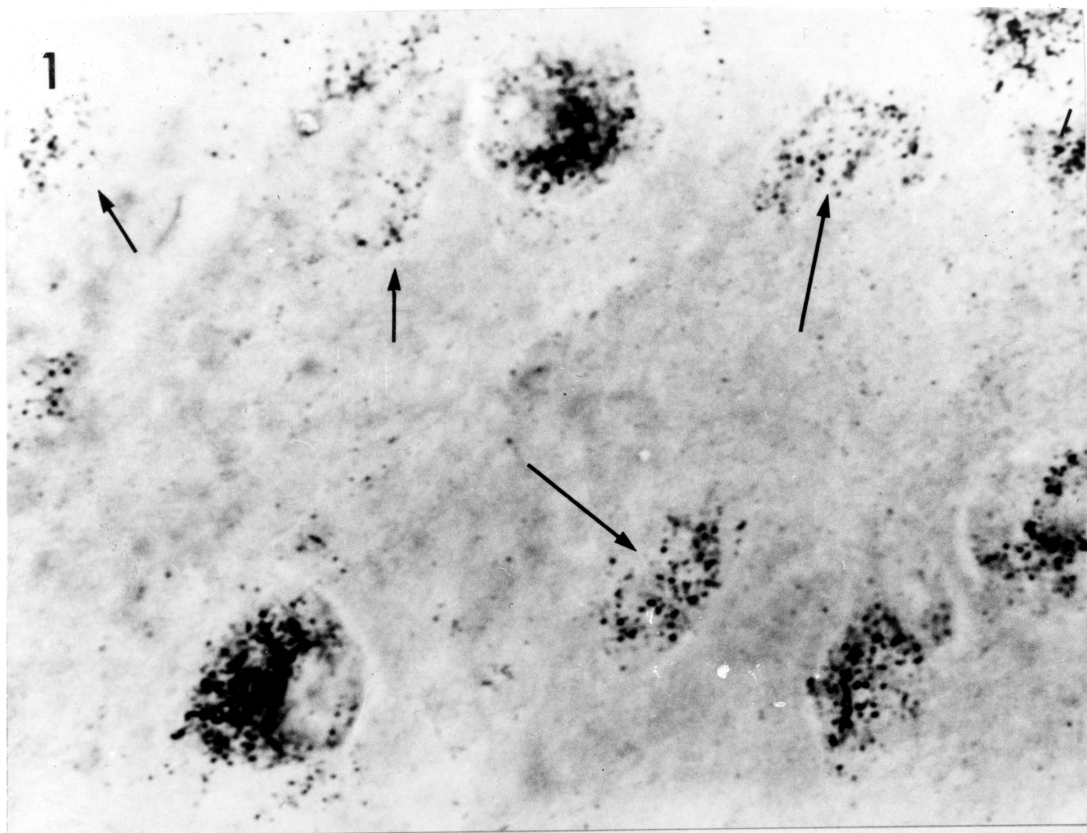


PLATE II

Figure 5. Left lateral geniculate body at fourteen days. Because of loss of stained lysosomes, neurons (arrows) are not clearly outlined. Note scattered large droplets. Compare with normal structure, Figure 1 (480X).

Figure 5 is of Gomori preparation.

5

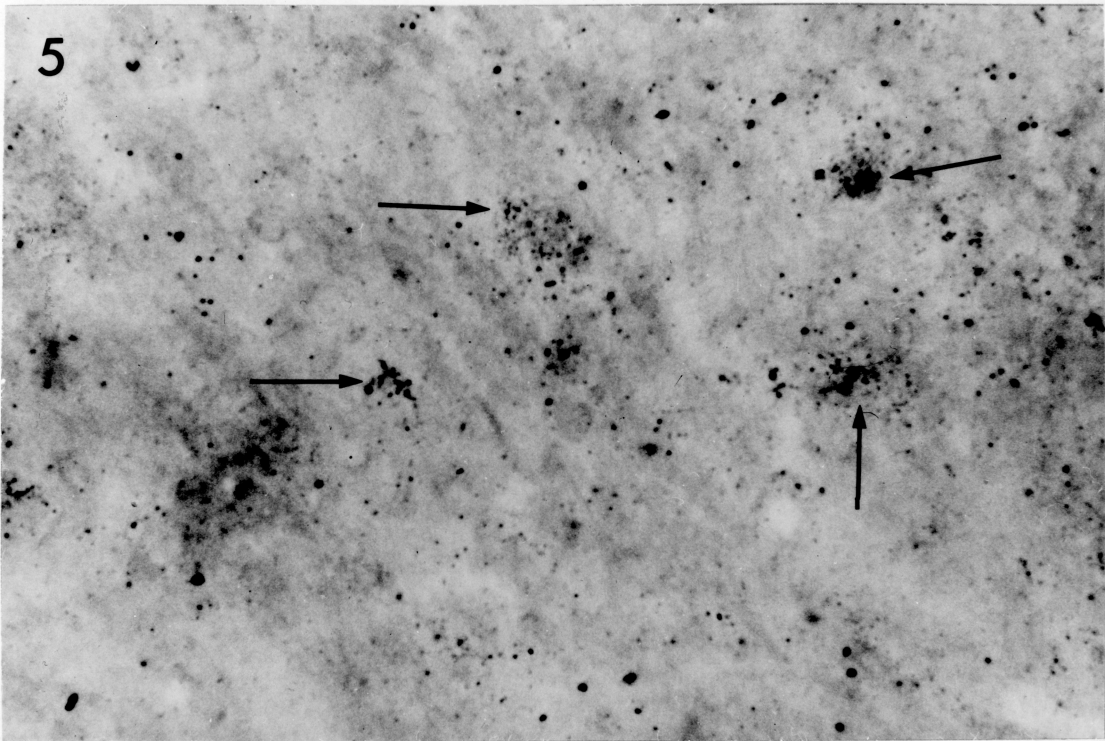


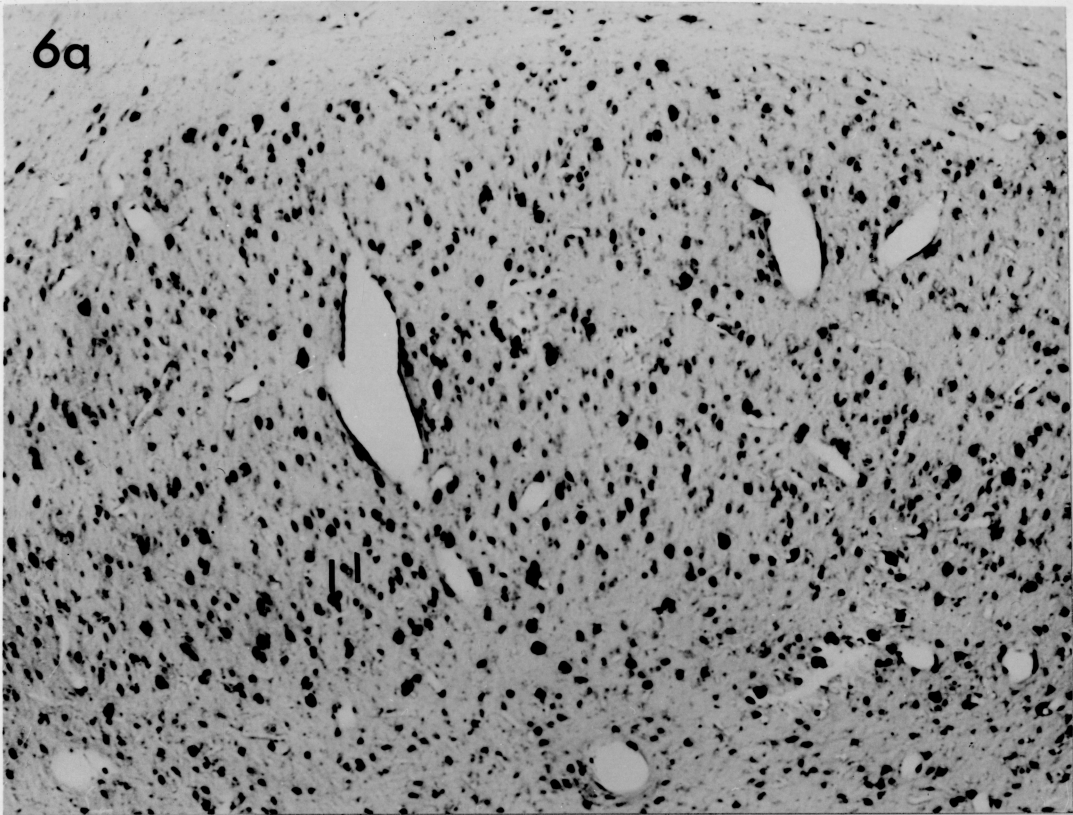
PLATE III

Figure 6a. Normal lateral geniculate body. Cytoplasm of neurons, especially the larger nerve cells, is stained deeply (about 25X).

Figure 6b. Left lateral geniculate body at 14 days. Atrophic neurons are difficult to discern at this low magnification (about 25X).

Figures 6a and 6b are of Barka-Anderson preparations.

6a



6b

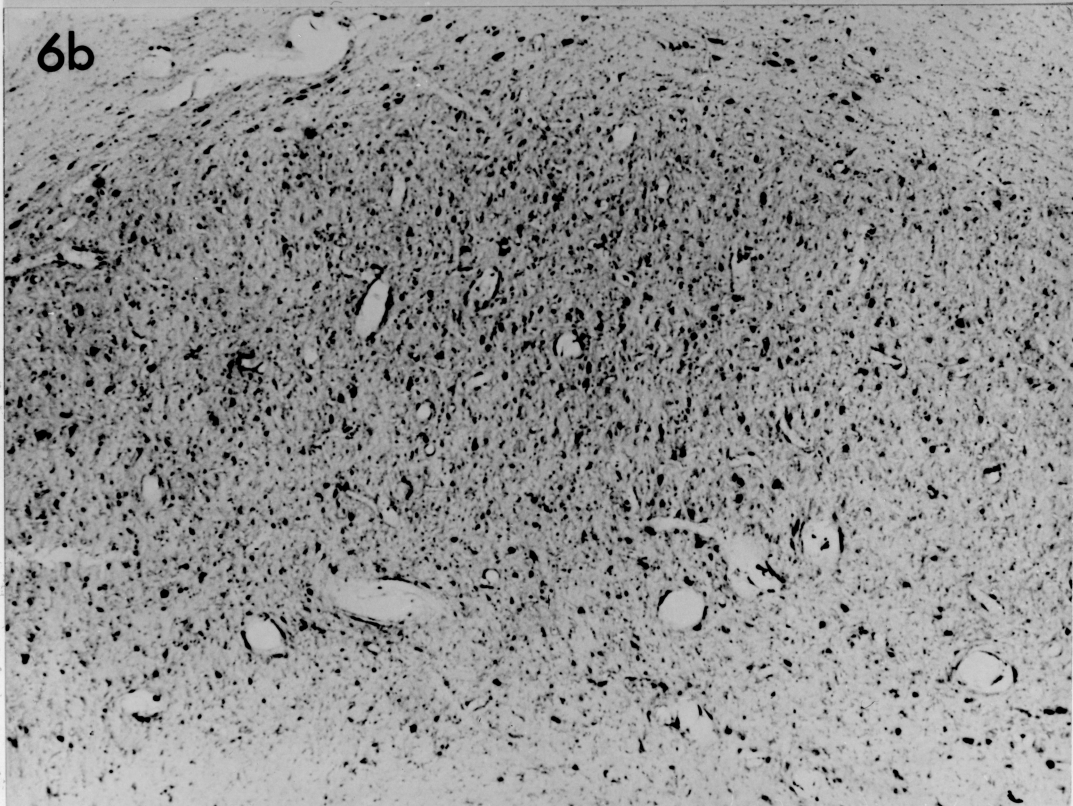


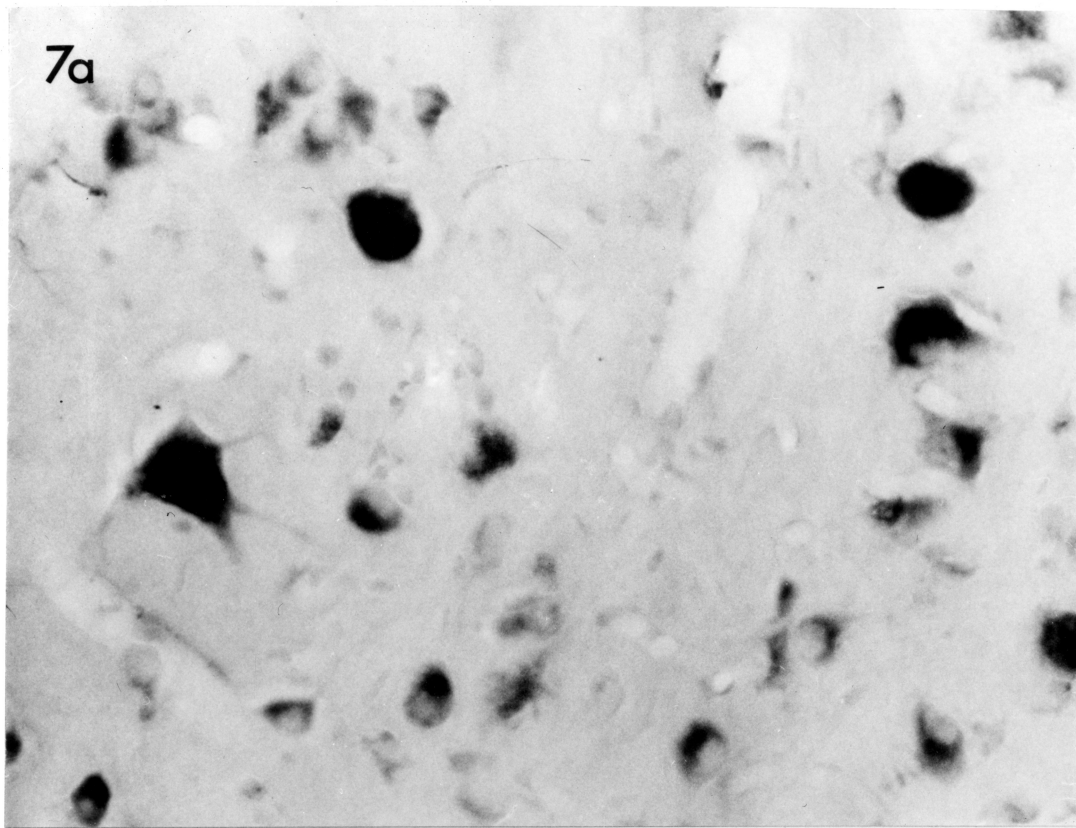
PLATE IV

Figure 7a. Normal lateral geniculate body. Note diffuse staining of neuronal cytoplasm (380X).

Figure 7b. Left lateral geniculate body, 96 days after cortical extirpation. Atrophic neurons are still visible, as at arrows, and their cytoplasm is pallid (380X).

Figures 7a and 7b are of Barka-Anderson preparations.

7a



7b

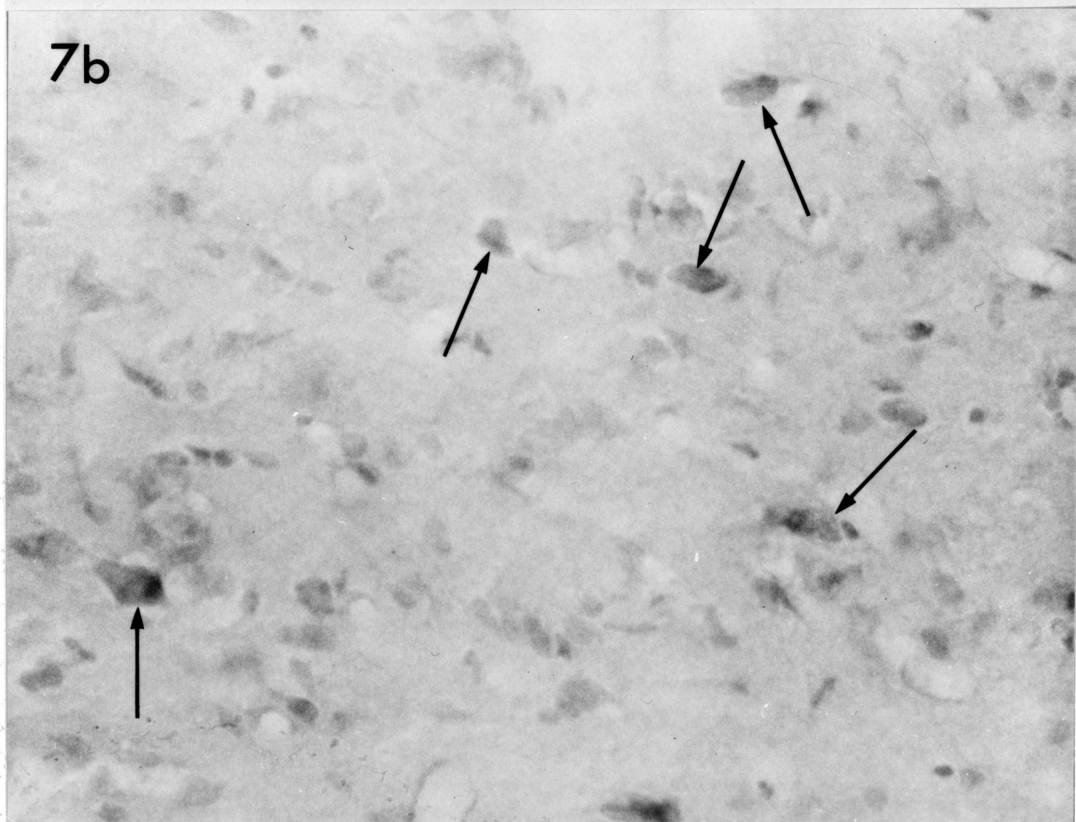


PLATE V

- Figure 8. Incubation for 60 minutes in solution containing $5 \times 10^{-3}M$ 5-cytidylic acid and $10^{-2}M$ manganese nitrate at pH 6.5. Note droplet activity in neurons at N and diffuse staining of neuropil (about 500X).
- Figure 9. Incubation for 90 minutes in solution containing $10^{-3}M$ 5-adenylic acid and $10^{-2}M$ Mn at pH 6.5. A large neuron contains numerous droplets. Neuro-pilar staining is dense (896X).
- Figure 10. Substrate solution as in Figure 9 but sections pre-incubated in pH 6.5 buffer containing $5 \times 10^{-3}M$ p-chloromercuribenzoic acid (PCMB). Neurons are unstained since nonspecific acid phosphatase activity in lysosomes is eliminated (about 500X).
- Figure 11. Substrate solution as for Figures 9 and 10 but sections pre-incubated in acetate buffer at pH 4.0. 5-nucleotidase activity in neuropil is inhibited completely but acid phosphatase activity in neurons appears to be activated (about 500X).

Figures 8 through 11 are of sections incubated in solutions containing 5-mononucleotides.

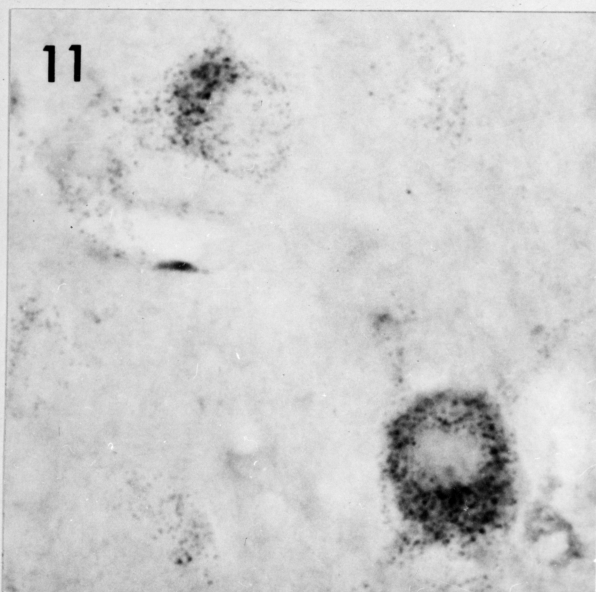
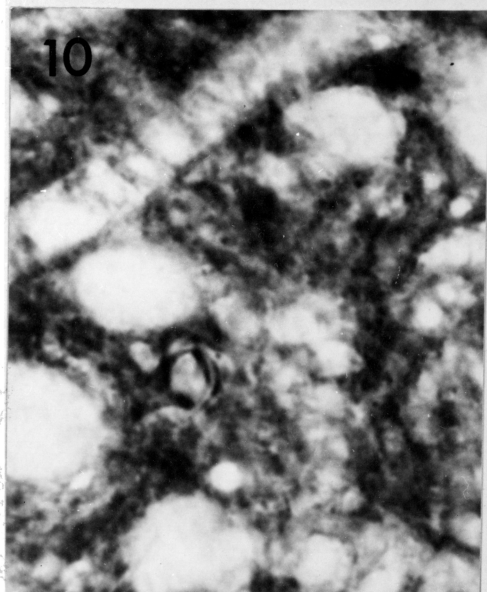
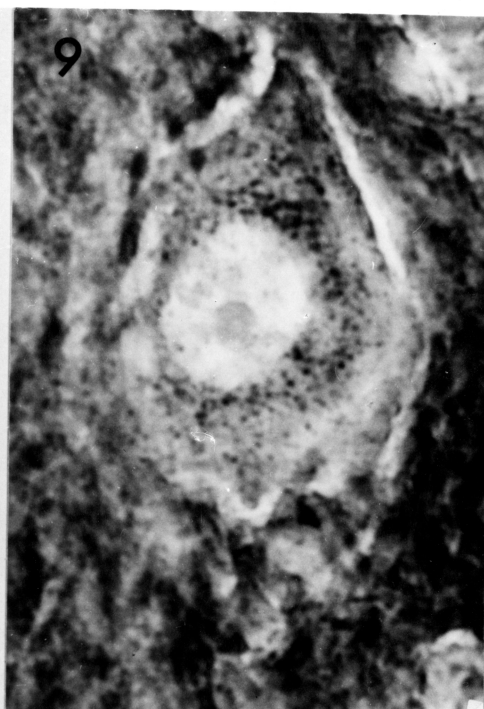
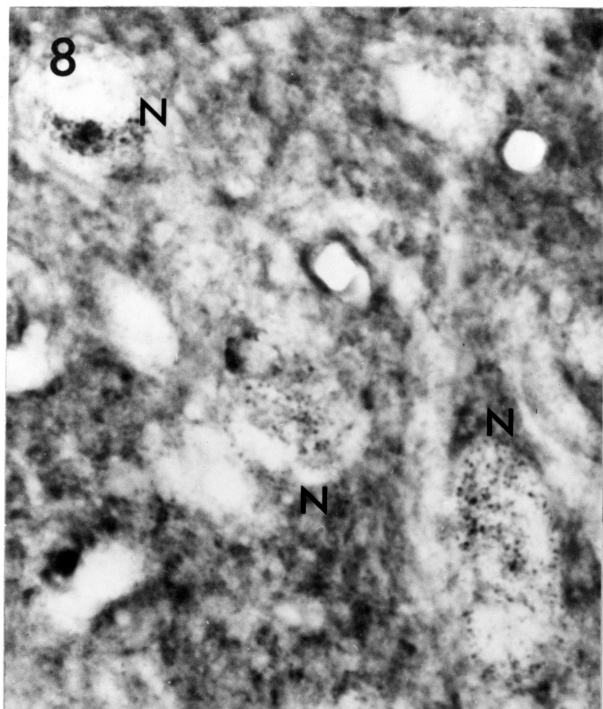


PLATE VI

Figure 12. Normal lateral geniculate body. A halo of peripheral unstained cytoplasm (arrows) bounds the Golgi reticulum of the large neuron. A row of four satellite glia (G) is virtually unstained in contrast to the small neuron above N. Note staining of cytoplasmic extensions of presumed astrocyte and diffuse capillary activity (896X).

Figures 13 and 14. Compare section incubated in solution containing Mn^{++} as in standard substrate medium (Figure 13) with one incubated in solution containing Mg^{++} (Figure 14). Glial processes are virtually absent in the latter but capillary and neuronal (Golgi) activities are sharply localized (about 500X).

Figure 15. Section incubated with Mn^{++} etc. in standard substrate solution but containing 0.75% CH_2O v/v. Glial activity is suppressed completely, vascular enzyme is inhibited but neuronal Golgi apparatus is easily discernible (about 500X).

Figures 12 through 15 derive from sections incubated in solutions containing thiamine pyrophosphate (Novikoff-Goldfischer).

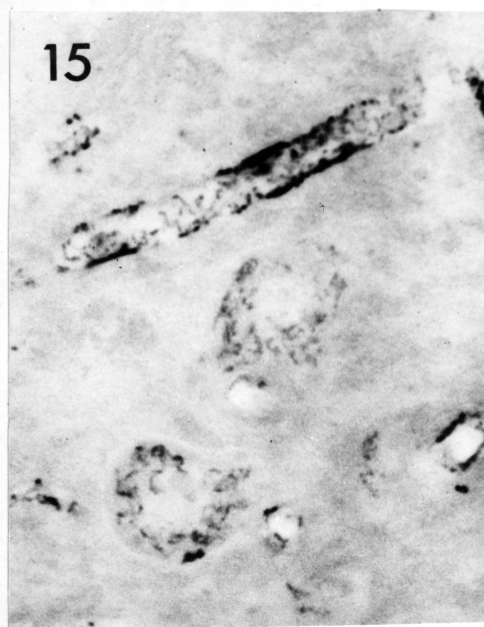
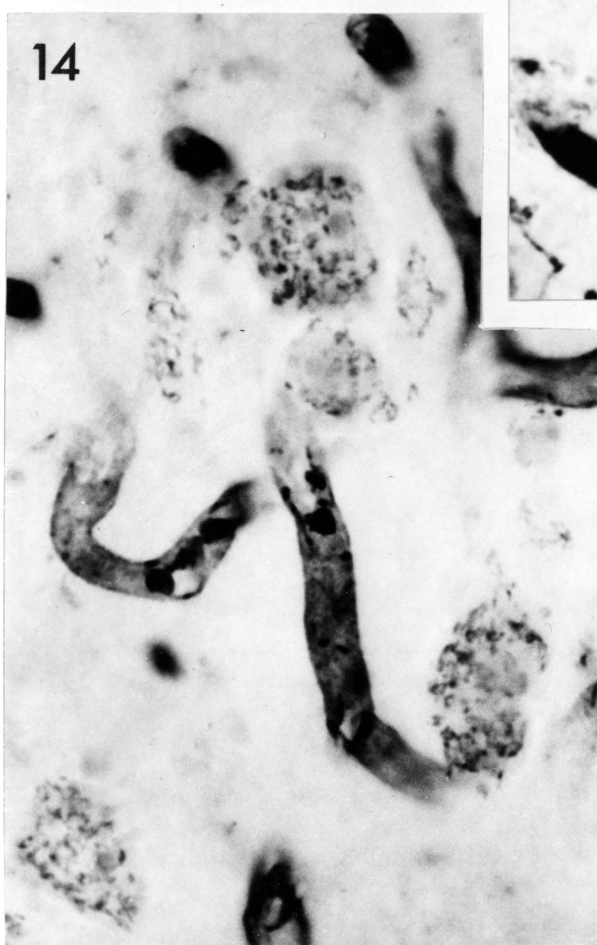
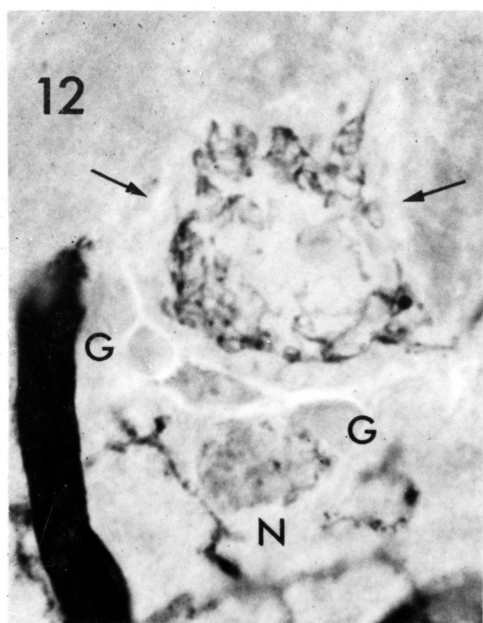


PLATE VII

- Figure 16. Left lateral geniculate body 33 days after operation. Thiamine pyrophosphate with Mn^{++} etc. Plasma membranes of two atrophic neurons are marked by arrows. The peripheral halo of cytoplasm devoid of nucleoside diphosphatase-positive lamellae seems proportionately increased when compared to normal cells and the lamellae appear attenuated. Neuro-pilar, glial and vascular activities are accentuated (896X).
- Figure 17a. Right lateral geniculate body of same animal as 17b. At this magnification vascular staining only is discernible (15X).
- Figure 17b. Left lateral geniculate body 14 days post-operatively. Note intense enzymatic reaction of atrophic lateral geniculate body. Arrow points to dorso-medial extremity of lateral geniculate body which is not involved by retrograde atrophy in these extirpations which are limited to striate cortex (15X).

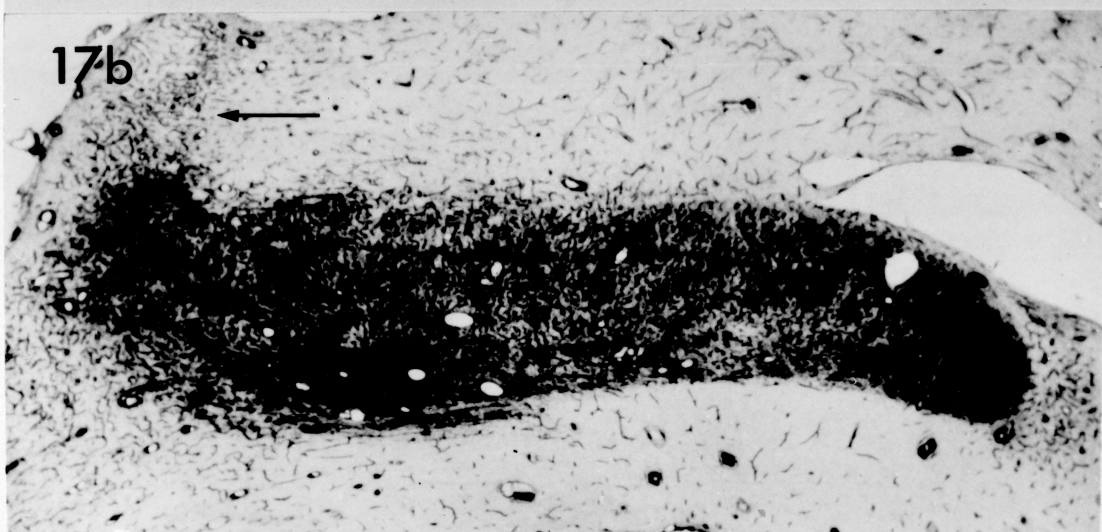
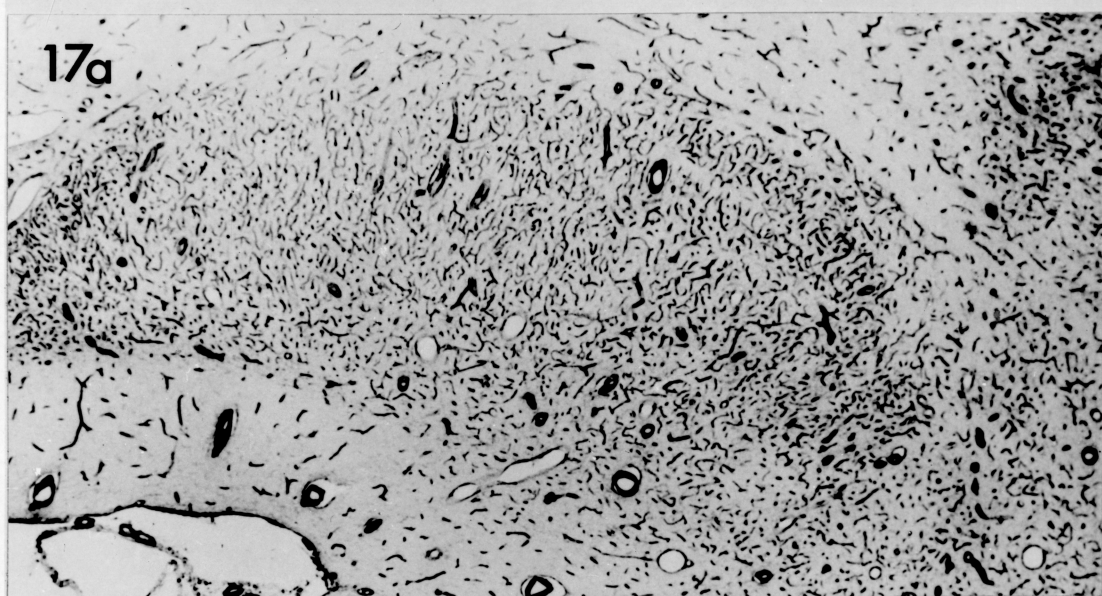
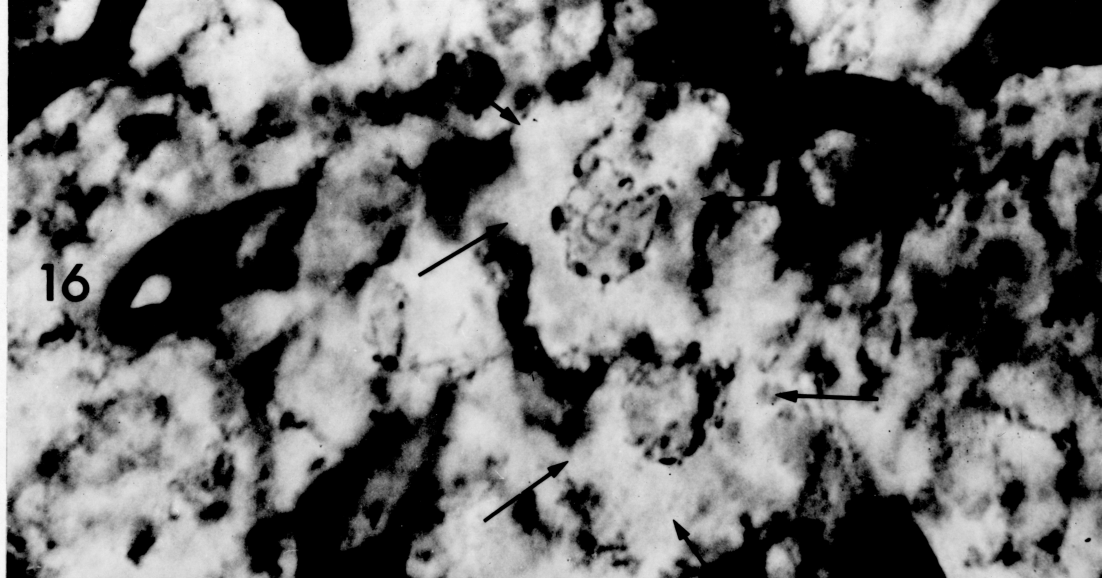


PLATE VIII

- Figure 18. Normal lateral geniculate body. Note glial, vascular and neuropilar staining, the last (as in upper right hand corner) showing patchy foci of concentration. Neurons are unstained (300X).
- Figure 19. Left lateral geniculate body 14 days after operation. There appears overall to be some enhancement of the reaction (300X).
- Figures 18 and 19 are of Wachstein-Meisel adenosine triphosphatase preparations.
- Figure 20. Normal lateral geniculate body. Droplet activity is discernible, especially in the smaller neuron at lower left, against a diffuse cytoplasmic coloration which is greatest in the largest nerve cell. Note droplets in satellite glia. Neuropilar staining is minimized in this high-contrast print (about 2100X).
- Figure 21. Normal lateral geniculate body. Effect of $5 \times 10^{-6}M$ E600. Outlines of neurons at N are only faintly visible as a result of suppression of cytoplasmic activity. Note large droplets of A-type organophosphate-resistant esterase in pericytes (P) and glia (G) (615X).
- Figure 22. Normal lateral geniculate body. Large vacuolated droplets of A-esterase associated with a capillary (about 1800X).
- Figure 23. Sudanophilic droplets of size and configuration similar to those of Figure 22. Oil Red O (about 1800X).

Figures 20, 21, 22 are from sections incubated for nonspecific esterase in the Lehrer-Ornstein substrate.

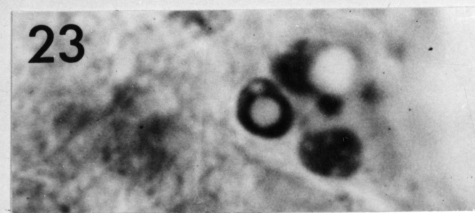
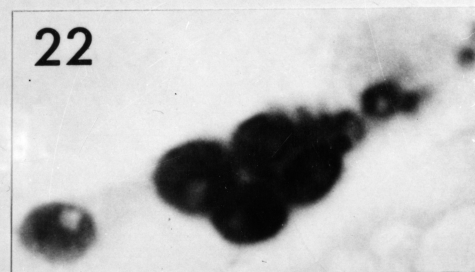
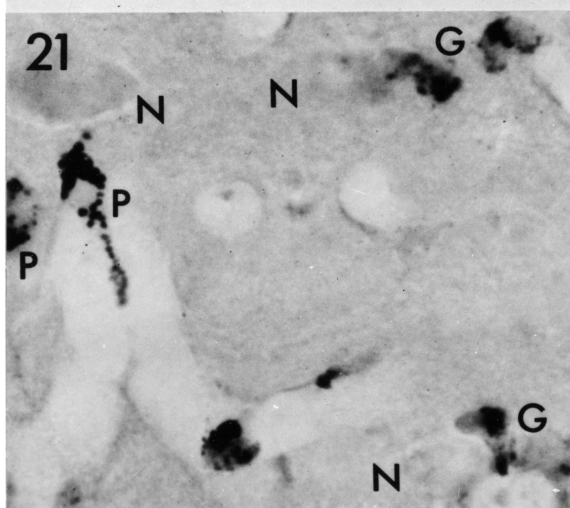
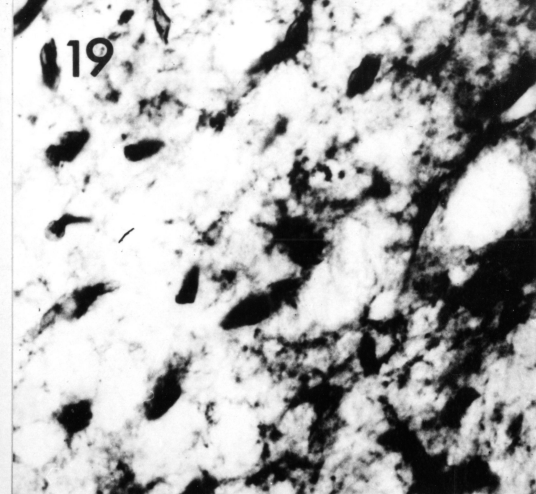
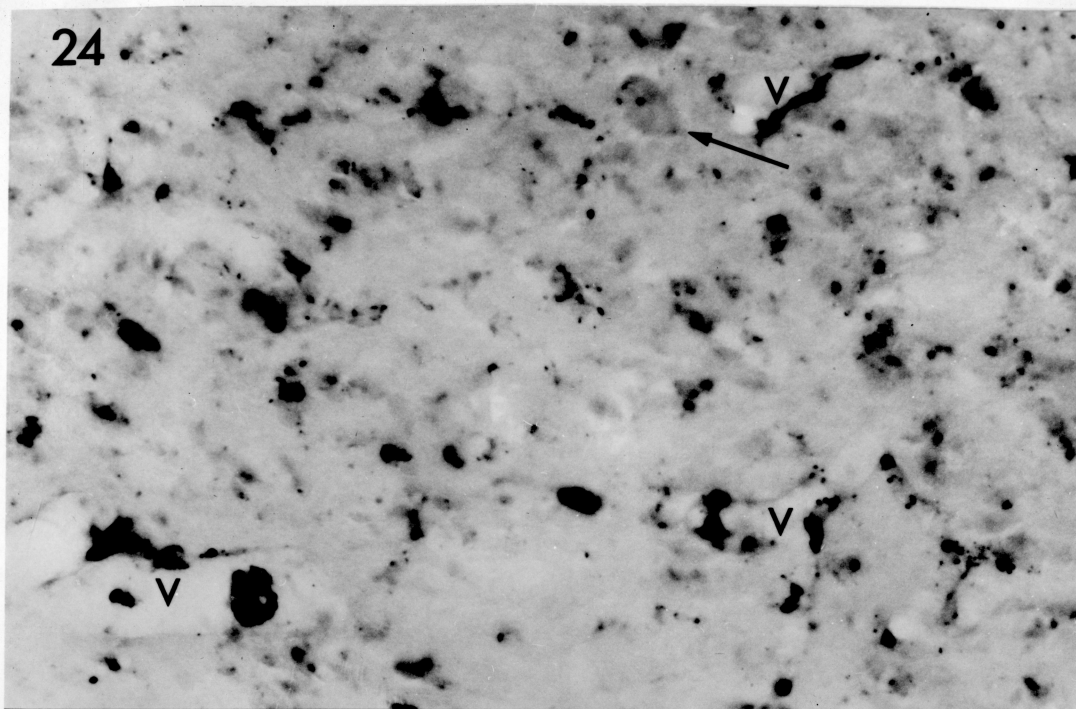


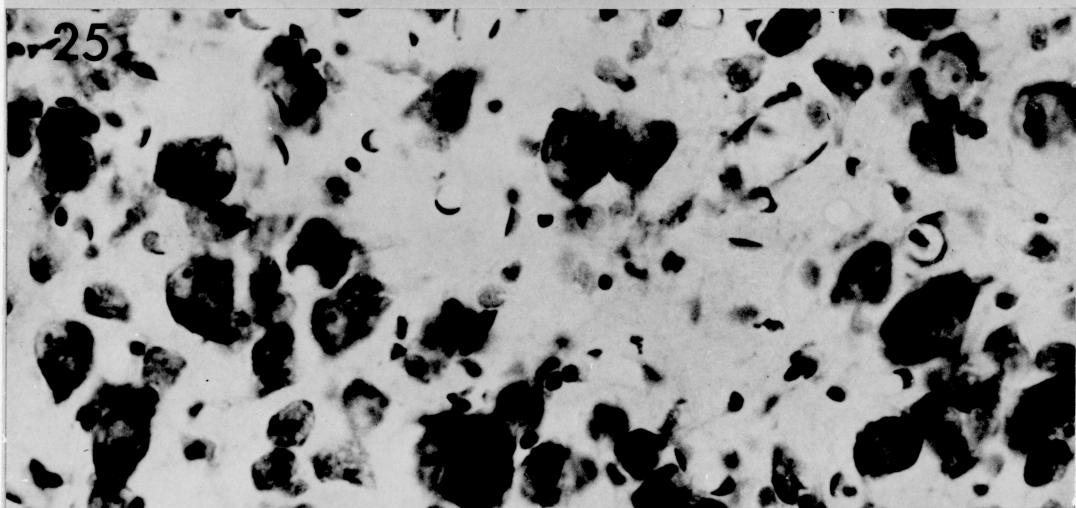
PLATE IX

- Figure 24. Numerous droplets of E600 ($5 \times 10^{-6}M$)-resistant esterase in vessels and parenchyma of left lateral geniculate body, 55 days after cortical extirpation. An occasional neuron lacking cytoplasmic activity is visible, as at arrow (350X).
- Figure 25. Right lateral geniculate body. Many neurons have eccentric nuclei and peripheral concentration of Nissl substance (350X).
- Figure 26. Left lateral geniculate body six days after operation. Neurons show loss of basophilia and beginning atrophy of perikarya (350X).
- Figures 25 and 26 are of buffered thionin preparations from the same animal.

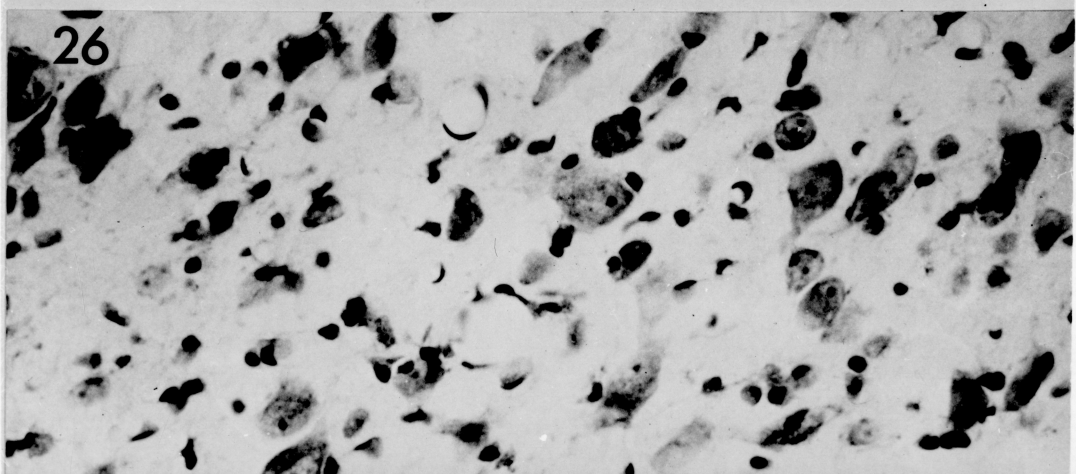
24.



25



26



APPROVAL SHEET

The thesis submitted by Doctor John B. Oldershaw has been read and approved by three members of the faculty of the Graduate School.

The final copies have been examined by the director of the thesis and the signature which appears below verifies the fact that any necessary changes have been incorporated, and that the thesis is now given final approval with reference to content, form, and mechanical accuracy.

The thesis is therefore accepted in partial fulfillment of the requirements for the Degree of Master of Science.

10-3-66

Date

David S. Jones

Signature of Adviser