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GLUTAMINE SYNTHETASE, GLUTAMOTRANSFERASE, AND GLUTAMINASE IN NEURONS AND NON-NEURAL TISSUE IN THE MEDICAL GENICULATE BODY OF THE CAT*

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Abstract—With the technique of retrograde degeneration to obtain tissue free from intact neurons it is found that glutamine synthetase is probably only in the glial–capillary wall portions of the central nervous system of the cat. Neurons have more glutamotransferase activity than have the glial–capillary wall portion of the CNS. Glutaminase is probably distributed equally between the neurons and the glial–capillary wall portions of the CNS.

Glutamine synthetase and glutamotransferase activities increase from the periphery to the cerebral cortex in the visual and somatic systems, except in the retina, which is high, and the cuneate nucleus, which is low. Glutaminase activity is highest in the dorsal thalamus in the visual system and in the cuneate nucleus in the somatic sensory system.

Glutamine synthetase, glutamotransferase, and glutaminase are apparently not related to cortical structures of different phylogenetic age.

LITTLE is known about the distribution of enzymes between CNS neurons and non-neuronal tissue. With the technique of retrograde degeneration in the central nervous system of the cat[1] one may obtain tissue consisting chiefly of astrocytic glia and capillaries without intact postsynaptic neurons. This paper will describe assays of glutamine synthetase in this tissue compared with that in normal. Glutamotransferase was similarly assayed. Although there is evidence that one enzyme may account for both these activities,[2, 3] some differences were found in distribution when the two substrates were used. Glutaminase was also assayed in these two tissues. The three activities in certain sensory systems will also be described.

METHOD

Tissue samples

Cats were prepared surgically[1] and each killed by electrical shock applied for 30 sec, since it has been demonstrated[4] that this stimulus affects endogenous NH_3 production in the CNS. All operated animals survived at least six weeks. Tissue samples were dissected free and weighed. Samples of white matter were taken from just under the cortex and referred to as visual or somatic "radiations" depending on the area. It is assumed that these were mainly fibers concerned with that particular modality,

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although other fibers were certainly present. Duplicate medial geniculate samples were used when sufficient material was available.

Glutamine synthetase assay

This enzyme was assayed by the method of Sellinger and De Balbian Verster.[5] Chilled tissue samples of less than 15 mg were homogenized in 0·5 ml of cold Tris buffer, 0·094 M, pH 7·2. The homogenizer was rinsed with another 0·5 ml of buffer, and then again with 0·75 ml containing 12·2 mg MgCl and 10·4 mg $NH_2OH \cdot HCl$; 17·6 mg glutamic acid in 0·75 ml; 22·1 mg ATP in 0·5 ml, and 0·186 mg British anti-lewisite in 0·03 ml ethanol were added to the mixture. Blanks were run without ATP. A standard curve was made with glutamohydroxamic acid. After 45-min incubation at 37° the reaction was stopped by 0·5 ml of 0·5 N HCl with 10% $FeCl_3$ and 8% trichloroacetic acid. The mixture was filtered with Schleicher and Schull filter paper 602, and read in the Beckman spectrophotometer at 520 mμ. The reaction rate was proportional to the amount of enzyme present.

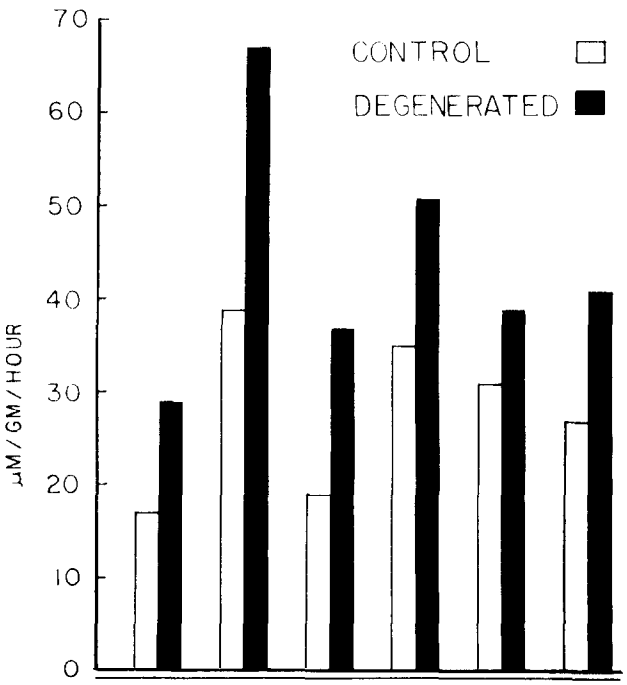

FIG. 1. Glutamine synthetase activity in medial geniculate bodies of cats operated unilaterally. Each pair of columns represents medial geniculates from one cat.

Glutamotransferase assay

Schou *et al.*[6] reported on the enzymatic formation of glutamohydroxamic acid from glutamine in mammalian tissues. Using their technique I found this activity in cat brain. Chilled tissue samples weighing 20 to 60 mg were homogenized in 0·5 ml cold 0·05 M acetate buffer, pH 5·5, and the homogenizer was rinsed with another 0·5 ml and then with 1 ml buffer containing 2 mg $MnCl_2 \cdot 4H_2O$ and 0·35 mg KH_2PO_4;

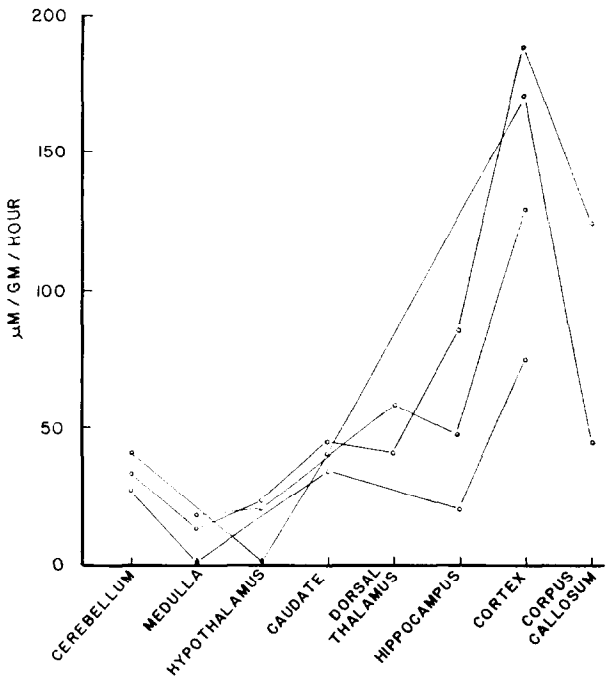

FIG. 2. Glutamine synthetase activity in cat brain. Each line connects samples from one cat. Sampling was random within a brain structure.

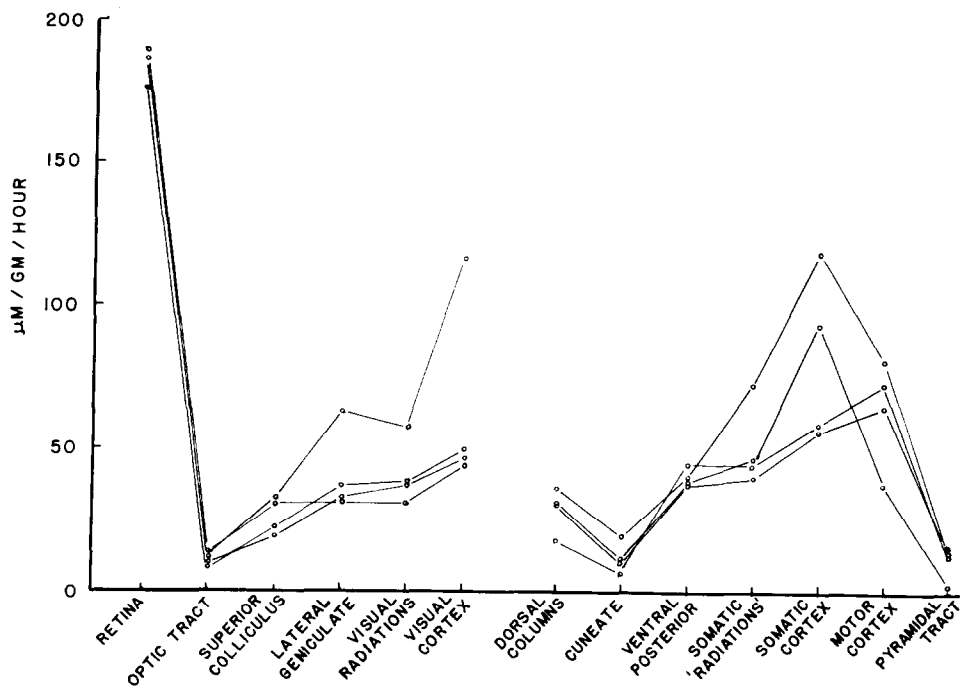

FIG. 3. Glutamine synthetase activity in the visual, somatic sensory, and motor systems of the cat. Each line connects samples from one cat.

6·3 mg glutamine in 0·5 ml buffer and 1·5 mg $NH_2OH \cdot HCl$ in 0·5 ml were added. Blanks contained no glutamine. A glutamohydroxamic acid standard curve was used. After 1-hr incubation at 37° the reaction was stopped with $FeCl_3$ in TCA and HCl, the mixtures were filtered with E & D filter paper 512 and read in the Beckman at 520 mμ. The reaction rate was proportional to the amount of enzyme present.

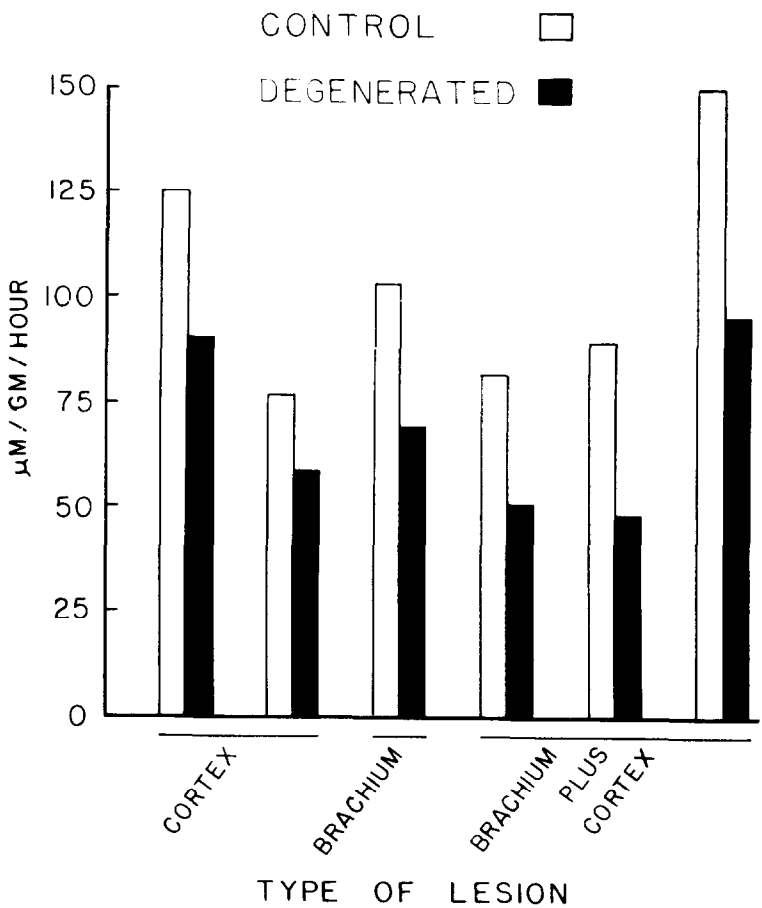

FIG. 4. Glutamotransferase activity in medial geniculate bodies of cats operated unilaterally. Each pair of columns represents medial geniculates from one cat.

Glutaminase assay

This enzyme was assayed by the method of Greenstein and Leuthardt.[7] NH_3 estimations were done by the distillation method of Speck.[8] Since Weil-Malherbe[4] reports that brain tissue releases NH_3 other than by the action of glutaminase, samples of tissue were incubated without glutamine as blanks. Chilled tissue samples weighing 20 to 40 mg were homogenized in cold 0·05 M veronal buffer, pH 8·6, and the homogenizer was rinsed with another 2 ml. To this was added 1 ml 0·264 M Na_2HPO_4, containing 0·014 M glutamine. After 1-hr incubation at 37° the reaction was stopped

by chilling, and NH_3 was estimated. The reaction rate was proportional to the amount of enzyme present.

All enzyme activities are expressed in micromoles product formed per gram of tissue per hour.

RESULTS

When intact neurons are lost, the specific activity of glutamine synthetase increases (Fig. 1). Figure 2 shows the activity of glutamine synthetase in various areas of the brain. Figure 3 shows the activity of the synthetase in the visual sensory system ascending from the peripheral receptor to the cortex. Except for the retina which is quite high, there is an orderly increase as the cortex is approached. Figure 3 also shows the synthetase activity in the somatic sensory system. As with the visual system the activity increases as the cortex is approached, except that the cuneate nucleus has less activity than the dorsal columns. On the efferent side the pyramidal tracts have much less activity than the motor cortex. The somatic sensory system has, in general, higher activity than the visual system.

When intact neurons are lost from the medial geniculate body, the glutamotransferase activity decreases (Fig. 4). Figures 5 and 6 show glutamotransferase activity in various parts of the brain. Figure 6 shows a high activity of the transferase in retina, and an increase from the optic tract to the cortex with the exception of the visual radiations.

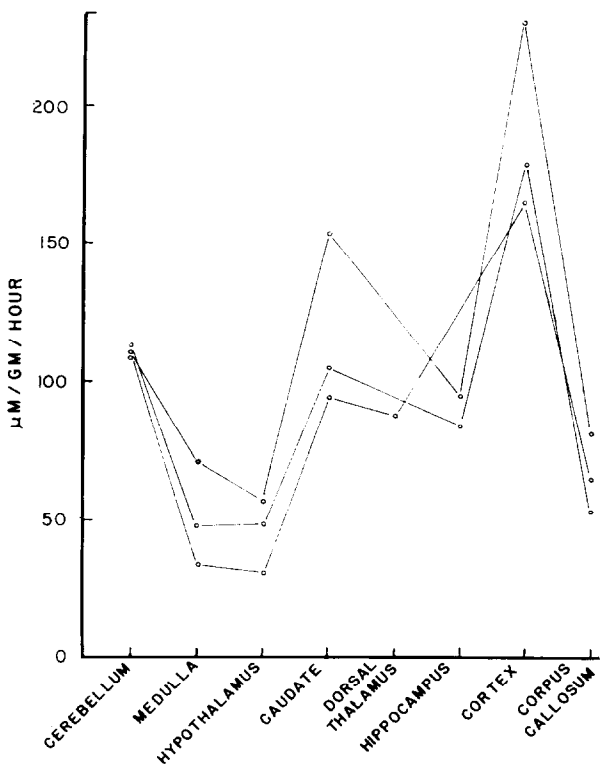

FIG. 5. Glutamotransferase activity in cat brain. Each line connects samples from one cat. Sampling was random within a brain structure.

There is also an increase in the enzyme activity in the somatic sensory system as the cortex is approached, except for low values in the somatic radiations and cuneate nucleus. On the motor side, activity drops peripheral to the cortex. This sensory system shows higher enzyme activity than does the visual system.

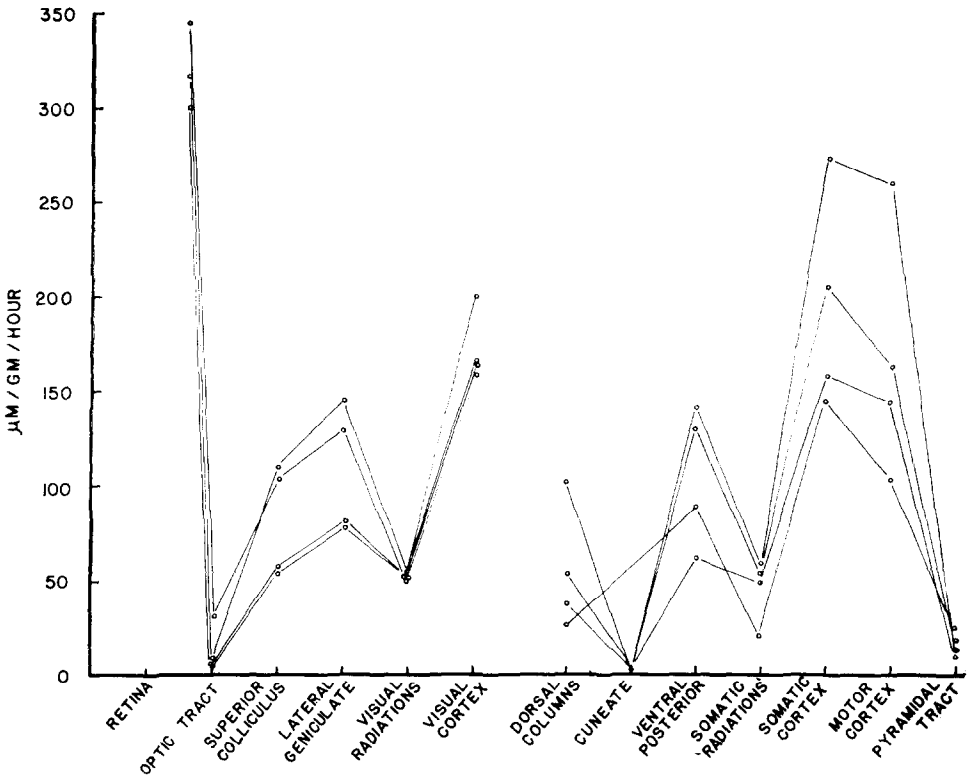

FIG. 6. Glutamotransferase activity in the visual, somatic sensory, and motor systems of the cat Each line connects samples from one cat.

When intact neurons are lost there is no change in activity of glutaminase (Fig. 7). Glutaminase activity in the cat brain is shown in Figs. 8 and 9. In the visual system glutaminase does not increase as the cortex is approached. The highest activity is in the dorsal thalamus. Retinal activity of this enzyme relative to the CNS is less than that of synthetase and transferase. A decline is seen in fiber tracts relative to adjacent cell groups. In the somatic sensory system, glutaminase is highest in the cuneate nucleus, which is not in the dorsal thalamus. A drop in enzyme activity occurs in the somatic radiations relative to adjacent cell groups. The motor cortex activity is higher than the efferent pyramidal tract activity. Glutaminase activity is approximately the same in the somatic sensory and visual systems, in contrast to the other two enzymes.

DISCUSSION

Certain assumptions must be made when interpreting these data. Six weeks after cortical lesions there are still some neurons either intact or in various stages of

chromatolysis, and there is an unknown but relatively small amount of gliosis.[1] One assumption is that the reactive glia do not introduce enzymes differing from those in normal glia. This assumption is probably justified in the case of an enzyme activity which is either decreased or unchanged six weeks after a cortical lesion. It may be justified in the case of an enzyme activity that increases, since the amount of gliosis is

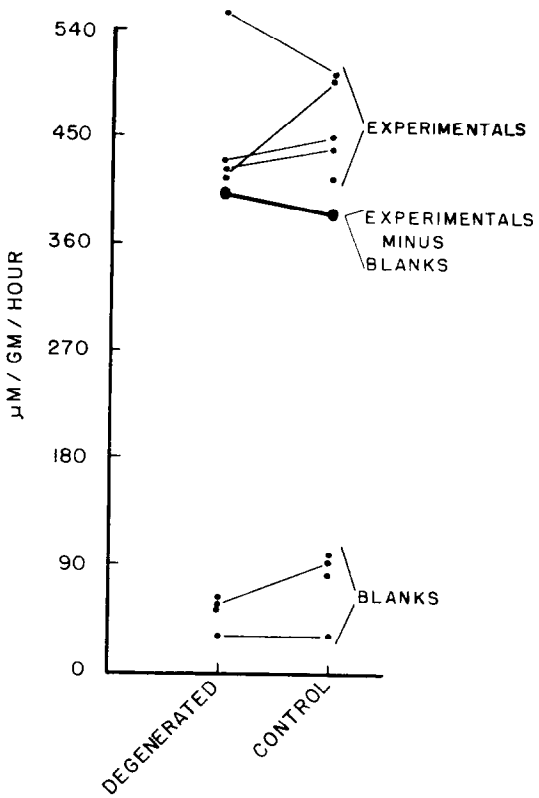

FIG. 7. Glutaminase activity in medial geniculate bodies of cats operated unilaterally. Each line connects control and degenerated medial geniculates from one cat. Each group of blanks was averaged and subtracted from the respective averaged experimentals to obtain single control and degenerated values.

relatively small, considering the tissue weight loss. Another assumption is that the glia present at six weeks have not preserved the enzymes from the degenerating neurons. The phagocytic microglia are supposed to lose their contents to the blood stream quickly. A third major assumption is that the remaining neurons have not significantly altered their enzyme pattern at six weeks. When these assumptions are made, the simplest account of the process of retrograde degeneration appears to be that most neurons affected by a cortical lesion degenerate, and their contents are lost to the circulation in less than six weeks, although a few neurons may degenerate more slowly.

The weight of the average control medial geniculate body is 28 mg and that of the average experimental one is 17 mg;[1] the change is a 39% decrease. This weight loss is

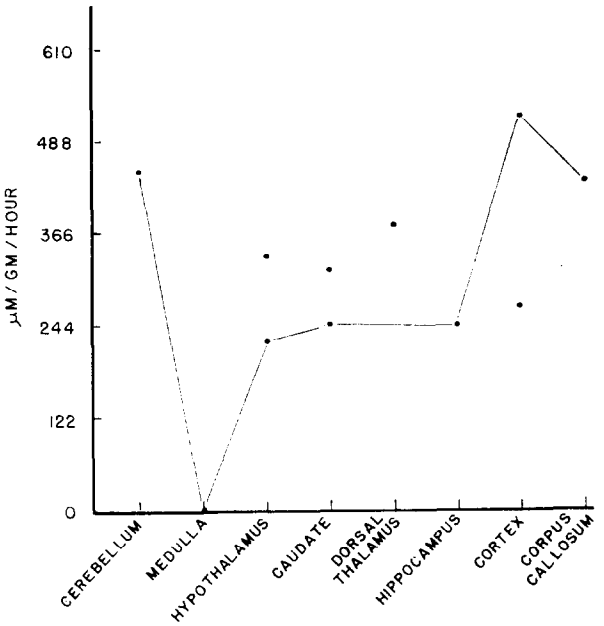

FIG. 8. Glutaminase activity in cat brain. Each line connects samples from one cat. Sampling was random within a brain structure.

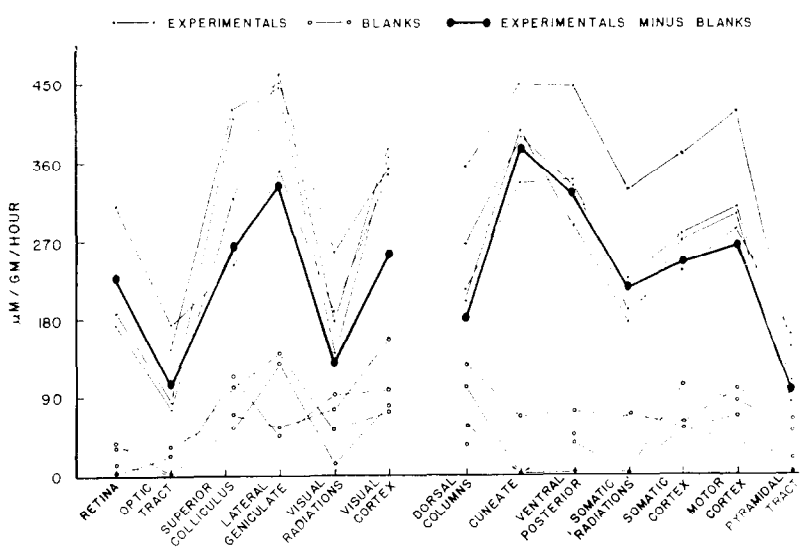

FIG. 9. Glutaminase activity in the visual, somatic sensory, and motor systems of the cat. Each line connects samples from one cat, and averaged blanks are subtracted from averaged experimentals to obtain one value for each brain area.

the result of the loss of postsynaptic neurons and is somewhat offset by the gliosis. If glutamine synthetase were only in glial cells and capillary walls one would expect an increase in enzyme specific activity of 63%. The observed increase is 58%. Since the synthetase specific activity increases to approximately the extent that neuron weight is lost, the enzyme may be only in astrocytes or the capillary wall. It is of interest to point out that these sites in the brain have been proposed as the blood–brain barrier.[9] Adams and Foley[10] suggest that the astrocytosis, which they see in cases of neurological disorder associated with liver disease, may be due to increased ammonium ions. By means of more precise techniques, other enzymes have been related to these tissues. Tschirgi suggests that carbonic anhydrase is required for hydration of CO_2 produced by the CNS, which is in turn required for passage of NaCl from the blood stream into the CNS.[11] Giacobini found most of the enzyme activity in glial cells.[12, 13] Bertler *et al.* report that brain capillaries contain DOPA–decarboxylase, which constitutes an obstacle to the penetration of the L-isomers of dihydroxyphenylalanine and 5-hydroxytryptophan.[14]

When intact neurons are lost from the medial geniculate body the glutamotransferase activity decreases. The enzyme activity of neurons is apparently higher than that of the glia and capillary walls. In the degenerated medial geniculate body the level of glutaminase activity is similar to that in control medial geniculate. Apparently the enzyme activity is evenly distributed between neural and non-neural tissue.

It has been reported that one enzyme is responsible for both glutamine synthetase and glutamotransferase activity in peas.[2, 3] Since the two activities have different distributions in cat brain, there are either different substrate affinities in different areas of the brain or two different enzymes actually exist. It is also possible that there are different glutaminases in the cat brain, since two are known in the liver of some animals, and other routes of ammonia release are possible. [15, 16] However, Errera and Greenstein have found relatively small amounts of ammonia released from rat, mouse, rabbit, and guinea pig brains containing added glutamine but no added phosphate.[17]

It is possible to explain the decrease in transferase and glutaminase activity in the white matter if myelin is assumed to be metabolically inactive.[18] White matter is composed mainly of capillaries, glia, and myelin, while relay nuclei and cortex have capillaries, glia, and neurons. Since the synthetase is in the glial–capillary wall space, one may suppose it is diluted similarly by neurons in gray matter and myelin in white matter. This would explain why the enzyme activity does not decline in the visual or somatic "radiations." On the other hand, the transferase and glutaminase are in both neurons and the glial–capillary wall space, but not in myelin. Myelin therefore may dilute the enzyme activity in the white matter.

Samples of pyriform cortex were analyzed for these three enzymes, and the activities were similar to those in neocortex. It was concluded that these enzymes were not related to structures of a particular phylogenetic age.

Acknowledgment—I am indebted to Dr. F. Bernheim for his interest and advice in this study.

Note added in proof

My attention has been called to a study by Koch *et al.*[19] to determine the ionic content of glia and one by Albers[20] to determine aldolase, glucose- 6- phosphate dehydrogenase, and *iso*-citric dehydrogenase activities in glia. Both procedures use the lateral geniculate. Koch *et al.* give a more extensive account

of the degenerative process. The process of retrograde degeneration in the lateral geniculate is probably not substantially different from that in the medial geniculate.

REFERENCES

1. J. D. UTLEY, *Biochem. Pharmacol.* **12**, 1228 (1963).
2. W. H. ELLIOTT, *J. biol. Chem.* **201**, 661 (1953).
3. L. LEVINTOW and A. MEISTER, *J. biol. Chem.* **209**, 265 (1954).
4. H. WEIL-MALHERBE, *Physiol. Rev.* **30**, 549 (1950).
5. O. Z. SELLINGER and F. DE BALBIAN VERSTER, *J. biol. Chem.* **237**, 1836 (1962).
6. M. SCHOU, N. GROSSOWICZ, A. LAJTHA and H. WAELSCH, *Nature (Lond.)* **167**, 818 (1951).
7. J. P. GREENSTEIN and F. M. LEUTHARDT, *Arch. Biochem.* **17**, 105 (1948).
8. J. F. SPECK, *J. biol. Chem.* **179**, 1387 (1949).
9. P. G. JEPPSSON, *Acta neurol. psychiat. scand.* **38**, Suppl. 160 (1962).
10. R. D. ADAMS and J. M. FOLEY, *Res. Publ. Ass. nerv. ment. Dis.* **32**, 198 (1953).
11. R. D. TSCHIRGI, in *Biology of Neuroglia*, W. Windle, Ed., p. 130. Springfield, Ill. (1958).
12. E. GIACOBINI, *Science* **134**, 1524 (1961).
13. E. GIACOBINI, *J. Neurochem.* **9**, 169 (1962).
14. A. BERTLER, B. FALCK and E. ROSENGREN, *Acta Pharmacol. (Kbh.)* **20**, 317 (1964).
15. F. W. SAYRE and E. ROBERTS, *J. biol. Chem.* **233**, 1128 (1958).
16. A. MEISTER, L. LEVINTOW, R. E. GREENFIELD and P. A. ABENDSCHEIN, *J. biol. Chem.* **215**, 441 (1949).
17. M. ERRERA and J. P. GREENSTEIN, *J. biol. Chem.* **178**, 495 (1949).
18. C. W. M. ADAMS, A. N. DAVISON and N. A. GREGSON, *J. Neurochem.* **10**, 383 (1963).
19. A. KOCH, J. B. RANCK, JR. and B. L. NEWMAN, *Exp. Neurol.* **6**, 186 (1962).
20. R. W. ALBERS, in *Biology of Neuroglia*, W. Windle, Ed., (Discussion) p. 226. Springfield, Ill. (1958).