EXPERIMENTAL NEUROLOGY 6, 142-151 (1962)

# Increased Utilization of an Amino Acid and Cellular Proliferation Demonstrated Autoradiographically in the Optic Pathways of Pigeons

JOSEPH ALTMAN AND ELIZABETH ALTMAN[1]

*Psychophysiological Laboratory, Massachusetts Institute of Technology, Cambridge, Massachusetts*

*Received April 23, 1962*

Utilization of a radioactively labeled amino acid (glycine-$H^3$) was investigated by autoradiography in the visual pathways of pigeons, one eye of which had been removed or blindfolded 1 month previously. The radiochemical was injected systemically and the animals were killed after an exchange period of 2 hours. In the enucleated animals, uptake of the labeled glycine was considerably greater in the severed than in the normal retinotectal pathway, indicating increased protein metabolism in the "degenerating" fiber tract. The differential incorporation was evident also in the stratum opticum of the optic lobes but ambiguous in the deeper layers. The increased uptake in the severed optic pathway was associated with an increase in the total number of neuroglia cells and the appearance of cells not observed in the undamaged pathway. No differential uptake was observed in any other parts of the central nervous system, the forebrain included. In the unilaterally blindfolded animal, no difference was observed in uptake of labeled glycine between the normal and "functionally deafferented" optic pathways. The increased protein metabolism in the distal stumps of the severed optic pathway, combined with proliferation of cells in this region, may reflect a regenerative attempt at this phase of Wallerian degeneration.

## Introduction

Several biochemical studies dealing with chemical changes accompanying Wallerian degeneration in peripheral nerves are available (8), and at least one of these is concerned with changes that follow sectioning of a central nervous tract, the optic nerve (7). These have provided some data (partially contradictory) on shifts in concentration of several cellular constituents (e.g., lipids, proteins, nucleic acids) during various phases

[1] The study was supported by a grant (M-5673) of the National Institutes of Health, U.S. Public Health Service, to Dr. H.-L. Teuber.

of degeneration and regeneration that follow nerve sectioning. Unfortunately, routine biochemical analyses provide little or no information regarding the exact cellular sites of the chemical alterations found, nor do they permit direct correlation of the chemical and histological changes. In the present study an attempt was made to utilize autoradiography for the localization of chemical changes in a severed fiber tract. Following enucleation of one eye in the pigeon, rates of incorporation of a radioactively labeled amino acid (glycine-$H^3$) in the optic tracts and lobes of both sides were compared. Although the autoradiographic technique fails to demonstrate the exact chemical fate of the incorporated radiochemical (after histological processing the retained radioactive amino acid is presumed to be mainly protein-bound), it does permit histological localization of the sites of metabolic alterations.

## Materials and Methods

Six domestic pigeons weighing about 350 gm were used. The left eye was removed in three, while in the others the left eye was occluded with an opaque plastic disc and the eyelid sutured over it. All except one were permitted to survive for 1 month. Two hours before killing them, two of the unilaterally enucleated and one of the unilaterally blindfolded pigeons were given 2 mc of glycine-2-$H^3$ intraperitoneally (dissolved in 1 ml of sterile water; specific activity 194 mc/mmole). The brains were fixed in 10 per cent neutral formalin and embedded in paraffin. Coronal sections 5 $\mu$ thick were cut serially from blocks extending from the caudal mesencephalon to the forebrain. The sections designed for autoradiography were coated with Ilford G-5 nuclear emulsion, exposed for varying periods up to 1 year, and developed in the usual manner. Parallel sections from the brain of the radioactive and nonradioactive animals were stained with gallocyanin-chromalum and with the Clark and Ward modification of the Pal–Weigert method to permit correlation of the autoradiographic information with the histological results. Some of the autoradiographic sections were also stained with gallocyanin-chromalum after developing, and some unstained sections were studied with the phase-contrast technique.

Since the projection of the optic nerve in birds in general, and in the pigeon in particular (4), is entirely contralateral, each eye projecting to the opposite optic lobe, the unilateral intervention allowed the normal half of the brain to control the effects of the experimental manipulations. The birds with one eye removed were intended to provide information of differential metabolic and cytological changes produced in the severed optic tract and optic lobe; unilateral blindfolding was designed to isolate the possible effects of eliminating normal visual stimulation without direct surgical damage to the optic pathways.

## Results

Figure 1 shows an unstained autoradiograph of a section from the brain at the level of the optic chiasm. As a consequence of removal of

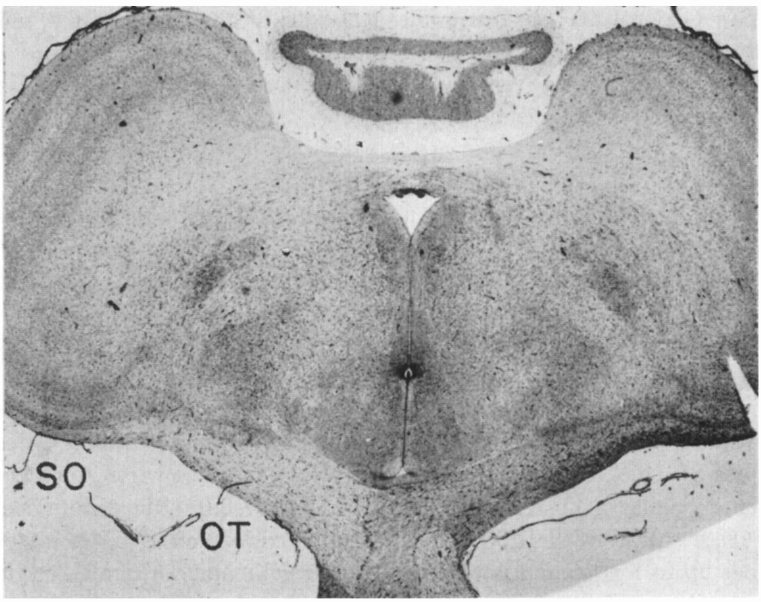


FIG. 1. Unstained autoradiogram of the optic lobes (upper left and right) at the level of the optic chiasm (lower center) of pigeon RP3. Left eye was removed 1 month prior to intraperitoneal administration of 2 mc of glycine-$H^3$; animal sacrificed 2 hours later. Magnification 8.5 ×. Abbreviations: OT, optic tract; SO, stratum opticum.

the left eye, the severed left optic nerve (bottom left) and the decussated right optic tract show higher uptake of the labeled glycine than the normal right optic nerve (bottom right) and the crossed left optic tract. Considerable difference in uptake of the radiochemical was seen also in the stratum opticum, the upper layer of the tectum which is formed by optic fibers coming from the retina. This is illustrated in Fig. 2, showing an autoradiograph of the midbrain region of another animal. The increased uptake of tagged glycine by the right stratum opticum, which receives its fiber supply from the severed optic tract, may also be seen at higher

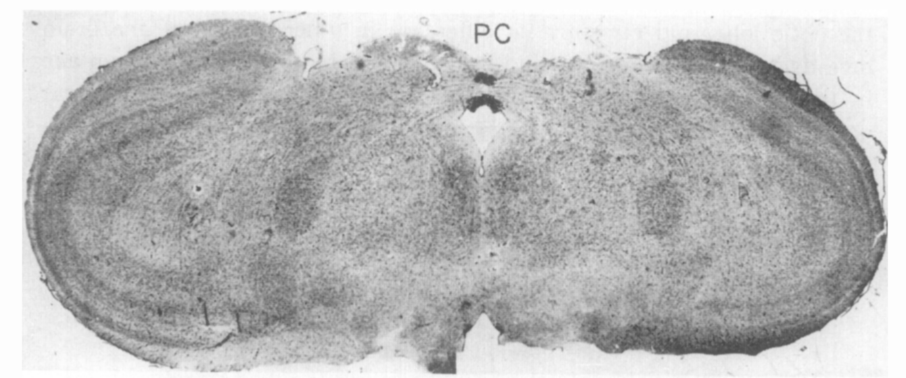


FIG. 2. Unstained autoradiogram of the optic lobes of pigeon RP4. Animal treated as RP3 (Fig. 1). Magnification 7.5 ×. Abbreviation: PC, posterior commissure.

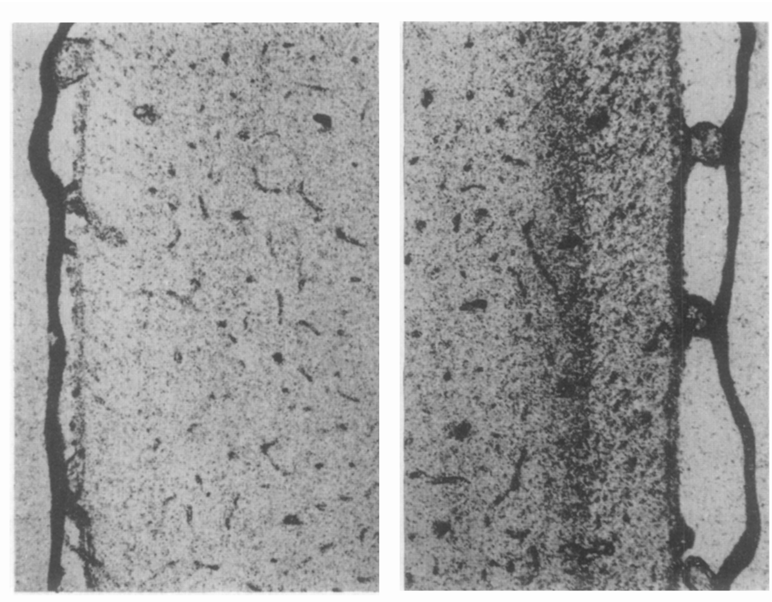

FIG. 3. Unstained autoradiogram of the superficial region of the normal (left) and affected (right) stratum opticum of the optic lobes of pigeon RP3. Note extreme darkening of the pia-arachnoid membranes (extreme right and left) due to the long exposure period. Magnification 90 ×.

magnification in Fig. 3. As may be observed in this photograph, the pia mater (but not the arachnoid) participates in the increased metabolic activity on the abnormal side. In contrast, there was no definite evidence of differential uptake on the right and left side in the deeper layers of the optic lobe, and certainly no difference in other regions of the brain investigated, the forebrain included. Furthermore, no difference in uptake in the right and left optic nerve, optic tract and stratum opticum was observed in a third animal (RP7) in which, instead of enucleation, the

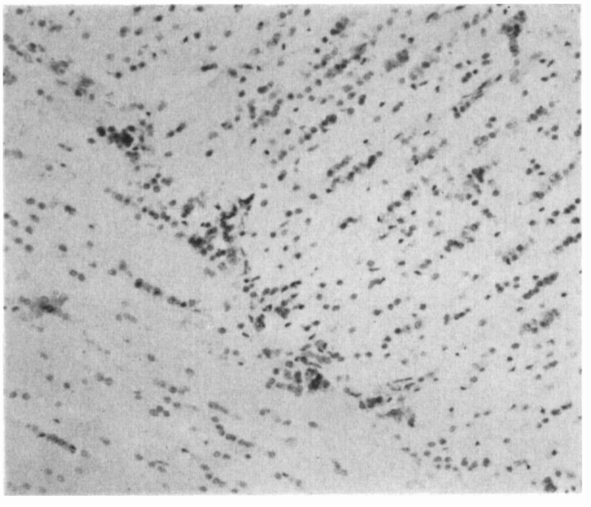

FIG. 4. Portion of the optic chiasm in pigeon RP3. Gallocyanin-chromalum stain. Right, crossed portion of degenerated optic tract with increased number of neuroglia cells. Magnification 161 ×.

left eye was merely occluded so that the corresponding optic pathway was deprived of direct visual stimulation.

Thus, the obtained autoradiographic evidence suggests increased utilization of an amino acid (presumably increased protein turnover) in the severed optic pathway. Investigation of the histological changes in autoradiographic sections stained with gallocyanin-chromalum, and in parallel unexposed sections stained either with gallocyanin-chromalum or by the Clark and Ward modification of the Pal–Weigert method, has thrown some light on the possible origin of the differential metabolic activity on the normal and abnormal sides. In the sections stained with Pal–Weigert method the left optic nerve and the decussating right optic tract showed degeneration of nearly all optic fibers; while the right optic nerve and the left optic tract appeared normal. The sections stained with gallocyanin-chromalum revealed an increase in the concentration of neuroglia cells (gliosis) in the affected optic pathway when compared with the normal one. Figure 4 illustrates this increase at the level of the chiasm.

Homologous areas in the right and left optic tracts were scanned at high magnification with a microviewscope. In twenty measurements on each side, in circular areas 150 $\mu$ in diameter, a mean count of 74 $\pm$ 8 neuroglia cells on the normal side, and 106 $\pm$ 7 on the severed side were

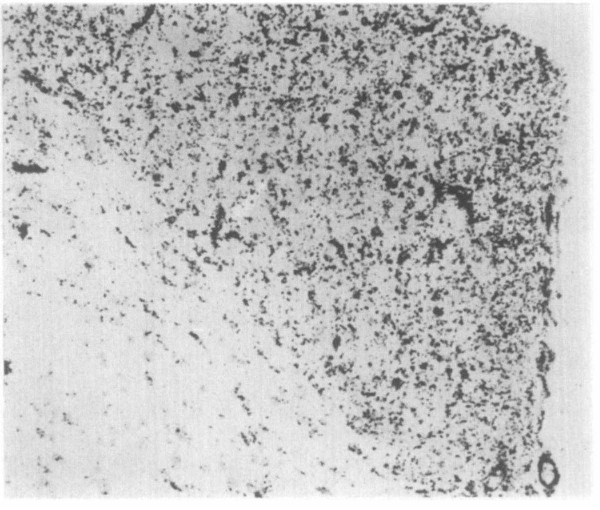

FIG. 5. Unstained autoradiogram of a portion of the optic chiasm in pigeon RP3. Right, crossed portion of degenerated optic tract. Magnification 121 $\times$.

obtained. Figure 5, taken from an unstained autoradiograph from a section neighboring that of Fig. 4, shows the increase in grain density paralleling the growth in the number of neuroglia cells. As may be observed, the increase in uptake of the radiochemical on the severed side greatly exceeds the relative increase of neuroglia cells, suggesting that the increased protein turnover can be attributed only in part to the greater number of neuroglia cells per unit area. The upper photomicrograph in Fig. 6 shows at higher magnification the appearance of neuroglia cells in the normal optic tract, all of which have small, densely stained nuclei. These are the only type of neuroglia cells (presumably oligodendrocytes) present on the normal side. The lower photomicrograph in Fig. 6 shows

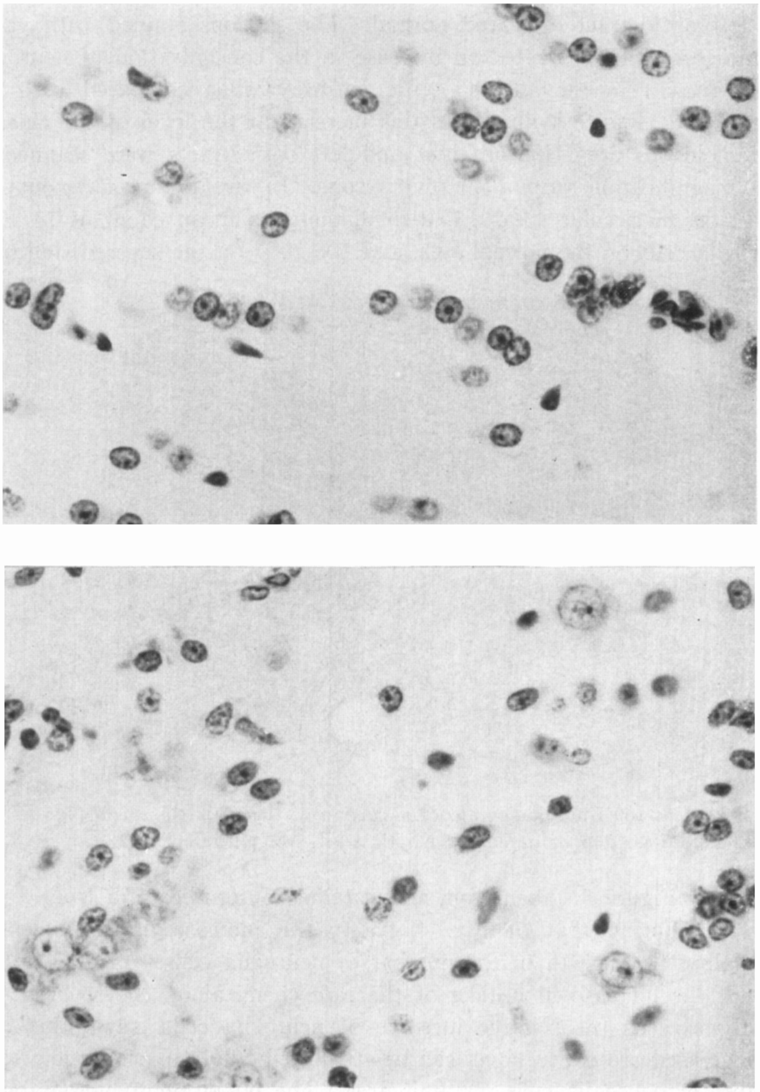

FIG. 6 Upper picture: portion of the normal stratum opticum in pigeon RP3. Lower picture: homologous region in affected stratum opticum. Gallocyanin-chromalum stain. Magnification 640 ×.

the appearance of neuroglia cells on the affected side where, in addition to the usual types of neuroglia cells other cell types also are present. These cells have larger but poorly stained nuclei and, unlike in "normal" neuroglia cells, their cytoplasm is often mildly stained. Some of these may be astrocytes, with an occasional microglia cell; others have the appearance of spongioblasts. Finally, in the region of the optic chiasm, but not in the stratum opticum, cells resembling neuroblasts were seen (Fig. 7).

FIG. 7. Cells resembling neuroblasts (large, pale cells) surrounded by neuroglia cells, presumably oligodendrocytes, in the degenerated portion of the optic chiasm. Gallocyanin-chromalum stain. Magnification 1600 ×; oil immersion.

## Discussion

The results suggest that the pathological changes produced in the retinotectal pathway as a consequence of removal of one eye are associated with increased amino acid utilization. This finding, indicating increased protein metabolism, is somewhat paradoxical; degeneration of a fiber tract—dissolution of the distal stumps following severance from their cell bodies—could be expected to lead to decreased protein turnover. The biochemical evidence on this point is somewhat ambiguous, for there are reports, not only of decreased (8), but also of increased protein concentration or turnover (1, 3, 6, 9, 10) during some phases of Wallerian degeneration. The only investigators who have studied the problem in the severed optic nerve (7) found no difference in protein concentration in the severed and normal nerves.

The evidence provided by standard biochemical analyses differs from that obtained by the radioactive tracer technique. Routine biochemical analyses give information about the concentration or mass of chemicals, whereas the radioisotope labeling technique provides data about their turnover rates. The higher concentration of a tagged substance in one region than in others need not imply a higher absolute concentration of the substance there; it only indicates a higher rate of turnover. If we are justified in assuming that cells are dynamic homeostatic systems which tend to restore losses due to work or injury, then the tracer method in general and the autoradiographic technique in particular are better suited to give information about dynamic events associated with different physiological and pathological states than biochemical measurements of absolute concentration of various substances. On the other hand, it has often been argued that the autoradiographic technique is not suited for the study of rates of regional protein turnover because it provides no information about the specific activity of regional amino acid pools. This objection was partially circumvented insofar as we studied the turnover rates of identical structures (e.g., right and left optic tracts). But the possibility remains that transportation and permeability changes occurred which made the labeled amino acid more freely available in the degenerating than in the normal optic tract. At any rate, the study does provide unambiguous evidence of active protein synthesis in a degenerating central nervous tract.

If oligodendrocytes function mainly in the myelination of central nervous fibers, then the presence of an increased number of such cells in the severed optic pathway would suggest a regenerative attempt. However, doubts have been expressed recently about this assumed function of oligodendrocytes (11). Furthermore, we have no information about the possible functional significance of the unusual types of cells present on the degenerated side. The demonstration of increased protein metabolism suggests that the role of the multiplied neuroglia cells may not be limited to phagocytosis. We can add little to the observation of the presence, in the region of the chiasm, of those cell types which resemble neuroblasts. According to available information, proliferation of nerve cells in birds ceases early in embryonic development, though neurogenesis may occur at somewhat later stages if parts of the spinal cord or brain are extirpated (5). In the absence of definite criteria for identifiying neuroblasts, we cannot say whether central neurogenesis (an abortive attempt by the optic lobe to regenerate the retina) could occur in adult birds. The recent demonstration of DNA turnover in some mammalian neurons (*2*), combined with the evidence obtained in the present study of increased protein synthesis in the severed optic pathways, may render such an assumption plausible.

## References

1. Abercrombie, M., and M. L. Johnson. 1947. Effect of reinervation on collagen formation in degenerating sciatic nerves of rabbits. *J. Neurol. Neurosurg. Psychiat.* **10**: 89-92.
2. Altman, J. 1962. Are new neurons formed in the brains of adult mammals? *Science* **135**: 1127-1128.
3. Bodian, D., and D. Dziewiatkowski. 1950. The disposition of radioactive phosphorus in normal as compared with regenerating and degenerating nervous tissue. *J. Cell. Comp. Physiol.* **35**: 155-177.
4. Cowan, W. M., L. Adamson, and T. P. S. Powell. 1961. An experimental study of the avian visual system. *J. Anat.* **95**: 545-563.
5. Hamburger, V. 1955. Regeneration in the central nervous system of reptiles and birds, pp. 47-53. *In* "Regeneration in the Central Nervous System," W. F. Windle (ed.). Thomas, Springfield, Illinois.
6. May, R. M. 1947. Études microchimiques sur le systèm nerveux; les combinaisons soufrées du nerf au cours de sa dégénérescence. *Bull. soc. chim. biol.* **29**: 1035-1042.
7. McCaman, R. E., and E. Robins. 1959. Quantitative biochemical studies of Wallerian degeneration in the peripheral and central nervous systems, I. *J. Neurochem.* **5**: 18-31.
8. Rossiter, R. J. 1961. The chemistry of Wallerian degeneration, pp. 207-227. *In* "Chemical Pathology of the Nervous System," J. Folch-Pi (ed.). Pergamon, London.
9. Samuels, A. J., L. L. Boyarsky, R. W. Gerard, B. Libet, and M. Burst. 1951. Distribution, exchange and migration of phosphate compounds in the nervous system. *Am. J. Physiol.* **164**: 1-15.
10. Takahashi, Y., M. Nomura, and S. Furusawa. 1961. *In vitro* incorporation of [$^{14}$C]-amino acids into proteins of peripheral nerve during Wallerian degeneration. *J. Neurochem.* **7**: 97-102.
11. Uzman, B. G. 1958. The role of neuroglia cells in myelin formation, pp. 73-74, *and* Round table discussion, pp. 74-82. *In* "Biology of Neuroglia," W. F. Windle (ed.). Thomas, Springfield, Illinois.