Distribution of Certain Enzymes in the Brain of the Pigeon.* (17332)

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In the course of investigations dealing with the functional significance of cerebral capillary patterns, a relationship was found between the vascularity of certain neuropils and the number of mitochondria present.[1] The relationship was considered significant because mitochondria are carriers of enzymes.[2-4] This fact and the availability of histochemical methods for the demonstration of enzymes in tissues suggested a study of the distribution of enzymes in the brain as one aspect of the relationship between cerebral vascularization and brain metabolism.

Materials and methods.† Pigeon brains were used in this study because it was found from a survey of the brains of various vertebrates that the histochemical methods available for the demonstration of enzyme activities gave the best over all results in pigeon brains. The methods used were those of Gomori[5] and Manheimer and Seligman[6] for alkaline phosphatase, and those of Gomori for acid phosphatase,[7] phosphamidase,[8] and cholinesterase.[9] Although these methods represent valuable additions to the existing histochemical methods, caution is required in the interpretation of the results that may be obtained from their application. Several investigators[10-12] have shown that there is a definite unavoidable destruction and loss of the phosphomonoesterases, particularly acid phosphatase, as a result of the fixing, embedding, and mounting processes to which the tissues are subjected. In fact, the validity of the assumption that the results obtained by the acid phosphatase method are wholly or at all due to the activity of this particular enzyme has been seriously questioned.[11,13,14] The accuracy of the localization of alkaline phosphatase activity has been doubted,[15] but the sharp boundaries in our pictures indicate that there occurred little if any diffusion. In regard to the demonstration of cholinesterase activity Koelle and Friedenwald[16] suggest that the method of Gomori localizes only a non-specific enzyme and that there may be other undemonstrated cholinesterases present which have different substrate specificities.

In preparation for the enzyme determinations the tissues were fixed in chilled acetone or absolute alcohol, were transferred to benzene and were infiltrated with and embedded in paraffin in a vacuum oven. The sections were cut at 5μ. The substrates used were sodium alpha glycerophosphate in the acid and alkaline phosphatase methods of Gomori,

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† We are greatly indebted to Dr. G. Gomori, University of Chicago, for helping us solve technical problems that arose in the course of the investigation.

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6 Manheimer, L. H., and Seligman, A. M., *J. Nat. Cancer Inst.*, 1948, **9**, 181.

7 Gomori, G., *Arch. Path.*, 1941, **32**, 189.

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15 Jacoby, F., and Martin, B. F., *Nature*, 1949, **163**, 875.

16 Koelle, G. B., and Friedenwald, J. S., PROC. SOC. EXP. BIOL. AND MED., 1949, **70**, 617.

calcium beta naphthol in the alkaline phosphatase method of Manheimer and Seligman, p-chloranilidophosphonic acid in the phosphamidase method, and palmitoyl choline chloride in preference to myristyl choline chloride in the cholinesterase method.

Results. The patterns of the different enzyme activities were consistent in the brains examined. Alkaline phosphatase was demonstrated in an apparently great quantity in the pigeon brain (Fig. 1) by both the methods of Gomori, and Manheimer and Seligman. This observation is noteworthy because Gomori[17] and Bourne[18] state that alkaline phosphatase is not found in brain tissue. On the other hand we could not demonstrate in the pigeon the great quantities of acid phosphatase which are reported by these workers to be present in the brains of other animals. However, the faint patterns which we did obtain were consistent. Although the results of this procedure are difficult to interpret, we have included them in our comparative analysis of the different enzyme patterns.

From observations restricted to a few clearly defined structures, we wish to point out some striking differences in the enzyme content of a few areas in the brain keeping in mind the questions raised in one of the preceding paragraphs concerning the validity of the methods employed.

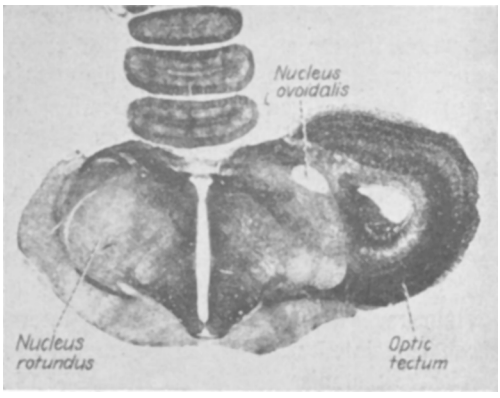

FIG. 1.

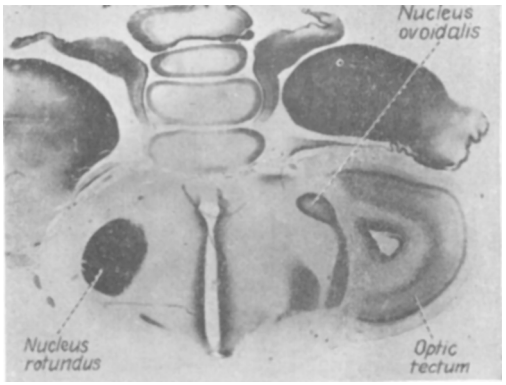

FIG. 2.

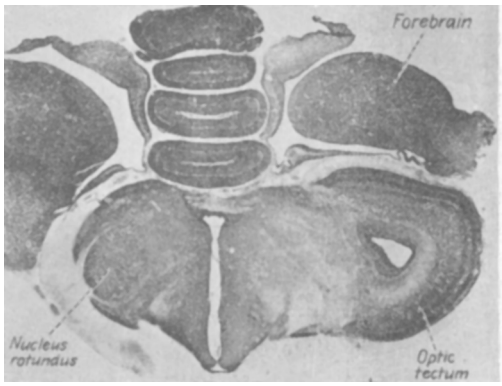

FIG. 3.

In adjacent sections the nucleus ovoidalis appears to be entirely lacking in alkaline phosphatase (Fig. 1), but seems very rich in cholinesterase (Fig. 2.). Its outline may be traced in a preparation which demonstrates acid phosphatase, but it can hardly be distinguished from other structures in a phosphamidase preparation (Fig. 3) inasmuch as its content of phosphamidase is about the same as that of the surrounding brain tissue.

The nucleus rotundus is similarly conspicuous in cholinesterase preparations (Fig. 2) whereas its outline can be barely identified in acid phosphatase preparations. Its content of alkaline phosphatase is markedly less than that of surrounding structures (Fig. 1); in phosphamidase preparations its boundaries are indistinct (Fig. 3).

The occipital pole of the forebrain shows some acid phosphatase and a high content of phosphamidase (Fig. 3) and of cholinesterase

[17] Gomori, G., *J. Cell. and Comp. Physiol.*, 1941, **17**, 71.

[18] Bourne, G., *Quart. J. Exp. Physiol.*, 1943, **32**, 1.

(Fig. 2); but it is entirely free of alkaline phosphatase (Fig. 1).

The observation of layers of enzymatic activity in the optic tectum (Figs. 1-3) is of particular interest in view of the corresponding arrangement of cells, fibers, blood vessels, and mitochondria in this part of the brain. A more detailed description of the distribution of enzymes in the optic tectum will be given in a separate paper.

Discussion. The statements "free of" or "rich in" as made in the description of our preparations should, of course, not be taken to mean absolute quantitative values, since there is, as has been pointed out above, an unavoidable loss of enzymatic activity that occurs in the process of fixing, embedding and mounting the tissues.

Consequently the results which we have obtained are only relative. For example the nucleus ovoidalis and the occipital pole of the forebrain appear in our preparations free of alkaline phosphatase. This may be only the result of the loss of the relatively small amounts of the enzyme which they contained in the living state. Other adjoining brain tissue may have had so much more alkaline phosphatase to begin with that even after having been subjected to the same technical procedures there is still enough of the enzyme left to give a marked reaction with the methods employed. The fact however remains that there are demonstrable differences in enzyme content.

The distribution of cholinesterase activity shown in our preparations is of interest in comparison with the results of Feldberg and Vogt.[19] These authors assayed very small amounts of tissue taken from 40 different regions of the central nervous system; they found pronounced differences in the content of the enzyme or enzyme system synthesizing acetylcholine in the different regions examined. The conclusion of Feldberg and Vogt that in the central nervous system cholinergic neurons alternate with noncholinergic ones suggests the possibility that our pictures of the distribution of cholinesterase in the pigeon brain may reflect the actual situation rather than inadequacies of the method.

It is evident that at present no conclusion should be drawn from these observations as to the differences in actual metabolic functions of various parts of the brain. But it is clear that such areas as the nucleus rotundus and the nucleus ovoidalis differ not only in anatomical structure but also in some way biochemically from the surrounding brain tissue. For instance processes which require an enzyme to catalyze the hydrolysis of phosphomonoesters within the pH range 9.0 to 9.4 evidently occur to a much smaller extent in the nucleus rotundus and nucleus ovoidalis than in the adjoining optic tectum. On the other hand the large amounts of cholinesterase in the nucleus rotundus and nucleus ovoidalis indicate that they consist largely of cholinergic neurons and that they are surrounded by brain tissue containing mainly non-cholinergic neurons. Whatever these differences may mean they are so pronounced that these structures are more conspicuous in histochemical preparations than in sections stained with ordinary histological stains.

Summary and conclusions. Histochemical methods were used to demonstrate the presence of alkaline and acid phosphatase, phosphamidase, and cholinesterase in the brain of pigeons. It was found that there are marked differences in the enzyme contents of various structures in the brain. This observation corroborates the concept of biochemical differences between different parts or different functional and anatomical systems that has evolved from the work of a number of investigators.‡ The use of histochemical methods makes possible the more detailed study of the "Chemoarchitectonics" of the vertebrate brain which will be of interest when correlated with the cyto-, myelo- and angioarchitectonics.

[19] Feldberg, W., and Vogt, M., *J. Physiol.*, 1948, **107**, 372.

‡ The relevant literature will be discussed in another paper.

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