# Quantitative Studies of White Matter

## I. *Enzymes involved in glucose-6-phosphate metabolism*

D. B. McDOUGAL, JR., D.W. SCHULZ, J.V. PASSONNEAU, J. R. CLARK, M. A. REYNOLDS, and O. H. LOWRY

From the Department of Pharmacology and the Beaumont-May Institute of Neurology, Washington University School of Medicine, St. Louis

ABSTRACT Total lipid and six enzymes closely related to the metabolism of glucose-6-phosphate have been measured in ten tracts of the rabbit. Lipid content appears to be a valid indicator of the degree of myelination. Heavily myelinated tracts have much larger amounts of glucose-6-phosphate dehydrogenase than lightly myelinated ones but there is no corresponding difference in 6-phosphogluconate dehydrogenase. In fact the ratios between the two enzymes were found to vary over a ninefold range. Hexokinase is found in largest amounts in tracts with relatively little lipid, and this tends to be true for phosphofructokinase as well. The fibrillar layer of olfactory bulb is exceptional with regard to both enzymes, and to glucose-6-phosphate dehydrogenase. The enzymes are present in amounts which are more than adequate to support glucose metabolism at a rate commensurate with the known rates of $O_2$ uptake by various tracts. The distribution of some of the enzymes is compatible with the notion that the nodes of Ranvier are regions of high metabolic activity. A simple algebraic relationship is found to hold fairly well for the distribution of four of the enzymes among the tracts.

### INTRODUCTION

The various tracts of the central nervous system are regions of relative simplicity within an exceedingly complex organ. Nevertheless there is considerable variation from tract to tract in the caliber and length of axons, degree of myelination, and probably in amount and even type of glia. Also, tracts vary widely in function and in synaptic transmitter agent.

Earlier studies (7, 23, 25) demonstrated rather surprising differences in enzyme activities among a limited group of tracts. Therefore, a more com-

prehensive study was undertaken in the hope of relating the chemical composition to structure or to function. This paper contains the results of analyses of ten fiber tracts of the rabbit for total lipid and for six enzymes intimately involved in the metabolism of glucose-6-phosphate. Because of the lack in most cases of quantitative data as to fiber composition of these tracts it is premature to attempt exact anatomical-chemical correlations. However, there appear to be certain obvious relationships between enzyme content and the extent of myelination.

## MATERIAL

Tissues were taken from adult laboratory rabbits of either sex. The tracts were dissected from frozen-dried sections 20 to 25 $\mu$ thick made at right angles to the long axis of the tract, and samples of suitable size (0.5 to 3 $\mu$g.) were weighed as previously described (18). Nine rabbits are represented. As many as five tracts have been analyzed in a single rabbit, and five of the six enzymes have been measured in four tracts from each of two rabbits. All the enzymes except phosphoglucoisomerase have been determined in one tract (pyramid) from three or four rabbits (Table I).

Dorsal columns were dissected from the region of the cervical enlargement of the spinal cord. The region selected for analysis is bounded laterally by Lissauer's tract, dorsally by the pial surface, and ventrally by a line running between the dorsomedial corners of the substantiae gelatinosae of each side. The dorsal spinocerebellar tract, also dissected from the cervical enlargement, was assumed to extend from Lissauer's tract ventrolaterally as far as a transverse line extending laterally from the dorsal aspect of the dorsal gray commissure. It was assumed to extend from the pia inward toward the central canal about as far as does Lissauer's tract. The pyramidal and olfactory tracts were taken from medulla and ventral surface of the frontal lobe, respectively, where they are easily distinguished from the surrounding tissues. The optic tract was taken just central to the chiasm. In this region there is a bundle of small fibers running along the ventrocaudal aspect of the tract which was analyzed separately. It is easily recognized as a grayer region in unstained sections, but its dorsal margin (next to the main body of the optic tract) is not sharp. We shall call it the commissural bundle.[1] The fornix and mamillothalamic tract were taken in the region between the mammillary bodies and the anterior thalamic nuclei. The full length of the habenulointerpeduncular tract was used except for that portion passing through the interpeduncular nuclei. Finally, the fibrillar layer of the olfactory bulb was used. In the case of those tracts lying at the surface of the nervous system, the membranes were stripped off each sample before weighing and assay.

[1] This is the region of the commissures of Gudden and Meynert. Because of many similarities between the enzyme contents of this region and those of the optic tract proper, it seems probable that the area contains many optic fibers. Whether it also corresponds to the small fiber portion of the optic tract described in the cat by Bishop, Jeremy, and Lance (4) and by Bishop and Clare (2), is not known. The location does not suggest it, but no other aggregation of small fibers has been found.

## METHODS

*Fat-Free Dry Weights* These were obtained by extracting with *n*-hexane, followed by absolute ethanol, and then more hexane (18). Total lipid is assumed to be represented by that fraction of the weight of each sample lost during the extraction.

*Hexokinase* This enzyme was assayed by a procedure in which the glucose-6-P first formed is converted to 6-P-gluconate with glucose-6-P dehydrogenase and $TPN^+$[2]. In a second step the 6-P-gluconate is further oxidized with 6-P-gluconate dehydrogenase and $TPN^+$. For each mole of original product, two moles of TPNH are produced. The TPNH is measured by its native fluorescence (19).

The procedure is essentially that described for analyzing samples of retina (21). Although 10 times larger samples were used in the present study, the total enzyme activity was not much greater. The incubation time was reduced from 60 to 30 minutes, consequently the concentration of glucose-6-P dehydrogenase was doubled. After incubation in 5 $\mu$l. of reagent there were added to each sample 25 $\mu$l. of 0.05 M tris buffer, pH 8.2, containing 2 mM EDTA and sufficient 6-P-gluconate dehydrogenase to give an activity (with substrate optimum) of at least 1 mmole per liter per hour at 38°. After 10 minutes at 38°, a 25 $\mu$l. aliquot was diluted with 1 ml. of 0.05 M $Na_2CO_3$:0.005 M $NaHCO_3$, and the fluorescence measured. The blank fluorescence of each tube with carbonate buffer was measured before adding the sample aliquot. In the original method, the second step was conducted in 1 ml. The change described reduced the blank reading, since the available 6-P-gluconate dehydrogenase was somewhat fluorescent. It was necessary as a result of this change to increase the EDTA concentration to block further hexokinase activity completely.

*Phosphoglucoisomerase* This was measured by a method of Buell *et al.* (7). Since that paper was published it has been found that fructose-6-P gives 122 per cent as much color as fructose in the resorcinol procedure used, rather than 80 per cent as had been assumed (13). Consequently the previous values are about 50 per cent too high. In an earlier paper (22) the assumed value for the color given by fructose-6-P was 62 per cent of that given by fructose.

*Phosphofructokinase* The method used (adapted from Ling *et al.* (16)) is based on the measurement of $DPN^+$ formed when the fructose-di-P first produced is converted to glycerol-P with the aid of auxiliary enzymes. The procedure is essentially that described for analyzing retinal samples (21) but because of the number of minor changes, the complete method is briefly described. To each sample were added 10 $\mu$l. of 0.1 M tris buffer, pH 7.9, containing 1.2 mM ATP, 5 mM $MgCl_2$, 100 mM KCl, 2 mM glucose-6-P, and sufficient phosphoglucoisomerase from muscle (13) to give an activity of 50 mmoles per liter per hour at 25° (substrate optimum). Also present were 0.8 mM DPNH, 0.05 per cent bovine plasma albumin, 0.01 per cent crystalline

[2] Abbreviations used in the text include $TPN^+$ for triphosphopyridine nucleotide, TPNH for its reduced form; $DPN^+$ for diphosphopyridine nucleotide, DPNH for its reduced form; EDTA for ethylenediamine tetraacetic acid, and tris for tris(hydroxymethyl)aminomethane.

aldolase (Sigma Chemical Company, St. Louis), and 0.003 per cent of a mixture of triosephosphate isomerase and glycerol phosphate dehydrogenase (Boehringer and Sons, Mannheim). (The phosphoglucoisomerase plus glucose-6-P produced an initial equilibrium concentration of about 0.5 mM fructose-6-P. The provision of substrate in this way was preferred to the use of less pure commercial preparations of fructose-6-P.)

Samples were incubated for 30 minutes at 38° in 10 μl. of the above reagent. The reaction was stopped with 3 μl. of 2 N HCl which also destroyed excess DPNH. A 10 μl. aliquot was added to 100 μl. of 7 N NaOH in a 3 ml. fluorometer tube. After 30 minutes at 38° 1 ml. of water was added and the fluorescence measured (19). Standards consisted of 0.25 mM fructose diphosphate, and the adequacy of the auxiliary enzymes was shown by the fact that extra standards gave 90 per cent or more of expected $DPN^+$ in 2 minutes' incubation.

*Phosphoglucomutase* The method used depends on measuring the product, glucose-6-P, with $TPN^+$ and glucose-6-P dehydrogenase. The procedure was similar to that described for retinal samples (21). The TPNH standard was appropriately increased, and in the second step reagent, which contained the dehydrogenase and phosphoglucoisomerase, the tris buffer was changed to 0.05 M, pH 8, and the EDTA was increased to 5 mM to suppress phosphoglucomutase more completely.

*Glucose-6-P Dehydrogenase* This enzyme was measured by fluorescence from the TPNH formed. The composition of the buffer–substrate–reagent was identical to that used in the retina study (21) except that no 6-P-gluconate dehydrogenase was added. Samples were incubated with either 10 μl. of reagent (group 1, Table II) or 25 μl. of reagent (group 2, Table II) for 30 minutes. In a few instances involving samples weighing less than 1 μg., incubation time was 60 minutes. In the case of group 1, a 9 μl. aliquot was diluted in 1 ml. of 0.05 M $Na_2CO_3$:0.005 M $NaHCO_3$ and the fluorescence of the native TPNH was read directly. Standards consisted of TPNH at a concentration of 0.05 mM during the incubation. Group 2 samples were treated with weak alkali to destroy excess $TPN^+$, and the TPNH was measured by the indirect procedure in strong alkali (21).

Since 6-P-gluconate dehydrogenase was omitted, only one mole of TPNH was formed per mole of glucose-6-P oxidized. Endogenous 6-P-gluconate dehydrogenase did not interfere because the activity of this enzyme in tracts is low relative to the activity of glucose-6-P dehydrogenase, and because the concentrations of 6-P-gluconate formed were low relative to the Michaelis constant (0.1 mM) at the pH of incubation (pH 9).

*6-Phosphogluconate Dehydrogenase* This enzyme was measured by a procedure very similar to that used for glucose-6-P dehydrogenase. The buffer–substrate–reagent was adapted to the present purpose from a reagent previously described (21). The samples were incubated 60 minutes at 38° in 6 μl. of 0.1 M tris buffer, pH 8.2, containing 1 mM 6-P-gluconate, 0.2 mM $TPN^+$, 0.3 mM EDTA, and 0.05 per cent bovine plasma albumin. A 5 μl. aliquot was diluted in 1 ml. 0.05 M $Na_2CO_3$:0.05 M $NaHCO_3$ and the fluorescence of the TPNH formed was measured. Because of the low final concentrations of TPNH, the blank fluorescence of each tube of carbonate buffer

was measured before adding the sample aliquot. Standards contained 0.05 mM TPNH during incubation.

## RESULTS

The enzymatic assays (Tables I and II) are presented on the basis of fat-free dry weight. Since most of the fat-free dry weight is protein, the figures given are approximately specific activities. In Table I the enzyme and lipid contents of pyramidal tracts from four rabbits are given to indicate the variability encountered. For averages of four to six determinations the standard errors ranged from 1 to 8 per cent of the mean values, and this agrees with the rest of the data to be presented. Except in the case of phosphoglucomutase the mean values are quite constant for the four animals.

TABLE I

LIPID AND ENZYME DATA FOR THE PYRAMIDAL TRACTS OF FOUR RABBITS

Enzyme activity is reported as moles of product per kilogram fat-free dry weight per hour ± S.E.M. Lipid values are reported as kilograms of lipid per kilogram fat-free dry weight ± S.E.M. Each figure is the mean of at least four (one instance—total lipid, rabbit 15) usually six, analyses.

| Rabbit, No. | Total lipid | Hexokinase | Phosphoglucomutase | Phosphofructokinase | Glucose-6-phosphate dehydrogenase | 6-phogluconic dehydrogenase | G6PDH / 6PGDH |
|---|---|---|---|---|---|---|---|
| 12 | 1.99 ±0.05 | 1.53 ±0.09 | 15.1 ±0.5 | 6.9 ±0.5 | 2.88 ±0.10 | 0.62 ±0.04 | 4.6 |
| 13 | 2.06 ±0.02 | 1.53 ±0.12 | 16.5 ±0.6 | 9.2 ±0.5 | 2.36 ±0.04 | 0.68 ±0.02 | 3.5 |
| 14 | 2.00 ±0.03 | 1.80 ±0.06 | 11.9 ±0.2 | 6.6 ±0.3 | 2.43 ±0.02 | 0.62 ±0.02 | 3.9 |
| 15 | 2.46 ±0.05 | | 11.0 ±0.3 | 7.8 ±0.3 | | | |

In Table II the tracts are arranged in order of increasing lipid content with the exceptions of the optic tract and commissural bundle, which are placed together to facilitate comparison between them. The tracts may be grouped anatomically thus: (*a*) the largely unmyelinated fibrillar layer of the olfactory bulb; (*b*) the tracts which are known to have rather small myelinated fibers: commissural bundle, fornix, mamillothalamic, and habenulointerpeduncular tracts; (*c*) those tracts with a somewhat larger average fiber diameter: optic, olfactory, and pyramidal tracts; and (*d*) tracts with large, heavily myelinated

fibers: dorsal columns and dorsal spinocerebellar tract. Grouped in this way, the tracts are seen to fall into coextensive groups according to lipid content, with means for each group of 0.6, 1.5, 2.3, and 3.5 kg. lipid per kg. fat-free dry weight respectively. It appears, therefore, that the gross lipid content may be a fair indicator of the degree of myelination, even though some of the lipid present is undoubtedly associated with protoplasm and despite the fact

TABLE II

LIPID AND ENZYME DATA FOR TEN TRACTS IN RABBIT

Units as in Table I. Data for pyramidal tract given as the mean of values in Table I.

| Group of animals§ | Total lipid* 1 + 2 | Hexokinase 1 | Phospho-glucomutase 1 | Phospho-glucoiso-merase 2 | Phospho-fructokinase 1 | Glucose-6-phosphate dehydrogenase‡ 1 | Glucose-6-phosphate dehydrogenase‡ 2 | 6-phospho-gluconate dehydro-genase 1 | G6PDH / 6PGDH 1/1 |
|---|---|---|---|---|---|---|---|---|---|
| Fibrillar layer olfactory bulb | 0.64 ±0.01 | 2.30 ±0.11 | 10.2 ±0.6 | 47 ±2 | 4.3 ±0.2 | 3.77 ±0.24 | | 0.95 ±0.05 | 4.0 |
| Fornix | 1.42 ±0.04 | 3.56 ±0.27 | 7.7 ±0.4 | 79 ±4 | 11.9 ±0.8 | 1.91 ±0.12 | 1.77 ±0.07 | 0.71 ±0.03 | 2.7 |
| Mamillothalamic tract | 1.49 ±0.02 | 3.22 ±0.23 | 8.5 ±0.3 | 70 ±3 | 11.5 ±0.3 | 1.89 ±0.06 | 1.44 ±0.12 | 0.79 ±0.03 | 2.4 |
| Habenulointerpeduncular tract | 1.63 ±0.04 | 3.02 ±0.11 | 9.3 ±0.3 | 58 ±3 | 7.9 ±0.7 | 2.21 ±0.10 | 1.76 ±0.15 | 1.22 ±0.09 | 1.8 |
| Commissural bundle | 1.54 ±0.04 | 3.92 ±0.54 | 12.8 ±0.9 | 117 ±5 | 13.8 ±0.9 | 2.16 ±0.11 | 2.35 ±0.05 | 1.03 ±0.07 | 2.1 |
| Optic tract | 2.51 ±0.02 | 2.60 ±0.31 | 10.2 ±0.4 | 178 ±11 | 11.1 ±0.2 | 2.78 ±0.08 | 2.07 ±0.17 | 0.73 ±0.01 | 3.8 |
| Pyramidal tract | 2.15 | 1.62 | 13.6 | 74 ±2 | 7.6 | 2.56 | 2.79 ±0.03 | 0.64 | 4.0 |
| Olfactory tract | 2.24 ±0.04 | 1.55 ±0.05 | 13.7 ±0.5 | 70 ±5 | 10.2 ±0.4 | 2.54 ±0.06 | 2.54 ±0.09 | 0.84 ±0.06 | 3.0 |
| Dorsal columns | 3.30 ±0.07 | 1.01 ±0.06 | 17.7 ±1.0 | 54 ±5 | 8.5 ±0.6 | 8.01 ±0.22 | 6.34 ±0.26 | 0.68 ±0.01 | 11.8 |
| Dorsal spinocerebellar tract | 3.76 ±0.05 | 1.33 ±0.04 | 12.9 ±0.5 | | 7.0 ±0.8 | 11.77 ±0.45 | | 0.68 ±0.02 | 17.3 |
| Average brain | 1.00 | 6 | 10 | 100 | 16 | 1.5 | 1.5 | 1 | 1.5 |

* Values from two or three animals except for dorsal spinocerebellar tract (one animal) and pyramidal tract (five animals).

‡ Tissues of group 1 were incubated in 10 μl. of substrate reagent, those of group 2 in 25 μl. of substrate reagent.

§ Group 1 includes animals 12, 13, 14, and 15, group 2 includes animals 5, 6, 7, and 10.

that no quantitative correlation with histologically demonstrable myelin is at present possible.

It may be seen that glucose-6-P dehydrogenase increases sixfold with increasing lipid and that phosphoglucomutase, though much more variable, tends in the same direction. On the other hand, hexokinase decreases to a third of the highest values with increasing lipid. Phosphofructokinase and

6-phosphogluconic dehydrogenase also decrease but not to the same degree. Isomerase remains rather constant with changes in lipid. It is of interest to note that the ratio of glucose-6-P dehydrogenase to 6-P-gluconate dehydrogenase varies tremendously, from 1.8 in habenulointerpeduncular to 17.3 in dorsal spinocerebellar tract. Although in general the enzyme contents of the tracts are similar within a group, it is seen that the optic tract contains more hexokinase, isomerase, and perhaps phosphofructokinase than the other members of its group. The high values for isomerase have been noted before (7) and may be related to the large amounts of isomerase in the cells of origin of this tract (20).

The fibrillar layer of the olfactory bulb may also be seen to contain amounts of some enzymes quite different from those expected on the basis of its lipid content. Thus it contains about one-half as much hexokinase and about one-third as much phosphofructokinase as expected, while the content of glucose-6-phosphate dehydrogenase is about three times that expected.

In comparing these data with values for average brain (Table II) it will be noted that in general those enzymes which are highest in the lightly myelinated group are even higher in whole brain, whereas glucose-6-P dehydrogenase, which is low in the lightly myelinated group of tracts, is even lower in whole brain.

## DISCUSSION

The enzyme activities found in the various tracts seem more than adequate to support the rates of $O_2$ consumption reported in the literature for separated tissues respiring in glucose-containing media, even assuming that all the oxygen consumed is used in the oxidation of glucose. Thus Bollard and McIlwain (5) report 29 millimoles $O_2$ per kg. wet weight per hour for subcortical white matter and 33 millimoles $O_2$ per kg. wet weight per hour for ventral slices of medulla, both in guinea pig. Rudin and Eisenman (24) report 19 millimoles $O_2$ per kg. wet weight per hour for dorsal columns of cat. In contrast the lowest hexokinase value is sufficient to provide enough glucose-6-P for oxygen consumption at 20 to 30 times these rates. In fact there is present sufficient 6-P-gluconate dehydrogenase, the least active of the enzymes measured, to transmit the entire flow of hexose with a ten-fold margin. Clearly the enzymes measured are present in abundance even though some of them cannot be as active in the cell as they are in the test tube in which pH and substrate concentrations are adjusted to give maximal rates.

In view of the evidence that many of the enzymes having to do with lipid metabolism are TPNH-dependent, it is perhaps not surprising to find a high concentration of glucose-6-P dehydrogenase in tracts with a high lipid content (see also reference 7). However, one would expect that for this en-

zyme to act as an efficient source of TPNH the rest of the pathway would have to be present in proportionately large amounts, and this is obviously not the case. In fact, the tremendous range in the ratio of the two dehydrogenases in the tracts suggests that the metabolism of 6-P-gluconate is not limited to direct conversion to ribulose-5-P. There is much evidence (see, *e.g.*, reference 9) which has been taken to mean that the hexose monophosphate shunt is not operative in the central nervous system, limited in part, by the low level of TPN there. But it is perhaps notable that the concentration of TPN in brain, which is at least 0.01 mM (19), is well above the $K_m$ of both glucose-6-P dehydrogenase and 6-P-gluconate dehydrogenase for this substrate.

If one can assume that the activities of the various enzymes measured *in vitro* are directly related to the activities *in vivo* and that the sum of activities leading toward glucose-6-P is equal to the sum of those leading away (*in vivo*), then some such relation as the following might be expected to hold:

$$a(\text{HK}) + b(\text{PGM}) = c(\text{PFK}) + d(\text{6-PGDH}).$$

The capital letters refer to the activity of the enzyme *in vitro* (hexokinase, phosphoglucomutase, phosphofructokinase, and 6-P-gluconate dehydrogenase, respectively). Actually, if one substitutes $a = 0.30$, $b = 0.030$, $c = 0.044$, and $d = 0.80$, the relationship holds fairly well for all the tracts (Table III). If, as suggested by some (9), $d$ should equal 0, solution for $a$, $b$, and $c$ gives 0.34, 0.04, and 0.13 respectively. In this case the fit is not as good and the corresponding value for column 5 for fibrillar layer of olfactory tract is 2.12, quite different from the mean, 1.12. The relationship above, with or without 6-P-gluconate dehydrogenase, is obviously a very crude approximation if for no other reason than that phosphoglucomutase is used in both directions, to and from glucose-6-P. It should be emphasized that the equation depends upon many assumptions, and is therefore in a very precarious position. If one may draw a conclusion from such a shaky source, it might be that one must have about twenty-five times as much phosphoglucomutase, twenty times as much phosphofructokinase, and two and one-half times as much hexokinase as 6-P-gluconate dehydrogenase in order to have equal activities *in vivo*. Differences in the levels of metabolic intermediates may not only affect the course of a complicated system, but may also have effects on the diffusion gradients of these substances within the cell. The significance of the above empirical relationship between enzyme activities may lie in the realm of biologically optimal levels of metabolites. In a complex, sequential reaction the effect of a change in an enzyme component would be to alter the steady state levels of the substrate and product of that enzyme.

In considering possible correlations between the biochemical data just

presented and available anatomical data it must be first admitted that the latter are on the whole inadequate for our purpose. Quantitative anatomical studies of tracts in rabbit have been relatively few. Bruesch and Arey (6) include the rabbit in their study of the number of fibers, myelinated and unmyelinated, in the optic tract of many vertebrates but do not give fiber population densities. Lassek and Rasmussen (15) also include the rabbit in their study of the number of fibers per unit area and per tract in the pyramidal tract, but do not include data for fiber size.[3]

TABLE III

THE DATA OF TABLE II FOR FOUR ENZYMES MULTIPLIED BY THE FACTORS GIVEN IN THE TEXT

| Column No. | 1 Hexokinase | 2 Phosphoglucomutase | 3 Phosphofructokinase | 4 6-Phosphogluconate dehydrogenase | 5 $\frac{1+2}{3+4}$ |
|---|---|---|---|---|---|
| Factor | 0.30 | 0.030 | 0.044 | 0.80 | |
| Fibrillar layer of olfactory bulb | 0.69 | 0.31 | 0.19 | 0.76 | 1.05 |
| Fornix | 1.07 | 0.23 | 0.52 | 0.57 | 1.19 |
| Mamillothalamic tract | 0.97 | 0.26 | 0.51 | 0.63 | 1.08 |
| Habenulointerpeduncular tract | 0.91 | 0.28 | 0.35 | 0.98 | 0.90 |
| Commissural bundle | 1.18 | 0.38 | 0.61 | 0.82 | 1.09 |
| Optic tract | 0.78 | 0.31 | 0.49 | 0.58 | 1.02 |
| Pyramidal tract | 0.49 | 0.41 | 0.33 | 0.51 | 1.07 |
| Olfactory tract | 0.46 | 0.41 | 0.45 | 0.67 | 0.77 |
| Dorsal columns | 0.30 | 0.53 | 0.37 | 0.54 | 0.91 |
| Dorsal spinocerebellar tract | 0.40 | 0.39 | 0.31 | 0.54 | 0.93 |
| Mean | | | | | 1.00 |
| ± S.E.M. | | | | | ±.04 |

Whether or not it be assumed that lipid content is a measure of degree of myelination, many enzymes vary with lipid content in a more or less regular way. It is not known whether there is more emphasis on glycolysis in tracts containing less lipid, as is suggested by the increase in amounts of hexokinase and phosphofructokinase with increasing fat-free dry weight. Since other enzymes also increase in this manner (*e.g.* fumarase and glutamic–oxalacetic transaminase (22)), it seems more likely that there is greater general metabolic capacity in tracts with high fat-free dry weight. An explanation for this might be sought in the probable distribution of nodes of Ranvier in various

[3] It is worth pointing out that an estimate of axoplasmic volume based on the mean fiber diameter of a tract may be very misleading because a proportionately larger contribution is made to total axonal volume by large fibers than by small ones. A satisfactory procedure is to use the root mean square of the fiber diameters. The mean fiber diameter of a tract *is* necessary in the estimation of node frequency, however, since the relation between fiber diameter and internodal length is nearly linear (12).

tracts. In the spinal cord the internodal distance increases with axon diameter (Hess and Young (12)). If this be true for other parts of the central nervous system, then the number of nodes per unit volume, other things being equal, would be greatest in the small fiber tracts. Carpenter (8) has suggested that the metabolic activity of axons is concentrated in the region of the nodes of Ranvier. One might expect, therefore, higher enzyme concentrations in the (small fiber) tracts with greater fat-free dry weight.

It has been seen that the fibrillar layer of the olfactory bulb is out of line with the other tracts. Its enzyme composition is approximately that expected for a tract with three or four times its lipid content. It may be that the differences between the actual enzyme contents and those expected reflect anatomical or physiological peculiarities. For example, the fibers of this layer are completely unmyelinated or nearly so. Furthermore, they appear to be considerably smaller than those elsewhere (11). The cells of origin of the tract have a peripheral location, rather than a central one, in contrast to all the other tracts studied here save only dorsal columns, some of whose cells of origin (probably relatively few in the rabbit) are located in dorsal ganglia. Finally, primary olfactory fibers in pike have the physiological properties of C fibers (11). The fibers in rabbit are probably C as well. The likelihood is that all the fibers in the other tracts are A fibers, with the possible exception of some of those in pyramid (26).

Buell *et al.* (7) have commented upon the high concentrations of glycolytic enzymes in the optic tract, and there is little to add here. It is probable, as they suggest, that the metabolic patterns in tracts are at least in part reflections of the patterns found in the parent neurons. It must be remembered, however, that a rather large but unknown amount of functional protoplasm is present in each tract which comes not from neurons but from glia. Using the fiber population densities kindly provided by Dr. Guillery (personal communication, 1960) and some estimates of fiber size and myelin thickness made upon electron micrographs generously provided by Dr. Sarah Luse, crude estimates[4] of proportions of axoplasm and myelin have been made for the fornix and mamillothalamic tracts. For both tracts, axoplasm occupies about 10 to 15 per cent of the total volume of the tract, while myelin occupies another 6 per cent. The amount of glial protoplasm present in these two tracts, therefore, is relatively large. No adequate estimate may be made of its enzyme activity at present.

The situation with regard to physiological data is at present no better than that for the anatomical. Physiological parameters which might be of interest

[4] No allowance has been made for shrinkage (which may be different for axon, myelin, and glia) in these estimates. Although the estimates of fiber population density made by Dr. Guillery and by electron microscopy are gratifyingly close, the correspondence may well be fortuitous since statistically adequate samples have not yet been obtained in electron micrographs.

from a biochemical point of view are those of conduction velocity, duration of absolute refractory period, and of supernormal and subnormal periods. In addition, some notion of the activity in a tract under conditions approaching those of everyday life for the animal would be interesting. One or more of these variables has been studied in the dorsal spinocerebellar tract (14, 17), pyramidal tract (1, 3, 26), optic tract (2, 4), and dorsal columns (10, 24) in cat; and in olfactory nerve (11) in pike. However, none of these variables has been adequately studied for many of the tracts in the rabbit. Unlike the other tracts studied, dorsal columns have no positive post-spike potential following a single stimulus (10, 24). Whether this is true in the rabbit as well as the cat is not known. If so, any possible enzymatic correlate of this fact is not yet identifiable.

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