Quantitative Studies of White Matter

II. *Enzymes involved in triose phosphate metabolism*

D. B. McDOUGAL, JR., ROBERT T. SCHIMKE,
ELIZABETH M. JONES, and ELIZABETH TOUHILL

From the Department of Pharmacology and Beaumont-May Institute of Neurology, Washington University School of Medicine, St. Louis. Dr. Schimke's present address is the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda

ABSTRACT Methods for measurement of glyceraldehyde-P dehydrogenase, triose-P isomerase, fructose 1,6-diphosphate aldolase, and the DPN-linked and flavin-linked α-glycero-P dehydrogenases in small amounts of tissue have been worked out. These enzymes have been measured in ten tracts in rabbit central nervous system. The activities of all the enzymes measured, except the flavin-linked α-glycero-P dehydrogenase, are present in larger amounts in lightly myelinated than in heavily myelinated tracts, but are relatively low in fibrillar layer of olfactory bulb, which is unmyelinated. Aldolase, like P-fructokinase (measured previously), is especially low in fibrillar layer. Taken together with relatively high 6-P-gluconate dehydrogenase activity found earlier this supports the hypothesis that the pentose-P shunt is particularly active in this tract. The activity of DPN-linked α-glycero-P dehydrogenase is inversely proportional to the lipid content of the myelinated tracts, which suggests that its primary role is not related to lipid synthesis in adult brain. The activities of flavin-linked α-glycero-P dehydrogenase are unrelated to those of the DPN-linked enzyme, which is contrary to expectation if the two enzymes function as partners in the "α-glycerophosphate shuttle."

INTRODUCTION

Previous studies of groups of closely related enzymes in white matter have raised a number of interesting problems regarding the metabolism of this relatively simple tissue (23, 24). The present paper is an extension of the earlier work to include five enzymes concerned with production and utilization of triose phosphate. To attain adequate sensitivity, new methods had to be developed. Although the methods have been devised particularly for brain, it is believed they are of general utility.

METHODS

Frozen-dried samples were obtained as previously described (24). Blocks of tissue containing the desired tracts (see legend, Fig. 1) were cut from the brains of rabbits. All ten tracts were obtained within 30 minutes after decapitation, and each was rapidly frozen in dichlorodifluoromethane (freon 12, E. I. du Pont de Nemours and Company) chilled in liquid nitrogen as soon as possible. Sections were cut in a cryostat at −25° and then dried *in vacuo* at −40° (16). Care was taken to avoid thawing until the samples were dry. The tracts were dissected freehand under a dissecting microscope at 18° and weighed on a quartz fiber balance (16). Storage of the tissues and handling during dissection were as described by Lowry *et al.* (19).

The lipid-free dry weights are in satisfactory agreement with values previously obtained (24).

The enzyme methods are based on the ultimate production and fluorometric measurement of DPN^+ or DPNH[1] (17). The assay conditions for each enzyme are listed in Table I. The auxiliary enzymes, glyceraldehyde-P and dihydroxyacetone-P, were obtained from California Corporation for Biochemical Research. The pyridine nucleotides and α-glycero-P were obtained from Sigma Chemical Company. Both imidazole and 2-methylimidazole as obtained from commercial sources contain fluorescent impurities. They may be adequately freed of those impurities by treatment with charcoal (norit A, Fisher Scientific Company) which has been washed with 0.01 N HCl and glass-distilled water. The brain samples are incubated in the reagent and for the time indicated. Standards used are DPN^+ for aldolase, the α-glycero-P dehydrogenases, and triose-P isomerase, and DPNH for glyceraldehyde-P dehydrogenase. In addition a dihydroxyacetone-P standard is carried through the entire α-glycero-P dehydrogenase (flavin) procedure. Dihydroxyacetone-P is also used for two secondary standards in the triose-P isomerase procedure. The first, with a blank, is incubated for 2 minutes at 38°, the second is carried through the 15 minute incubation with the tissue samples. A similar pair of glyceraldehyde-P standards is used in the aldolase assay. These standards are used to provide assurance that the auxiliary enzymes are sufficiently active. After the incubation the samples are chilled in ice and prepared for reading in the fluorometer as follows:

Aldolase and α-Glycero-P Dehydrogenase (DPN) HCl is added to a final concentration of 0.2 to 0.4 N. All of each sample is transferred to a 75 μl aliquot of 7 to 8 N NaOH, and the tubes are incubated at 60° for 10 minutes to develop the fluorescence of DPN^+ (17). 1 ml of water is added and the samples are read. *Triose-P isomerase* HCl is added as above. A 10 μl aliquot is added to 75 μl of 8 N NaOH and incubated at 60° for 10 minutes. 1 ml of water is added before reading.

[1] DPN^+ and DPNH are used for oxidized and reduced diphosphopyridine nucleotide respectively. DPN is used when the state of oxidation need not be defined. Throughout this report the term α-glycero-P dehydrogenase (DPN) is used for enzyme 1.1.1.8 L-glycero-3-phosphate:NAD oxidoreductase and α-glycero-P dehydrogenase (flavin) for the flavoprotein 1.1.2.1 L-glycero-3-phosphate: cyt *c* oxidoreductase (28).

TABLE I

ASSAY CONDITIONS

Incubations were for 15 minutes at 38° for triose-P isomerase and glyceraldehyde-P dehydrogenase, 30 minutes at 38° for aldolase and α-glycero-P dehydrogenase (DPN), 60 minutes at 38° in reagent I for α-glycero-P dehydrogenase (flavin). The second step (reagent II, assay of dihydroxyacetone-P) was carried out at room temperature for 10 minutes. All concentrations are given as they occur in the final reagent.

The following abbreviations are used: DHAP for dihydroxyacetone-P, EDTA for ethylenediaminetetraacetic acid, FDP for fructose 1,6-diphosphate, GAP for D-glyceraldehyde-3-P, D,L-α-GOP for D,L-α-glycero-P, α-GOPDH for α-glycero-P dehydrogenase (DPN), Imid for imidazole, Me-imid for 2-methylimidazole, and TPI for triose-P isomerase.

		Substrates	Cofactors	Added Enzymes	Buffer and pH	Other additions*	Tract weight	Reagent volume
		mM	*mM*	*μg/ml*	*mM*	*mM*	*μg*	*μl*
Aldolase		FDP 1	DPNH 0.5	TPI-α-GOPDH 12.5	Imid 100 pH 7.45	Nicotinamide 20	0.2–1.3	10
Triose-P isomerase		GAP 2	DPNH 0.5	α-GOPDH 10	Imid 100 pH 7.45	Nicotinamide 20	0.02–0.12	100
Glyceraldehyde-P dehydrogenase		GAP 2 K_2HPO_4 50	DPN^+ 0.5		Me-imid 75 pH 8.25	EDTA 2 mercaptoethanol 5 Na amobarbital 4	0.03–0.30	100
α-Glycero-P dehydrogenase (DPN)		DHAP 1	DPNH 0.5		Imid 100 pH 7.45	Nicotinamide 20 Na amobarbital 5	0.5–3.0	10
α-Glycero-P dehydrogenase (flavin)	Step I	D,L α-GOP 40	Methylene blue 0.5		PO_4 50 pH 7.9		0.5–4.0	3
	Step II		DPNH 0.003	α-GOPDH 3.5	PO_4 50 pH 7.4	Nicotinamide 20 Na amobarbital 5		300

* The first four reagents contain 0.05 per cent bovine serum albumin. α-Glycero-P dehydrogenase (flavin) reagent I contains 0.02 per cent albumin, II contains 0.01 per cent.

Glyceraldehyde-P Dehydrogenase A 90 μl aliquot is transferred to 1 ml of 0.02 N NaOH. The DPNH fluorescence may then be read (17).

α-Glycerophosphate dehydrogenase (flavin) is assayed in a two-step reaction:

$$\text{I. } \alpha\text{-Glycerol-P} + \text{methylene blue (oxidized)} \xrightarrow[\text{(brain sample)}]{\alpha\text{-glycero-P dehydrogenase (flavin)}} \text{dihydroxyacetone-P} + \text{methylene blue (reduced)}$$

$$\text{I}a\text{. Methylene blue (reduced)} + H^+ + O_2 \xrightarrow{\text{non-enzymatic}} \text{methylene blue (oxidized)} + H_2O_2$$

$$\text{II. Dihydroxyacetone-P} + \text{DPNH} \xrightarrow[\text{(purified enzyme)}]{\alpha\text{-glycero-P dehydrogenase (DPN)}} \alpha\text{-glycero-P} + DPN^+$$

After incubation of the brain samples in step I reagent (Table I), the reaction is stopped with HCl (final concentration, 0.3 N), and the entire mixture is transferred to fluorometer tubes containing step II reagent which contains no DPNH. DPNH is added separately under reduced illumination to yield the appropriate final concentration. The tubes are incubated in the dark at room temperature for 10 minutes. In dim light, HCl is added to each tube to a final concentration of 0.2 to 0.4 N to destroy the remaining DPNH. Then 1 ml of 8 N NaOH is added, and the tubes are heated at 60° for 10 minutes to develop the fluorescence of the DPN^+ produced in step II. As pointed out by Lowry *et al.* (17), in strong alkali the fluorescence is more sensitive to destruction by light, and special care must therefore be taken to avoid direct irradiation of the samples before they have been read.

COMMENTS ON METHODS

Aldolase The method is a modification of one used by Wu and Racker (34). The assay is linear with enzyme concentration up to 2 μg (wet weight) of average brain, while 0.4 μg gives a reading which is double the blank after a 30 minute incubation. ("Average brain" refers to an homogenate of whole rabbit brain, exclusive of olfactory bulbs and all the tissue caudal to the intercollicular sulcus.) Conversion of the glyceraldehyde-P standard to α-glycero-P, with corresponding DPN^+ production, was always 95 per cent complete or better in 2 minutes. The activity at 4° is given as 3 per cent of that at 38° by Lowry *et al.* (20).

Triose-P Isomerase The choice of buffer is of some significance. Oesper and Meyerhof (25) have shown that the enzyme is inhibited by phosphate ions. Sulfate, arsenate, and carbonate also inhibit the enzyme. Imidazole is a buffer with an appropriate pK which does not inhibit the enzyme nor react with its substrate (see below).

The reaction is linear with brain concentrations up to 0.15 μg of average brain and for at least 30 minutes at 38°, provided that less than 5 per cent of the substrate is utilized. The Michaelis constant of partially purified enzyme

from rabbit brain for glyceraldehyde-P was 0.12 mM which is one-third of that given by Beisenherz (3) for the enzyme from calf muscle. The K_m for dihydroxyacetone-P was not measured. The activity of the enzyme at 0° is 9 per cent of that at 38°. Rabbit brain contains four times as much activity as observed by Oesper and Meyerhof (25) for a brain from an unnamed species. They found a ratio of triose-P isomerase to aldolase activity of 300, which is similar to the ratio found here.

Glyceraldehyde-P Dehydrogenase Optimal conditions for measuring this enzyme are attained by taking into account a series of interrelated circumstances, many of which appear to conspire against the experimenter. In the first place, it is clear that triose-P isomerase will compete for glyceraldehyde-P. Since rabbit brain contains seven times as much isomerase activity as dehydrogenase activity, sufficient substrate must be included to saturate the dehydrogenase throughout the incubation period in the face of this competition. Raising the P_i concentration helps to minimize interference from this source, and permits linearity with a wider range of brain concentrations (up to 0.8 μg (wet weight) of average brain, where in 15 minutes 0.2 μg gives an uncorrected reading equal to twice the 15 minute blank) and incubation times (up to 1 hour).

Amelunxen and Grisolia (2) have shown that glyceraldehyde-P dehydrogenase is inactivated by DPNH. In the present method, this problem has been met by keeping the final product concentration below 0.05 mM.

Another potential source of trouble is α-glycero-P dehydrogenase (DPN). Since the substrates for this enzyme accumulate during the incubation, α-glycero-P dehydrogenase (DPN) will become increasingly active. However, difficulty from this source is not appreciable except in tissues in which the activity of the two enzymes is more nearly equal than it is in brain.

Finally, two non-enzymatic reactions may occur which must be minimized. The first of these is the reaction of glyceraldehyde-P with primary amino groups. Cori *et al.* (7) found that glyceraldehyde-P will react with cysteine. According to Segal and Boyer (30) the half-time of the reaction with tris(hydroxymethyl)aminomethane or cysteine is 10 to 20 minutes. (Apparently a Schiff base is formed. The reaction can be shown to be reversible by adding triose-P isomerase, α-glycero-P dehydrogenase (DPN), and DPNH.) This reaction is avoided by using 2-methylimidazole as a buffer and β-mercaptoethanol as a source of sulfhydryl groups. The second reaction to be avoided is that of DPN^+ with carbonyl-containing compounds (in this case glyceraldehyde-P) which was studied by Burton and Kaplan (5). The reaction does not occur rapidly enough to deplete substrate significantly, but it does produce a material which, as Burton and Kaplan have shown, has some of the properties of DPNH, namely acid lability, alkali stability, and enhanced absorption of

light at 340 mμ. As might be expected the material also fluoresces under the same conditions as DPNH, and consequently contributes materially to the blank. It is for this reason that the reaction is run at pH 8.23, well below that given by Cori *et al.* (7) as optimum for the enzyme. At this pH the blank is lower, apparently because ionization of the hydrogen atom on carbon 2 of glyceraldehyde-P is suppressed. Furthermore, at this pH the K_m for glyceraldehyde-P (0.18 mM) is about half what it is at pH 9.0, so that the glyceraldehyde-P concentration may be reduced without reducing the degree of saturation of the enzyme with substrate. At pH 7.1 the K_m for glyceraldehyde-P is even lower (0.05 mM).

Under the conditions of assay (Table I), the Michaelis constants for DPN^+ and P_i are 0.02 mM, and 1 mM respectively. Cori *et al.* (7) gave a K_m of 0.05 mM for glyceraldehyde-P and of 0.039 mM for DPN^+ with the crystalline enzyme from rabbit muscle at pH 8.5.

The activity of the enzyme at 0° is 1.5 per cent of the activity at 38°.

α-Glycero-P Dehydrogenase (DPN) The DPNH dehydrogenase (oxidase) activity of brain homogenates is of the same order of magnitude as the activity of α-glycero-P dehydrogenase (DPN). The problem in rat brain has been defined by Laatsch (15) who successfully used amobarbital to block DPNH oxidation, as has been done here. The DPNH dehydrogenase activity is also readily blocked by cyanide and antimycin A, and is much reduced in frozen-dried tissues. Cyanide cannot be used in the assay of α-glycero-P dehydrogenase (DPN), however. This is because of the occurrence of a contaminant in solutions of dihydroxyacetone-P. The contaminant is transformed, in the presence of cyanide, into a substance, perhaps pyruvate, which is a substrate for lactate dehydrogenase. The contaminant is present in freshly prepared solutions of dihydroxyacetone-P, and increases in concentration with the age of the solution. Therefore it is probably a decomposition product of dihydroxyacetone-P. The substance appears to be methylglyoxal. This interpretation is strengthened by Smythe's demonstration that methylglyoxal is changed in the presence of cyanide to pyruvate (32). The result is that the large amounts of lactate dehydrogenase in brain interfere with the assay of α-glycero-P dehydrogenase (DPN) if cyanide is used.

The pH optimum for the enzyme in brain is 7.4. The Michaelis constant for dihydroxyacetone-P is 0.1 mM, for DPNH, 0.01 mM. The activity of the enzyme at 0° is 7 per cent of that at 38°.

There has been some question about the occurrence of this enzyme in brain (see review by Greville (13), also 14 and 15). However, Laatsch (15), Delbrück *et al.* (9), Pette and Luh (27), and Garcia *et al.* (12) have all found that the rate of enzyme action measured *in vitro* is high relative to the probable

rate of triose consumption by brain *in vivo*, but there are large species differences. The amount present in rabbit brain is only one-seventh of that in rat.

α-Glycero-P Dehydrogenase (Flavin) Methylene blue, required in the first step, can oxidize DPNH non-enzymatically, and the reaction is powerfully catalyzed by light. Therefore, to keep the blank low the reaction mixture from step I must be diluted 100-fold in step II reagent, reducing the methylene blue concentration to 5 μM at this stage. DPNH is then added under subdued illumination, and light is avoided as much as possible until after the excess DPNH has been destroyed by acid.

Recovery of a dihydroxyacetone-P standard carried through all the steps was 90 to 100 per cent. The enzyme withstands freeze-drying well. In fact, there was considerable activation of the enzyme in frozen-dried homogenates. The reaction is linear for at least 1 hour as long as the product concentration is kept below 0.2 mM.

The pH optimum for the enzyme in rabbit brain homogenates is 7.9–8.1, similar to that given by Ringler and Singer (29) for an acetone powder preparation of pig brain under the same conditions used here. The K_m for methylene blue is 0.02 mM, that for α-glycero-P is 3 mM, calculated as the L form. Ringler and Singer obtained a K_m for α-glycero-P of 9.5 mM for enzyme obtained from pig brain. There was no inhibition of the enzyme by 0.07 mM dihydroxyacetone-P at an α-glycero-P concentration of 40 mM, although under different conditions inhibition has been observed (29). The activity of the enzyme at 0° is 4 per cent of that at 38°. Pette and Luh (27) found two-thirds as much α-glycero-P dehydrogenase (flavin) activity in rat cerebral cortex as found in whole rabbit brain in the present study.

DISTRIBUTION OF ENZYMES IN WHITE MATTER

It can be seen that in general aldolase activity is higher in tracts with low lipid content (Fig. 1). There are two exceptions. The unmyelinated fibrillar layer of olfactory bulb has much less aldolase activity, and optic tract has more, than would be predicted on the basis of lipid content. On comparing aldolase with P-fructokinase (Fig. 2) it can be seen that the activities of the two enzymes vary together, without any striking exceptions. Triose-P isomerase and glyceraldehyde-P dehydrogenase activity (Figs. 3 and 4) also vary with the aldolase activity. The fibrillar layer of olfactory bulb is exceptional in both cases, but optic tract is seen to fall rather close to the mean for myelinated tracts.

α-Glycero-P dehydrogenase (DPN) activity is distributed in much the same way as aldolase (Fig. 5) except that optic tract is not out of line with the rest. Many of the tracts contain more of this enzyme than does average brain. In marked contrast, α-glycero-P dehydrogenase (flavin) activity (Fig. 5) varies

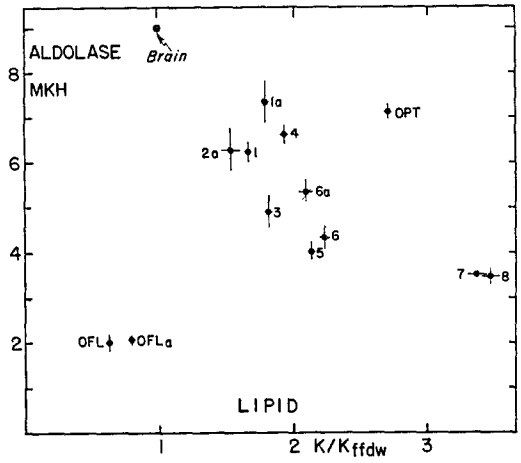


FIGURE 1. Aldolase activity of the various tracts plotted against the lipid content of the tracts. In all figures, the symbol for fibrillar layer of olfactory bulb is designated by OFL, for optic tract by OPT, and for average brain by Brain (for definition see Comments on Methods) The other tracts are designated by number, as follows: 1, fornix; 2, mammillothalamic tract; 3, habenulointerpeduncular tract; 4, commissures of Gudden and Meynert; 5, pyramidal tract; 6, olfactory tract; 7, dorsal columns; 8, dorsal spinocerebellar tract. The number or abbreviation alone indicates a tract from one rabbit; when followed by "a" it indicates a tract from a second rabbit. Enzyme activities are given as moles of substrate acted upon per kilo lipid-free dry weight and hour (MKH). Lipid content is expressed as the ratio weight of lipid/unit weight of lipid-free dry weight. Vertical lines are 2 × SEM for the assay on the ordinate; horizontal lines are 2 × SEM for the assay on the abscissa. These lines are absent when the symbol used to represent the datum is as large as or larger than 2 × SEM.

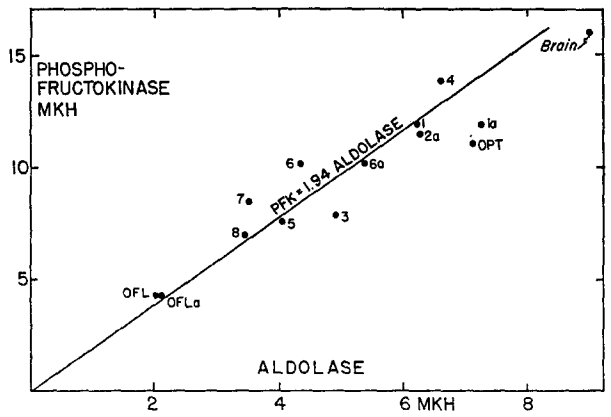


FIGURE 2. Phosphofructokinase activity plotted against aldolase. SEM's are omitted, symbols and units are as in Fig. 1 except that the P-fructokinase assays were on tracts from four rabbits not represented in Fig. 1 (24). The line represents the mean of the ratio P-fructokinase/aldolase for all the tracts (the ratio for average brain was omitted from the calculation).

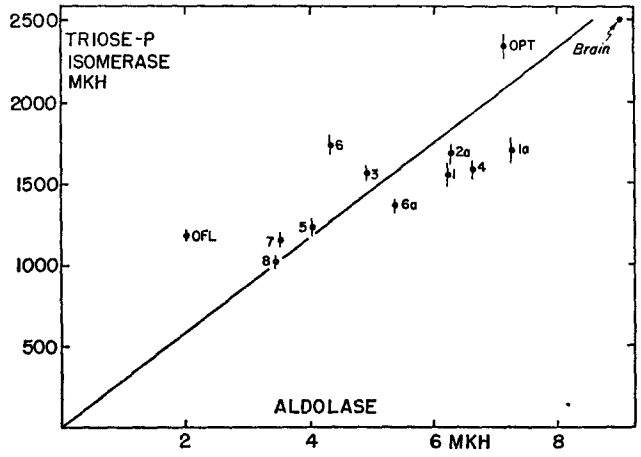


FIGURE 3. Triose-P isomerase plotted against aldolase. SEM for aldolase omitted. The line represents the mean of the ratio triose-P isomerase/aldolase for all myelinated tracts studied (fibrillar layer of olfactory bulb was omitted from the calculation). For significance of symbols see Fig. 1.

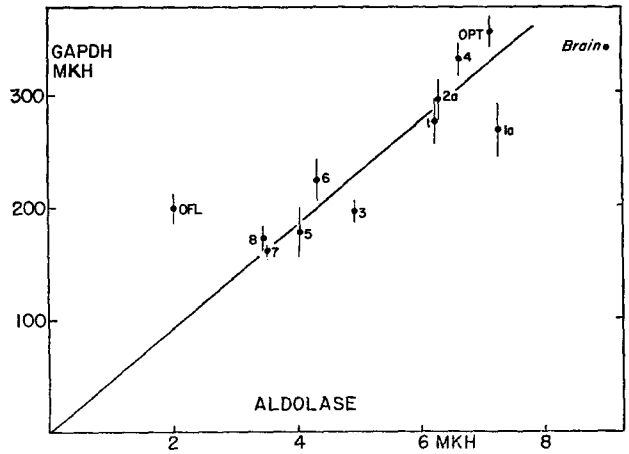


FIGURE 4. Glyceraldehyde-P dehydrogenase plotted against aldolase. The line is plotted for myelinated tracts. For significance of symbols see Fig. 1.

little from tract to tract except in the fibrillar layer of olfactory bulb where it is present in large amounts, although there is not as much present as in average brain.

DISCUSSION

The close association of glyceraldehyde-P dehydrogenase, triose-P isomerase, aldolase, and P-fructokinase is most striking. The distribution of this group of enzymes resembles the distribution of hexokinase (24), fumarase, malate

dehydrogenase, and glutamate–oxalacetate transaminase (23). That is to say, the enzymes are more active in (lightly myelinated) tracts with low lipid content, less active in heavily myelinated tracts, and less active than would be predicted on the basis of lipid content in the fibrillar layer of olfactory bulb. An interesting difference between the distributions of these two groups of enzymes is the smaller range of activities of the glycolytic group among myelinated tracts. For the glycolytic enzymes the highest values are only 2 to 2.5 times the lowest. For the citric acid cycle enzymes mentioned, and for hexokinase, the range is fourfold. It is tempting to relate this difference in the distribution of these two groups of enzymes, both of which are directly involved in the supply of energy to the tissue, to differences in intracellular distribution. Johnson (14) has shown that the P-fructokinase, aldolase,

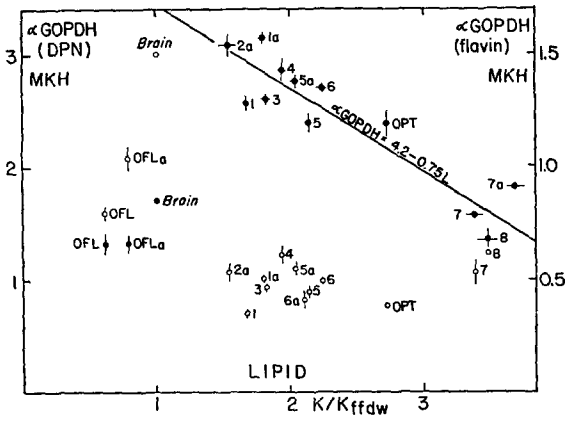


FIGURE 5. α-Glycero-P dehydrogenase (DPN) (filled symbols) and α-glycero-P dehydrogenase (flavin) (open symbols) plotted against lipid. For significance of symbols see Fig. 1.

triose-P isomerase, and glyceraldehyde-P dehydrogenase of rat brain are largely soluble. On the other hand, most of the hexokinase in brain is particulate (8). The studies of intracellular distribution of malate dehydrogenase (1), glutamate–oxalacetate transaminase (21, 22), and fumarase (31) in brain are somewhat difficult to interpret, perhaps partly because they were done with methods which have only recently been found to be inadequate for brain. However, it seems reasonable to suppose that, as with other tissues, brain mitochondria will be found to contain a significant fraction of the total tissue content of these enzymes. In retina all three are concentrated in a layer where mitochondria are densely congregated (18; O. H. Lowry, personal communication). It may be, then, that the process of concentration of enzymes at nodes of Ranvier, postulated earlier (24), is accomplished more efficiently by the cell when the enzymes are located in particles. Friede *et al.* (11), using

staining methods, found no evidence for the hypothesis that enzymes may be concentrated at the nodes of Ranvier in the central nervous system. Their evidence does support the same hypothesis made earlier with reference to peripheral nerve by Carpenter (6), and Williams and Landon (33) have recently found large numbers of Schwann cell mitochondria adjacent to the nodes. They found the remainder of the internodal Schwann cell cytoplasm to be "devoid of organelles." There are as yet no direct quantitative enzyme measurements pertinent to the subject. It should be pointed out that α-glycero-P dehydrogenase (flavin), also a particulate enzyme, shows very little variation among the myelinated tracts. Clearly the distribution of at least two different sorts of particles is being observed. It may be that the enzymes studied by Friede *et al.* are associated in the central nervous system with particles distributed like those containing α-glycero-P dehydrogenase (flavin).

The earlier finding (4, 24) of relatively high activity of glycolytic enzymes as a group in rabbit optic tract is extended to include the glycolytic enzymes triose-P isomerase and glyceraldehyde-P dehydrogenase. In contrast the glycero-P dehydrogenases, which do not serve on the pathway to lactate, are not exceptional in optic tract.

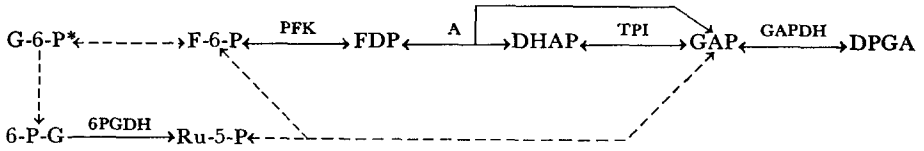
The triose-P isomerase/glyceraldehyde-P dehydrogenase ratio is about the same in the (unmyelinated) fibrillar layer of olfactory bulb as in myelinated tracts (Table II), but the ratios of aldolase and P-fructokinase to glyceraldehyde-P dehydrogenase are each about one-half in the unmyelinated tissue what they are in myelinated ones. If the activities of glyceraldehyde-P dehydrogenase and of aldolase or P-fructokinase in the two kinds of tissue may be taken as indirect measures of the rates of flow of triosephosphates and hexosephosphates respectively, one might conclude that the glyceraldehyde-P dehydrogenase of fibrillar layer is receiving triose-P from a source other than the main glycolytic path. The identity of this other source is suggested by the finding that the amount of 6-P-gluconate dehydrogenase (24) is increased relative to glyceraldehyde-P dehydrogenase in the unmyelinated tract. In fact, the ratio of 6-P-gluconate dehydrogenase to aldolase in fibrillar layer is three times what it is in myelinated tracts. Although 6-P-gluconate dehydrogenase is less active than enzymes in the main glycolytic path, one-tenth of the activity present would probably be sufficient to transmit the entire flow of hexose which occurs *in vivo*, as has been mentioned (24). In addition to 6-P-gluconate dehydrogenase and glucose-6-P dehydrogenase, transketolase has also been demonstrated in nervous tissue (10). The fact that the rate of conversion of ribose-5-P to sedoheptulose-7-P was higher in spinal cord than in cerebral cortex was interpreted as a sign of high activity of the shunt in white matter. In addition, under normal steady-state conditions P-fructokinase is probably rather severely inhibited (26). Therefore, the pentose-P shunt may well contribute significantly to the over-all utilization of glucose by nervous

tissue, perhaps particularly by white matter (compare myelinated tracts with average brain in Table II). On the basis of the present data, the shunt may be most active in fibrillar layer of olfactory bulb, among the tracts studied.

With regard to the α-glycero-P dehydrogenases the results are somewhat confusing. Since there appears to be no glycerokinase in brain, α-glycero-P dehydrogenase (DPN) must be a prime supplier in the synthesis of glycero-

TABLE II

The activities of 6-P-gluconate dehydrogenase and several glycolytic enzymes expressed as percentage of glyceraldehyde-P dehydrogenase activity for each tract and average brain in 1 unmyelinated and 9 myelinated tracts, and in average brain.



	6-P-gluconate dehydrogenase (6PGDH)	P-fructo-kinase (PFK)	Aldolase (A)	Triose-P isomerase (TPI)	Glyceralde-hyde-P-dehy-drogenase (GAPDH)
1. Fibrillar layer of olfactory bulb	0.48	2.2	1.0	595	100
2. Myelinated tracts	0.36	4.2	2.2	656	100
3. Myelinated tract deviating farthest in direction of 1	0.62‡	3.1§	1.9‖		
4. Average brain	0.29	4.7	2.6	735	100

Data for 6-P-gluconate dehydrogenase and P-fructokinase from (24).

* Abbrevations for metabolites in the diagram are G-6-P for glucose-6-P, F-6-P for fructose-6-P, FDP for fructose diphosphate, DHAP for dihydroxyacetone-P, GAP for glyceraldehyde-P, DPGA for 1,3-diphosphoglycerate, 6-P-G for 6-P-gluconate, Ru-5-P for ribulose-5-P.

‡ Habenulointerpeduncular tract.

§ Optic tract.

‖ Olfactory tract.

phospholipids. One might, therefore, predict that this enzyme would be related to myelination. Indeed, the work of Laatsch (15) on α-glycero-P dehydrogenase (DPN) in developing dorsal columns shows this relationship quite clearly. However, in adult tracts there is actually no more α-glycero-P dehydrogenase activity in dorsal spinocerebellar tract, the most heavily myelinated of them all, than in fibrillar layer of olfactory bulb, which is nearly completely unmyelinated. There is, furthermore, little difference between the distribution of this enzyme and that of the main glycolytic enzymes.

One might therefore inquire about the relation of the two α-glycero-P dehydrogenases, since much work has pointed toward the cooperation of the two enzymes in maintaining the extramitochondrial DPN^+ concentration. However, no correlation between the two enzymes is apparent. With regard to the flow of metabolites, if the α-glycerophosphate shuttle were the sole mechanism involved in the reoxidation of the DPNH produced by glyceraldehyde-P dehydrogenase (which seems unlikely), about one-fourth to one-sixth of the α-glycero-P dehydrogenase (flavin) present would be required to support the flow of trioses. This is a lower margin of safety than has been found in tracts for any other enzyme studied.

This research was supported by Grants R-96-59C and R-96-61C of the United Cerebral Palsy Research and Educational Foundation, by Grant B-2452 from the National Institutes of Health, and by Grant P-78 of the American Cancer Society.

Dr. McDougal started this work as Sister Elizabeth Kenny Scholar in Pharmacology. He is the recipient of a Research Career Development Award (NB-K3-18,005) of the National Institutes of Health.

Received for publication, July 12, 1963.

REFERENCES

1. ABOOD, L. G., GERARD, R. W., BANKS, J., and TSCHIRGI, R. D., *Am. J. Physiol.*, 1952, **168**, 728.
2. AMELUNXEN, R., and GRISOLIA, S., *J. Biol. Chem.*, 1962, **237**, 3240.
3. BEISENHERZ, G., *in* Methods in Enzymology, (S. P. Colowick and N. O. Kaplan, editors), New York, Academic Press Inc., 1955, **1**, 387.
4. BUELL, M. V., LOWRY, O. H., ROBERTS, N. R., CHANG, M.-L. W., and KAPPHAHN, J. I., *J. Biol. Chem.*, 1958, **232**, 979.
5. BURTON, R. M., and KAPLAN, N. O., *Arch. Biochem. and Biophysics*, 1957, **70**, 107.
6. CARPENTER, F. G., *Am. J. Physiol.*, 1958, **195**, 33.
7. CORI, G. T., SLEIN, M. W., and CORI, C. F., *J. Biol. Chem.*, 1948, **173**, 605.
8. CRANE, R. K., and SOLS, A., *J. Biol. Chem.*, 1953, **203**, 273.
9. DELBRÜCK, A., SCHIMASSEK, H., BARTSCH, K., and BÜCHER, T., *Biochem. Z.*, 1959, **331**, 297.
10. DREYFUS, P. M., and MONIZ, R., *Biochim. et Biophysica Acta*, 1962, **65**, 181.
11. FRIEDE, R. L., FLEMING, L. M., and KNOLLER, M., *J. Neurochem.*, 1963, **10**, 263.
12. GARCIA-BUNUEL, L., McDOUGAL, D. B., JR., BURCH, H. B., JONES, E. M., and TOUHILL, E., *J. Neurochem.*, 1962, **9**, 589.
13. GREVILLE, G. D., *in* Neurochemistry, (K. A. C. Elliott, I. H. Page, and J. H. Quastel, editors), Springfield, Illinois, Charles C Thomas, 1962, 238.
14. JOHNSON, M. K., *Biochem. J.*, 1960, **77**, 610.
15. LAATSCH, R. H., *J. Neurochem.*, 1962, **9**, 487.
16. LOWRY, O. H., *J. Histochem. and Cytochem.*, 1953, **1**, 420.
17. LOWRY, O. H., ROBERTS, N. R., and KAPPHAHN, J. I., *J. Biol. Chem.*, 1957, **224**, 1047.
18. LOWRY, O. H., ROBERTS, N. R., and LEWIS, C., *J. Biol. Chem.*, 1956, **220**, 879.

19. LOWRY, O. H., ROBERTS, N. R., SCHULZ, D. W., CLOW, J. E., and CLARK, J. R., *J. Biol. Chem.*, 1961, **236**, 2813.
20. LOWRY, O. H., ROBERTS, N. R., WU, M.-L., HIXON, W. S., and CRAWFORD, E. J., *J. Biol. Chem.*, 1954, **207**, 19.
21. MAY, L., MIYAZAKI, M., and GRENELL, R. G., *J. Neurochem.*, 1959, **4**, 269.
22. MCARDLE, B., THOMPSON, R. H. S., and WEBSTER, G. R., *J. Neurochem.*, 1960, **5**, 135.
23. MCDOUGAL, D. B., JR., JONES, E. M., AND SILA, U. I., *Research Pub. Assn. Research Nerv. and Ment. Dis.*, 1962, **40**, 182.
24. MCDOUGAL, D. B., JR., SCHULZ, D. W., PASSONNEAU, J. V., CLARK, J. R., REYNOLDS, M. A., and LOWRY, O. H., *J. Gen. Physiol.*, 1961, **44**, 487.
25. OESPER, P., and MEYERHOF, O., *Arch. Biochem.*, 1950, **27**, 223.
26. PASSONNEAU, J. V., and LOWRY, O. H., *Biochem. and Biophysic. Research Commun.*, 1962, **7**, 10.
27. PETTE, D., AND LUH, W., *Biochem. and Biophysic. Research Commun.*, 1962, **8**, 283.
28. Report of the Commission on Enzymes of the International Union of Biochemistry, New York, Pergamon Press, **20**, 1961.
29. RINGLER, R. L., and SINGER, T. P., *J. Biol. Chem.*, 1959, **234**, 2211.
30. SEGAL, H. L., and BOYER, P. D., *J. Biol. Chem.*, 1953, **204**, 265.
31. SHEPHERD, J. A., and KALNITSKY, G., *J. Biol. Chem.*, 1954, **207**, 605.
32. SMYTHE, C. V., *Ber. chem. Ges.*, 1932, **65B**, 819.
33. WILLIAMS, P. L., and LANDON, D. N., *Nature*, 1963, **198**, 670.
34. WU, R., and RACKER, E., *J. Biol. Chem.*, 1959, **234**, 1029.