THE QUANTITATIVE HISTOCHEMISTRY OF THE RETINA*

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The retina is not only an exceedingly interesting structure in its own right, but it presents an exceptional opportunity for study of the central nervous system, of which it is an integral part. In the retina a number of nervous elements which are usually intimately mixed together have been sorted out into discrete layers. Analysis of each of these layers could provide information as to the composition of several kinds of cell bodies, dendrites, naked axons, and even synapses (in the outer reticular layer) in addition to the rods and cones themselves.

Anfinsen measured cholinesterase and DPN[1] in a number of layers in beef retina (2). Wald isolated the pigment epithelium in the frog and analyzed it for vitamin A and xanthophyll (3). Saito made preparations of outer rod segments by vigorous shaking of retinas in saline, followed by differential centrifugation in strong sucrose or fructose solutions (4). Such preparations have been analyzed for rhodopsin and related substances and for five phosphorus fractions (5). Otherwise, except for preliminary data for MDH and LDH in rabbit retina (6) there do not appear to have been analyses of individual retinal layers. (Some valuable histochemical information has been obtained by staining methods (7–9).)

To be reported are analyses of the layers of monkey and rabbit retina by a modified (10) procedure of Linderstrøm-Lang *et al.* (11), which incorporates some of the steps employed by Anfinsen *et al.* (12). Data for dry weight, total lipides, lactic, malic, and glutamic dehydrogenases, phosphoglucoisomerase, glutamic-aspartic transaminase, and glutamate are reported, as well as the new methods used for measuring some of these.

Materials

Retinas were obtained from monkeys (*Macaca mulatta*) and from white rabbits. The eyeball was frozen in liquid nitrogen, and blocks of retina

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[1] Abbreviations used in this paper include glutamic dehydrogenase, GDH; malic dehydrogenase, MDH; lactic dehydrogenase, LDH; tris(hydroxymethyl)aminomethane, Tris; oxidized and reduced diphosphopyridine nucleotide, DPN^+ and DPNH, respectively.

were mounted, sectioned while frozen, and dried as previously described (10). Special care was required to mount the thin blocks without partial thawing. Usually the sclera and a little of the vitreous humor were left attached, and sections were cut at a tangent to the layers, starting at either surface. Sections were cut at 6 μ to give good separation of the layers and to facilitate identification of the layers during subsequent dissection. It was possible in the frozen-dried material, after study of stained sections, to identify not only the eight major retinal layers but a number of sublayers as well. Pure samples of each layer weighing in the order of 0.1 γ were readily obtained, usually in one piece except from the thinnest layers. It was possible to make a more distinct separation of the inner and outer segments of the rods (and cones) in monkey retina than in rabbit retina.

The material analyzed came from within a few mm. of the fovea. Since the relative thickness of individual layers is different at different points on the retina, several blocks were required to obtain good specimens of all the layers. For example, the ganglion cell layer is thin and the cells are sparse, except for a narrow zone close to the fovea. The rod and cone layers in the monkey were chiefly obtained from a zone in which there were about twenty-five rods per cone. The cones were evenly spaced 11 μ apart. The diameters of rods and cones were found to be 1.45 and 8 μ, respectively, in fixed sections from this area. From these measurements it may be calculated that the rods and cones occupied 38 and 42 per cent, respectively, of the total volume of the zone analyzed. This ignores possible volume changes during fixation. Rabbit retina contains only rods.

Analytical Procedures

The general analytical techniques and tools have been described (13). A few changes were required, since the samples were 10 or 20 times smaller than those previously used in brain studies.

Weighing—Samples were weighed on a balance of the quartz fish-pole variety (10) with a fiber length of 30 mm., a pan 0.7 mm. in diameter, a sensitivity and reproducibility of 0.001 γ for a 0.005 mm. displacement, and a load limit of about 1 γ. The balance case is 15 mm. in diameter and 60 mm. long. To facilitate handling these light samples, they were transferred to and from the balance with hair points (13) sharpened to about 10 μ at the tip.

Defatting—The weights of hexane-soluble lipides and total lipides were measured by the loss of weight upon successive extraction with hexane and ethanol (10). Because of the small size of the samples and their fragility, they were extracted in special tubes made by sealing a 5 mm. length of 2 mm. bore capillary tubing onto a glass rod handle 30 or 40

mm. long. After extraction, it is easier to recover samples from these short tubes than from the longer ones used for chemical analysis.

Glutamic Dehydrogenase—The GDH activity was assessed by the DPN^+ formed upon incubation with a reagent containing DPNH (0.05 to 0.1 mM), α-ketoglutarate (3 mM), ammonium sulfate (7.5 mM), and nicotinamide (20 mM) in Tris-HCl buffer at pH 7.6. Crystalline bovine albumin was added to give a 0.01 per cent solution. It has since been found preferable to use 0.05 per cent albumin, particularly with very small volumes or very high tissue dilutions. The DPN^+ was measured by the method of Kaplan *et al.* (14) (see below).

The reagent was stored frozen without the DPNH (Sigma Chemical Company, St. Louis) which was added before use as a 10 mM solution in pH 8.5, Tris buffer. This DPNH solution was also stored in the frozen state.[2]

The tubes (1.6 to 1.8 mm. bore, 55 mm. long), each containing a sample of about 0.1 γ of dry weight, were placed in a rack in an ice bath, and to each tube were added 5 μl. of substrate reagent with no mixing until 10 or 15 minutes of soaking in the ice bath. The tubes were then gently tapped, and the rack was transferred to a 38° bath for exactly 30 minutes. After returning the rack to the ice bath, 1 μl. of 1 N HCl was added to each sample to destroy DPNH and to stop the reaction. After "buzzing" to mix, a 5 μl. aliquot was added to 100 μl. of 6.6 N NaOH in the bottom of a 3 ml. fluorometer tube and left 60 minutes at room temperature. The sample was then diluted by the addition of 1 ml. of water, and the fluorescence was measured against a quinine working standard, with a Corning glass No. 5860 as primary filter, to isolate the 365 mμ mercury line, and, as secondary filter, Corning glasses No. 4308 and 3389 (transmission maximum at about 480 mμ). The blank may be reduced slightly at the expense of sensitivity by using two primary filters and substituting for the secondary filter Corning glasses No. 4308, 5562, and 3387.

Blanks were provided, together with DPN standards consisting of 5 μl. samples of substrate reagent which contained 0.05 mM added DPN^+; *i.e.*, a total of 0.25×10^{-9} mole. In calculating the activity, 6 minutes in ice water were taken as the equivalent of 1 minute at 38°.

Comment on Glutamic Dehydrogenase Method—The substrate reagent contains the reactants at optimal concentrations for rabbit brain. These concentrations differ somewhat from those found to be best by Olson and

[2] Storage tests were made with DPNH at various pH levels in several buffers. It was found that in Tris or 2-amino-2-methylpropanediol at pH 8 to 9 DPNH was stable for several days at 4° and at least several weeks at −20°. In contrast it was quite unstable, even when frozen, at more acid pH levels or at pH 8 in phosphate buffer. In phosphate the instability was actually greater when frozen than at 4°. Instability refers to conversion to DPN^+.

Anfinsen (15) for liver glutamic dehydrogenase. They used approximately 3 times higher concentrations of DPNH and α-ketoglutarate, both of which have been found to be inhibitory if used in higher concentrations than those prescribed here. There was a 60 per cent inhibition with 0.4 mM DPNH and a 20 per cent inhibition with 10 mM α-ketoglutarate.

The Michaelis constant for α-ketoglutarate with GDH was found to be 0.22 mM with rabbit brain homogenate. The Michaelis constant for DPNH is probably very small since 90 per cent of the maximal activity was found with 1.5×10^{-5} M DPNH (15 per cent conversion to DPN^+). Therefore, without affecting the rate, the DPNH concentration may be varied from 0.025 to 0.1 mM, depending on the activity expected. The blank value is largely determined by the amount of DPNH present. Olson and Anfinsen (16) reported a much larger K_m (10^{-4} M) for DPNH with liver GDH.

The method is more convenient and much more sensitive than that previously published (6). The reaction is measured in the more favorable direction, and the DPN^+ formed can be measured fluorometrically at a dilution of 10^{-8} M compared to a limit of about 2×10^{-5} M for DPNH measured by its ultraviolet absorption.

The enzyme has a small temperature coefficient; it is 16 and 58 per cent as active at 0.5° and 25.5°, respectively, as it is at 38°. For this reason the time in ice water ought to be taken into account in calculating the activity of tissue samples.

Since the method depends on the measurement of DPN formed, it was necessary to see how much DPNH is oxidized in the absence of substrates. With neither α-ketoglutarate nor ammonia present, fresh brain homogenates under the prescribed conditions oxidize 50 to 75 mmoles of DPNH per kilo of brain per hour. The addition of α-ketoglutarate alone does not result in a detectable increase in DPN^+ formation. The addition of ammonium sulfate alone results in oxidation of DPNH at a rate of 100 to 150 mmoles per kilo of brain per hour. This effect of ammonium ion has been noted by Huennekens *et al.* for pig heart DPNH oxidase (17). Aging brain homogenates for several days (frozen) results in loss of much of the ability to oxidize DPNH, and in frozen-dried tissue sections the oxidation is not detectable. GDH activity is unimpaired. In working with fresh homogenates, the ability to oxidize DPNH directly may be considerably suppressed with little decrease in GDH activity by increasing the buffer strength to 0.4 M.

Nicotinamide is included in the reagent to protect DPN^+ formed from DPNase. However, if omitted, the results are only 5 or 10 per cent low, since, under the analytical conditions prescribed and the DPN^+ concentrations encountered, brain DPNase splits only 50 to 100 mmoles of DPN^+ per kilo per hour.

Reproducibility and proportionality with time and brain concentration are satisfactory (Table I).

Simultaneous measurements were made of DPN^+ formation and the disappearance of α-ketoglutarate, measured with quinolyl hydrazine (18). The α-ketoglutarate concentration was reduced to 0.6 mM, and DPNH was added as the reaction progressed to avoid inhibition. With the forma-

TABLE I

Glutamic Dehydrogenase Activity with Variation of Time and Tissue Concentration (Rabbit Brain Homogenate)

Incubation volume, 12 μl. The values are recorded as moles per kilo of wet weight per hour (MKH).

Incubation time	Wet weight	Activity	Incubation time	Wet weight	Activity
min.	γ	*MKH*	*min.*	γ	*MKH*
15	0.44	0.94	60	0.44	0.91
15	0.44	0.92	60	0.44	0.92
15	0.44	1.03			
15	0.44	0.92	Average		0.90
Average		0.95	30	0.22	1.10
			30	0.22	1.04
30	0.44	0.99	30	0.22	1.09
30	0.44	1.00	30	0.22	1.04
30	0.44	0.93			
30	0.44	0.93	Average		1.07
Average		0.96	30	0.88	0.99
			30	0.88	1.02
60	0.44	0.90	30	0.88	0.99
60	0.44	0.86	30	0.88	1.05
			Average		1.01

tion of 100×10^{-9} mole of DPN^+, 98×10^{-9} mole of α-ketoglutarate disappeared.

Lactic and Malic Dehydrogenases—These were measured by a method previously described which depends on the color produced with quinolyl hydrazine and the pyruvate or oxalacetate formed (18). The samples weighing 0.05 to 0.2 γ were incubated for 15 minutes at 38° with 10 μl. of substrate reagent. The final volume for color measurement was 100 μl. The general procedure was similar to that given for GDH. The over-all reproducibility of the LDH method as applied to different layers of the retina seems adequate to the problem (Table II). The observed values reflect errors in weighing, errors in dissection, and biological varia-

tion within each retinal layer, as well as variation due to the enzyme method itself. Comparable data for MDH are even more consistent.

DPN—The GDH determination is based on the formation of DPN^+ from DPNH, and exploitation of the fluorometric method of Kaplan, Colowick, and Barnes (14) for DPN^+. DPNH as purchased (Sigma) gives only about 1 per cent as much fluorescence as DPN^+, provided it is destroyed by acidification prior to the strong alkaline treatment. Otherwise it will give about a seventh as high a reading as DPN^+, because of

TABLE II

Lactic Dehydrogenase in Monkey Retina

The dry weight of each sample is given. The values are expressed as moles of substrate oxidized per kilo of dry weight per hour (MKH).

Layer			Layer			Layer		
	γ	*MKH*		γ	*MKH*		γ	*MKH*
Pigment	0.069	13	Outer nuclear	0.095	53	Inner plexiform	0.159	30
	0.088	12		0.101	63		0.134	32
	0.067	15		0.099	58		0.141	34
	0.083	7		0.104	52		0.137	34
	0.069	13		0.084	52		0.164	35
	0.058	13		0.100	50		0.132	34
Outer rods and cones	0.070	9	Outer plexiform	0.073	104	Ganglion cell	0.111	32
	0.069	10		0.073	101		0.108	35
	0.065	10		0.082	102		0.118	32
	0.061	10		0.062	121		0.092	37
	0.071	41		0.080	104		0.114	32
	0.046	23		0.073	113		0.114	32
Inner rods and cones	0.061	38	Inner nuclear	0.112	37	Optic nerve	0.169	14
	0.070	50		0.114	47		0.176	19
	0.055	48		0.108	52		0.130	26
	0.070	39		0.122	48		0.150	23
	0.074	39		0.126	47		0.160	21
	0.072	39		0.095	54		0.082	20

the natural fluorescence of unchanged DPNH. As reported by Kaplan *et al.*, the fluorescent product in the strongly alkaline solution used to produce it is very unstable to the exciting ultraviolet light. It has been found, however, that, by diluting 10- or 20-fold with water after the alkaline reaction is finished, the instability to light is decreased to a point where it is not troublesome. It is obvious that innumerable sensitive enzyme methods might be based on this means of DPN^+ measurement.

Transaminase—To measure this enzyme, a substrate reagent was used which contained an excess of purified MDH, aspartate, α-ketoglutarate, and DPNH. In the presence of transaminase, oxalacetate is formed, and

this is immediately converted to malate, which may be measured fluorometrically (19). The MDH was purified from hog heart according to Straub's procedure (20), with omission of the last two steps which involve alcohol precipitation. The MDH activity was assayed by measuring the rate of oxidation of DPNH with oxalacetate under conditions approximating those of the transaminase assay. To the buffer alone was added DPNH to give a concentration of 0.15 mM, and oxalacetate to give a concentration of 0.2 mM. The rate of disappearance of DPNH was measured in the Beckman spectrophotometer. An enzyme concentration sufficient by calculation to oxidize at least half of this level of DPNH in 2 minutes was found suitable for the final transaminase reagent. Stronger oxalacetate than indicated was not used, since it is inhibitory. The MDH must be nearly free of transaminase activity which would increase the blank value.

The complete transaminase substrate reagent consisted of 40 mM aspartate, 8 mM α-ketoglutarate, 4 mg. per cent pyridoxal phosphate, 3 mM DPNH, and MDH as indicated above, all in 0.1 M Tris-HCl buffer at pH 7.7. The DPNH was added as a powder. The MDH and the balance of the reagent were stored separately at $-20°$. The MDH was added last, just before use, in case it contained a little transaminase activity. It is desirable to make the reagent 0.05 per cent in bovine plasma albumin.

The incubation of 0.1 γ of retina samples was carried out in the manner described for GDH, except that an incubation time of 60 minutes was employed.

After the enzyme reaction was arrested in ice water, a 4 μl. aliquot was transferred to a 3 ml. fluorometer tube containing 1 ml. of β-naphthol sulfuric acid reagent for malate measurement, as previously described for fumarase assay (19).

Standards consisted of 5 μl. of 0.5, 1, and 2 mM malate made up in the complete substrate reagent. They were carried through the entire procedure together with blanks.

Comment on Transaminase—The method is based on the same principle as that of LaDue *et al.* (21), except that these authors measured the disappearance of DPNH rather than the appearance of malate.

With brain homogenate, no apparent malate formation is observed in the transaminase assay if aspartate, α-ketoglutarate, or DPNH is omitted. Omission of pyridoxal phosphate reduces malate formation only slightly. Omission of MDH results in a 25 per cent reduction. That the reduction is not greater is owing to the fact that MDH activity in average brain is higher than transaminase activity, and the Michaelis constant for oxalacetate is exceedingly low. Therefore the MDH already present is nearly sufficient for the assay without supplementation.

The complete system was tested with added oxalacetate to assess the yield of malate. The addition of 0.5, 1.0, and 1.55 mM oxalacetate (1.65 mM DPNH present) resulted in the formation of 0.46, 0.94, and 1.49 mM malate. With oxalacetate in excess (1.0 mM), the addition of 0.84 mM DPNH led to formation of 0.80 mM malate.

The assay is conducted at the pH optimum for brain transaminase. The aspartate concentration is 15 times the K_m and the α-ketoglutarate is 12 times the K_m; therefore the rate is about 85 per cent of the theoretical maximum. Further increase in substrates would increase the blank value. (The K_m was evaluated for rabbit brain transaminase under the assay conditions and found to be 0.59 and 2.60 mM for α-ketoglutarate and aspartate, respectively. These may be compared to the corresponding values of about 0.4 and 2 reported for hog heart transaminase by Nisonoff and Barnes (22).)

Brain transaminase withstands freezing, drying from the frozen state, and considerable periods of storage after drying. The average of two experiments gave values of 7.6, 7.8, and 7.8 moles per kilo per hour for fresh, frozen, and frozen-dried rabbit brain homogenate. Frozen-dried homogenates lost 0, 20, and 66 per cent of their initial activity when stored at 25° for 1, 7, and 41 days, respectively. At −20° a 15 per cent loss occurred in 41 days. It is probable that frozen-dried tissue sections are even more stable than this, since 1 year-old sections from the molecular layer of rabbit cerebellum had 80 per cent of the activity of sections stored for only a few days.

Replicates had a coefficient of variation of about 1 per cent (10 γ of brain, wet weight, incubated in a volume of 25 μl. for 30 minutes, or 1.5 γ incubated in 5 μl. for 60 minutes). Malate formation is not strictly proportional with tissue or brain concentration. With 10 γ of brain, wet weight, incubated for 15, 30, and 60 minutes in a volume of 50 μl., values of 7.53, 6.54, and 6.56 moles per kilo per hour were observed, whereas 10, 20, and 40 γ of brain incubated for 15 minutes gave values of 7.53, 7.34, and 6.38 moles per kilo per hour, respectively. The deviation from linearity is attributed in part to accumulation of glutamic acid, and in part to some fumarate formation from the fumarase present.

If it is necessary to measure smaller amounts of enzyme than in the present instance, the formation of DPN^+ rather than malate may be measured (fluorometrically) with a 50- to 100-fold enhancement in sensitivity.

Phosphoglucoisomerase—This was measured by an unpublished procedure[3] in which the fructose phosphate formed from glucose-6-phosphate is determined with a modification of the method of Roe *et al.* (23). The mod-

[3] Buell, M. V., Lowry, O. H., Kapphahn, J. I., Roberts, N. R., and Giese, A. W., Jr., to be published.

ified procedure yields a final colored product that is stable to light. The incubation volume was 2 μl., and the final color was measured at a volume of 200 μl.

Glutamic Acid—This was measured by a method to be published later which is based on the ability of glutamic acid to catalyze the conversion of aspartic acid to malic acid in the presence of added GDH, glutamic-

TABLE III

Dry Weight and Total Lipide of Monkey Retina

Each value is the average of four samples. The heptane- and ethanol-soluble fractions are expressed as per cent of total dry weight. The standard error of the mean is indicated.

Layer No.	Total dry weight	Heptane-soluble	Ethanol-soluble heptane-insoluble	Heptane- and ethanol-soluble
	gm. per l.	*per cent*	*per cent*	*per cent*
1. Pigment	404 ± 25	4.0 ± 1.0	18.7 ± 1.2	22.7 ± 0.4
2a. Outer portion of outer rod-cone segments	148 ± 7	2.3 ± 1.2	22.3 ± 1.1	24.6 ± 1.2
2aa. Inner portion of outer segments	198 ± 7	4.7 ± 0.7	20.6 ± 0.8	25.3 ± 0.6
2b. Outer portion of inner segments	222 ± 8	3.4 ± 0.7	20.6 ± 0.8	24.0 ± 0.3
2bb. Inner portion of inner segments	203 ± 8	6.9 ± 0.6	15.1 ± 1.8	22.0 ± 2.2
4. Outer nuclear	240 ± 9	4.8 ± 0.5	7.2 ± 0.4	12.0 ± 0.2
5. " reticular	196 ± 8	4.3 ± 0.5	13.7 ± 2.0	18.0 ± 1.8
6. Inner nuclear	263 ± 11	6.3 ± 2.1	11.1 ± 1.5	17.4 ± 3.2
7. " reticular	248 ± 5	14.4 ± 4	22.6 ± 1.2	37.0 ± 0.8
8. Ganglion cell	215 ± 5	5.3 ± 0.3	14.7 ± 0.4	20.0 ± 0.2
9. Fiber	213 ± 6	11.8 ± 0.8	21.8 ± 0.4	33.6 ± 0.5
Comparative values from rabbit brain*				
Dendritic zone	233	18.7	19.2	37.9
Cell body "	170	9.7	12.3	22.0
Myelinated fiber zone	303	31.0	30.7	61.7

* Strata radiata, pyramidalis, and alveus, respectively, of Ammon's horn (24).

aspartic transaminase, MDH, DPN^+, and DPNH. Under the assay conditions, for each mole of glutamic acid present, 20 to 40 moles of malic acid were produced. The malic acid was finally measured by the fluorescence produced with β-naphthol (see the transaminase method above).

RESULTS AND DISCUSSION

Dry Weight and Total Lipides (Table III)—The outer layers of the retina are all low in lipides compared to the rest of the brain. This is

particularly true of heptane-soluble lipides, which are virtually absent from the outer rod and cone segments. The relatively low value for lipide in Layer 5 is perhaps to be expected for a layer of naked axons. This low value may be compared with the higher levels in fiber Layers 7 and 9 and the very high level in a myelinated tract of rabbit brain. The lipide percentages in the three cell body layers are comparable to that found in a cell body layer in rabbit brain, but the values for total dry weight are considerably greater. Although the values for dry weight are believed to be accurate relative to each other, there is a possibility that all of the retinal values are somewhat too high, since it is difficult to avoid compression when cutting such thin frozen sections. The very low value for total dry weight in the outer rod and cone layer is attributed to the lace-like structure of this region, resulting from the tapered shape of the cones which leaves a good deal of space around each outer cone segment.

Collins *et al.* (5) found that beef outer rod segments, separated in a sucrose gradient, contained 30 per cent phospholipides. Sjöstrand (25) reported that a similar preparation from guinea pig contained 40 per cent lipide. Either guinea pig and beef rod outer segments contain more lipide than those of the monkey, or the isolation process used by others extracted constituents low in lipide, leaving a lipide-rich residue. On the basis of staining reactions, Lillie (9) suggests that rod outer segments contain a cerebroside together with phospholipides. Probably both cerebrosides and gangliosides would give similar staining reactions and both would be insoluble in heptane. The outer segments appear to contain a large amount of insoluble carbohydrate other than glycogen (8). This would be the case if much of the lipide present was ganglioside.

LDH, MDH, and Phosphoglucoisomerase (Figs. 1, 2)—There are rather dramatic differences in LDH and MDH activity in different retinal layers. In the monkey there is 30 times more MDH in the inner rod and cone segments than in the outer. In general, when MDH is high, LDH is low. In the monkey retina, the peak MDH and LDH values are respectively four and seven times greater than those found in average monkey brain. The peak enzyme levels have the enormous values of 430 and 230 moles of substrate oxidizable per 100,000 gm. of protein per minute for MDH and LDH, respectively. This would indicate that the enzymes constitute several per cent of the total protein present. Both rabbit and monkey have the highest MDH levels in Layer 2b, which consists of the inner segments of rods in the rabbit or of rods and cones in the monkey. Sjöstrand (26), by electron microscopy, has demonstrated dense aggregates of slender mitochondria almost filling the apical portion of the rod inner segments. Presumably this is the locus of the MDH and perhaps of associated members of the citric acid cycle. Wislocki and Sidman (8), using a staining method, found this layer to be rich in succinoxidase.

There is more LDH and less MDH in the inner layers of rabbit retina than in the monkey. This is in keeping with the differences in blood supply in the two species. The monkey has one set of vessels in the choroid and another set on the inner surface of the retina which send capillaries as far as the inner nuclear layer. This second set of vessels is missing over most of the rabbit retina, and therefore a glycolytic metabolism might be required for the inner layers. The outer reticular layer, which is rich in

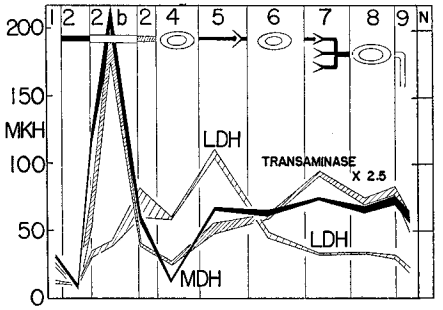

FIG. 1

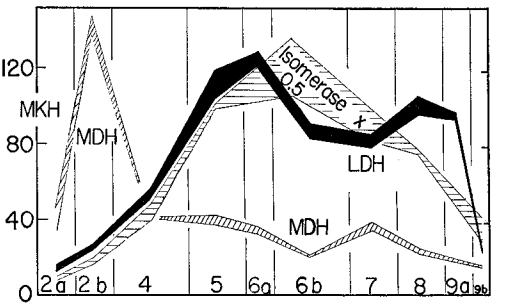

FIG. 2

FIG. 1. LDH, MDH, and glutamic-aspartic transaminase of monkey retina. The values are recorded as moles of substrate oxidized or transaminated per kilo of dry weight per hour (MKH). The breadth of each line at the center of each zone is twice the standard error of the mean for that zone. Each mean is the average of six determinations. Each zone represents one retinal layer, as indicated by numbers which correspond to the names given in Table III and by a schematic representation of the composition of each zone. Zone N is optic nerve tissue obtained inside of the lamina cribrosa; *i.e.*, before the fibers become myelinated. The zone widths are proportional to the approximate thickness of the respective retinal layers. The midpoints of each zone are connected for better visualization, but this does not imply a gradual transition from one zone to the next. There is instead a sharp transition between zones (see Table I).

FIG. 2. LDH, MDH, and phosphoglucoisomerase of rabbit retina. The values, line widths, and zone widths have the same significance as in Fig. 1. Layer 9*a* is from a band of myelinated fibers characteristic of rabbit retina. Note that isomerase values have been plotted on a scale of one-half.

LDH in both monkey and rabbit, is avascular in both species. A few samples were dissected from the outer reticular layer so as to contain only the naked axon portion of this layer. The LDH values were not appreciably different from those for the whole layer.

The parallelism between LDH and phosphoglucoisomerase (Fig. 2) strengthens the belief that LDH is a valid measure of glycolytic capacity. The enormous absolute value for isomerase in the richest layers will be noted. The maximal values are about $2\frac{1}{2}$ times those of average brain. The peak value corresponds to conversion of about 560 moles of substrate per 100,000 gm. of protein per minute.

These findings concur with earlier preliminary data (6) and strengthen the conclusion that the long known high rate of aerobic glycolysis in whole retina is a kind of artifact resulting from the mixture of cells, or parts of cells, of high oxidative capacity with cells of high glycolytic capacity (6).

The low values for LDH, MDH, and isomerase in the outer rod-cone segments are to be noted.

Transaminase, GDH, and Glutamate—It is seen (Fig. 1) that glutamic-aspartic transaminase has almost exactly the same distribution as MDH. Furthermore, the ratio of MDH to transaminase is about the same in whole monkey brain. This suggests a metabolic relationship between the two

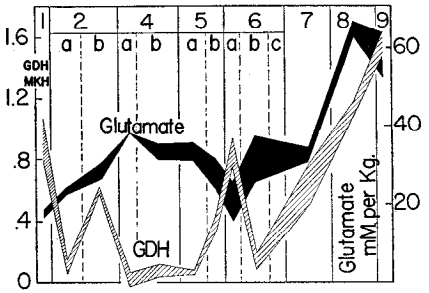

FIG. 3. GDH and glutamate of monkey retina. See Fig. 1 for the meaning of line and zone widths. GDH is recorded as moles of α-ketoglutarate reduced per kilo of dry weight per hour, and glutamate is recorded as millimoles per kilo, dry weight. Layer 4*a* contains nearly pure cone nuclei, Layer 4*b* rod nuclei, 5*a* almost pure naked axons from rods and cones, and Layer 5*b* consists of synapses and dendrites. No values are recorded for Zone 6*c*.

enzymes. MDH and transaminase might, for example, cooperate in the conversion of glutamate to aspartate plus CO_2 as follows:

(1) $$\text{Malate} \xrightarrow{\text{MDH}} \text{oxalacetate}$$

(2) $$\text{Oxalacetate} + \text{glutamate} \xrightarrow{\text{trans.}} \text{aspartate} + \alpha\text{-ketoglutarate}$$

(3) $$\alpha\text{-Ketoglutarate} \longrightarrow \text{- - -} \longrightarrow \text{malate} + CO_2$$

This could be an emergency mechanism for the use of glutamate when other metabolites are lacking. Dawson (27) has in fact found that with hypoglycemia there occur in brain a fall in glutamate and a rise in aspartate. Attempts to separate brain transaminase from MDH by differential centrifugation or ammonium sulfate fractionation have failed; nevertheless it seems unlikely that a single enzyme is involved.

Monkey retina, especially in the outer layers, is very low in GDH compared to the rest of the central nervous system (Fig. 3). The outer granular

and reticular layers contain less than 2 per cent of the activity of average brain. Analysis of a few layers of rabbit retina has confirmed these low GDH values; in fact the ganglion cell layer had an activity of only 0.19 mole per kilo per hour. This difference between rabbit and monkey may be due to the fact that the portion of Layer 8 that was analyzed in the rabbit contained only a few ganglion cell bodies, whereas Layer 8 in the monkey was chosen from a zone of packed ganglion cell bodies.

In the outer retinal layers, *glutamate* (Fig. 3) is only half to two-thirds as concentrated as in average brain (60 mmoles per kilo, dry weight). There is a suggestion of a reciprocal relationship between glutamate and GDH in the outer retinal layers. The implication that the presence of greater GDH concentrations causes the lower glutamate levels is, however, probably not valid, since both enzyme and substrate are so much higher in the rest of the central nervous system. The naked axon portion of the outer reticular layer is not remarkable in its glutamate concentration, although it contains much less GDH than the synaptic and dendritic portion of the layer. GDH has been found to be especially rich in certain molecular layers of the central nervous system and relatively rich in myelinated fiber tracts (6). Both types of nervous tissue are absent from the retina.

The outer segments of the rods and cones are as deficient in transaminase and GDH as they are in LDH, MDH, and isomerase. These specialized structures may depend on adjacent layers for sources of energy.

SUMMARY

1. New methods are presented for measuring GDH and glutamic-aspartic transaminase in samples of brain and retina weighing 0.1 γ (dry).

2. The activities or concentrations of MDH, LDH, transaminase, GDH, and glutamate have been measured in each of the eight major retinal layers of the monkey. In some cases the major layers were further subdivided for analysis. Similarly the amounts of MDH, LDH, and phosphoglucoisomerase have been determined in rabbit retinal layers.

3. MDH is exceedingly rich in the inner segments of the rods and cones. LDH bears a reciprocal relationship to MDH. It is especially rich in the outer reticular layer of the monkey and all inner layers of the rabbit. Glutamic-aspartic transaminase is distributed in close parallelism with MDH. Phosphoglucoisomerase and LDH parallel each other in distribution.

4. GDH is very low in most retinal layers, and the glutamate concentration varies from 50 to 100 per cent of that found in average brain.

5. All enzyme activities are low in the outer segments of the rods and cones.

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