Histochemie 4, 330—335 (1964)

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HISTOCHEMISTRY OF THE GLYCOGEN BODY OF THE TURKEY SPINAL CORD*

By

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With 3 Figures in the Text

(Received July 17, 1964)

Introduction

The glycogen body is a gelatinous structure composed of vesicular, glycogen-storing cells, located between the dorsal tracts in the lumbo-sacral region of the spinal cord in birds. No glycogen body has been found in other vertebrates; its biological significance is unknown. The high glycogen content of the tissue was recognized histochemically by TERNI (1924) and confirmed by DOYLE and WATTERSON's biochemical studies (1949); the latter authors reported up to 75% glycogen per lipid-free dry weight. This paper presents a comparative histochemical study of 13 enzymes in the relatively large glycogen body of the turkey.

Materials and methods

Turkeys, 23—25 weeks old, were killed by cutting the jugular vein. The portion of spinal cord which contained the glycogen body was removed within 2 hours after death. Twenty three turkeys were used for histochemical studies of the following enzymes: lactic dehydrogenase (LDH), NAD[1]-diaphorase, succinic dehydrogenase (SDH), cytochrome oxidase (CYO), malate dehydrogenase (MDH), glucose-6-phosphate dehydrogenase (G-6-PDH), 6-phosphogluconate dehydrogenase (6-PGDH), alcohol dehydrogenase (ADH), isocitrate dehydrogenase (IDH), acetyl cholinesterase (AChE), nonspecific cholinesterase (ChE), acid phosphatase (AcP), alkaline phosphatase (AlkP). Three turkeys were used for biochemical assays of SDH and LDH activity.

a) Histochemistry. Twelve μ cryostat sections were cut from fresh tissue that had been frozen in an isopentane-dry ice mixture. Thirty μ frozen sections were cut from tissue that had been fixed in cold formalin for 12 hours. For the study of LDH, NAD-diaphorase (SCARPELLI, HESS and PEARSE, 1958; FRIEDE, FLEMING and KNOLLER, 1963a, 1963b), AChE, ChE (GEREBTZOFF, 1959), AcP, and AlkP (PEARSE, 1961), cryostat sections were fixed in cold formalin for 1 hour prior to incubation; also, loose frozen sections of formalin-fixed tissue were stained for these enzymes. Cryostat sections of unfixed tissue were incubated for SDH (FRIEDE, FLEMING and KNOLLER, 1963b), CYO (BURSTONE, 1959), and MDH (PEARSE, 1961). All incubations were carried out in an Eberbach water-bath shaker; incubation times were 1 hour at 38° C for AChE and ChE, and 1 hour at room temperature for AlkP. It was found that certain enzymes (G-6-PDH, 6-PGDH, ADH and IDH) could not be adequately studied in cryostat sections because of enzyme loss due to solubilization and leakage. For these enzymes, 1 to 3 mm thick blocks of fresh tissue were incubated in the substrate media (FRIEDE, FLEMING and KNOLLER, 1963b; PEARSE, 1961); then fixed in formalin and sectioned at 30 μ with a freezing microtome.

* This investigation was supported by U.S. Public Health Grant B 3250.

** Submitted in partial fulfillment of Masters' degree requirements.

[1] Nicotinamide-adenine dinucleotide or DPN.

Periodic acid-Schiff (PAS) studies were done on 12 μ cryostat sections post-fixed in Carnoy's fixative, and on 12 μ paraffin sections of Carnoy-fixed tissue. Thirty μ frozen sections of formalin-fixed tissue and 12 μ cryostat sections fixed in formalin were stained with hematoxylin and eosin.

b) Biochemical Assay Methods. The glycogen body was easily separated from the spinal cord without contamination from cord tissue, and the wet weight was determined. A 2 mm thick slice of spinal cord, taken just proximal to the glycogen body served as a control specimen. Homogenates were prepared using a tissue concentration of 20 mg/ml tris-HCl buffer, pH 7.4. For SDH and LDH determinations, 0.1 ml of homogenate per 3 ml of incubation medium was used. Incubation time was 20 minutes at 38° C. All determinations were done in triplicate. No-tissue and no-substrate blanks were run for each sample. For details of methodology, see FRIEDE, FLEMING and KNOLLER, 1963b.

Results

For all histochemical enzyme reactions, the staining intensity in the gray and white matter of the spinal cord served for comparison with the staining intensity in the cells of the glycogen body. The various enzyme patterns observed in the spinal cord generally were consistent with previous data from this laboratory (FRIEDE, FLEMING and KNOLLER, 1963b) and with reports in the literature.

Periodic acid-Schiff Reaction. The glycogen body was intensely stained with the PAS reaction due to the high concentration of glycogen in the vesicular, glycogen-storing cells; there was no glycogen in the spinal cord. Most of the glycogen was removed by a very short exposure of the sections to water, indicating that there was little protein-binding of the glycogen. There was no PAS staining of the glycogen body in diastase-treated control sections.

Lipids. Sections stained with Sudan black and Fettrot 7B showed no lipid-staining in the glycogen body cells. In contrast, the white matter of the spinal cord stained intensely with the lipid stains; fiber bundles of the dorsal commissure, which often traversed the ventral part of the glycogen body, were clearly demonstrated.

Oxidative Enzymes. In glycogen body cells, the oxidative enzymes were localized primarily in the knob-shaped perikaryon, which pro ruded into the vesicular lumen of the cell (Fig. 1); the enzyme activity was always higher in the perikaryon than in the cell membrane. The levels of LDH, NAD-diaphorase and MDH activity (Fig. 2b) in the glycogen body cells were comparable to the high levels of activity of these enzymes in the cytoplasm of oligodendroglia of white matter (FRIEDE, 1961), and in the neurons and neuropil of gray matter. SDH activity was weak in the glycogen body cells (Fig. 2a), moderate in oligodendroglia, and very strong in neurons and neuropil. CYO was not demonstrated in the glycogen body. Minimal CYO activity in the white matter did not permit distinction of glial cells, but there was very strong activity in neurons and neuropil of gray matter.

As stated above, the histochemical reactions for G-6-PDH, 6-PGDH, ADH, and IDH were too weak in cryostat sections to permit study; in sections cut from the blocks of tissue that were incubated in substrate media for these enzymes, high levels of activity were demonstrated in the glycogen body cells. However, in these preparations, the media diffused more readily into the glycogen body than into the cord tissue; for this reason, the levels of enzyme activity in the two structures were not compared.

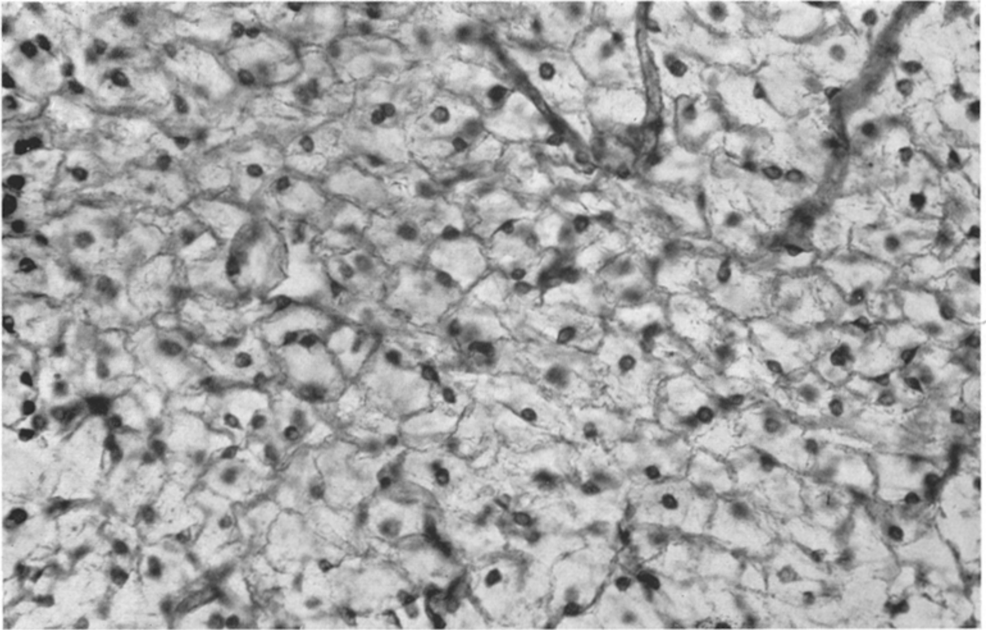
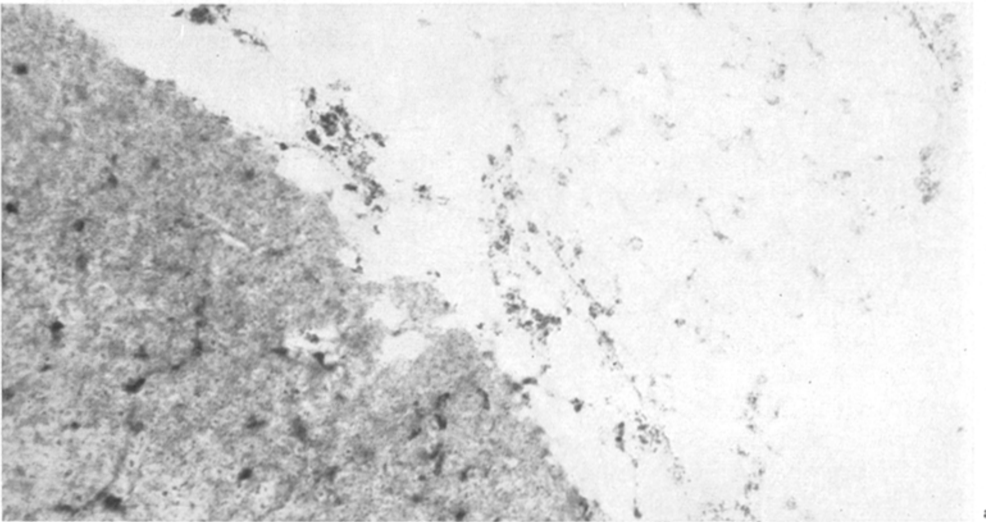


Fig. 1



a

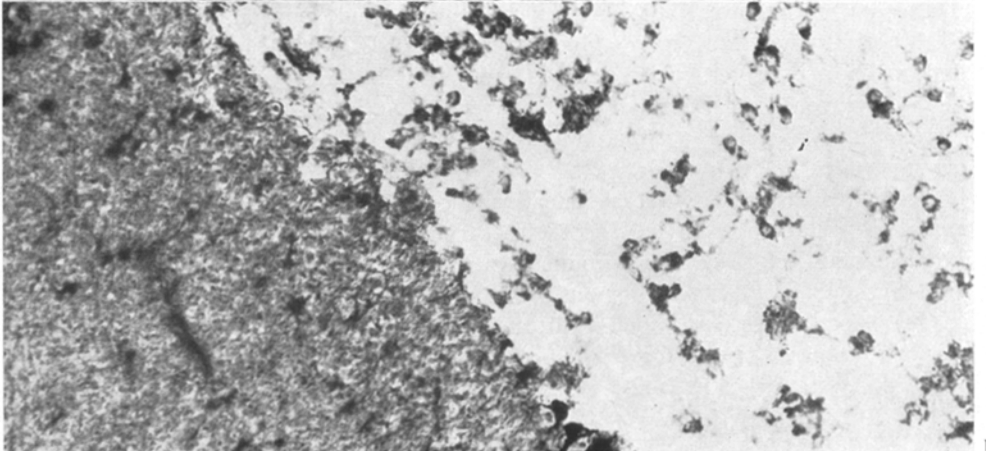


Fig. 2 b

Figs. 1 and 2 (Captions see page 333)

Cholinesterases. AChE and ChE activity was not demonstrated in the glycogen body. This complete lack of activity was in marked contrast to the observation of moderate activity of these enzymes in white matter and high levels of activity in the neurons, particularly in motor neurons of the anterior column.

Phosphatases. The only evidence of AlkP in the glycogen body was a weak staining in some capillaries. In the spinal cord, this enzyme was diffusely distributed, the activity being weak in white matter and moderate in gray matter. High levels of AlkP activity in the capillaries of nervous tissue have been reported for many mammalian species; AlkP was not demonstrated, however, in capillaries of the turkey cord. This species difference in AlkP distribution was also observed by Shimizu (1950) in the chick brain.

There was moderate AcP activity throughout the perikarya and membranes of the glycogen body cells (Fig. 3). In the cord, the enzyme activity was weak in the white matter, somewhat stronger in the gray matter, and very high in the neurons of the anterior column.

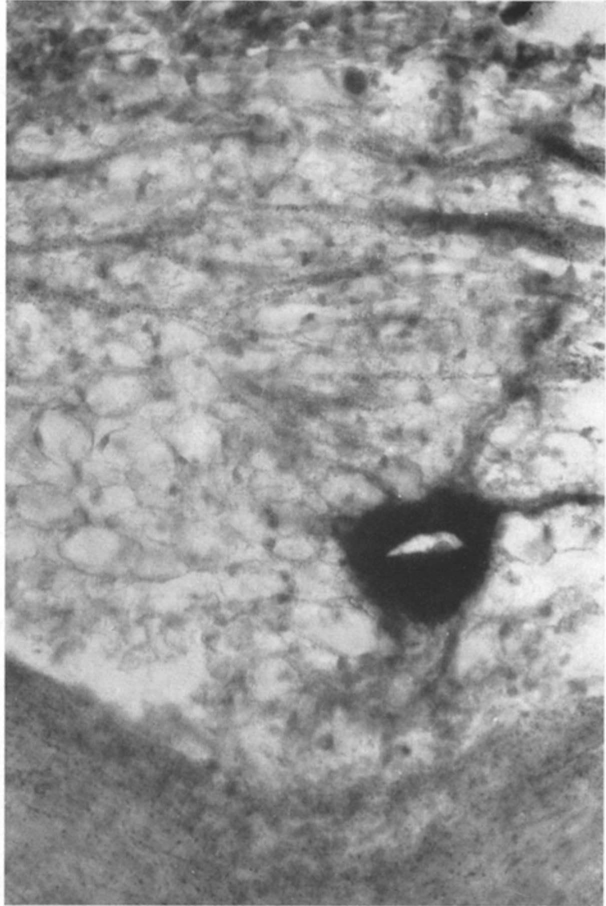


Fig. 3. Acid phosphatase in the glycogen body which encompasses the canalis centralis at this level. The reaction is distributed uniformly throughout perikaryon and cytoplasm of the glycogen body cells. An intense reaction is present in the ependyma. 30 μ; 200 ×

Additional Histochemical Observations. The ependyma of the canalis centralis usually bordered the ventral surface of the glycogen body, but occasionally the tissue of the glycogen body encompassed the canalis centralis (Fig. 3). The

Fig. 1. Lactic dehydrogenase reaction in the cells of the glycogen body. The reaction is very intense in the knob-like perikarya, moderate in the membranes. No reaction is observed inside the cell, where glycogen is stored. 30 μ section; 200 ×

Fig. 2a and b. Comparison of the reactions for succinic dehydrogenase (a) and malate dehydrogenase (b) in the glycogen body (right) and the adjacent white matter (left) and its oligodendroglia cells. The succinic dehydrogenase reaction is very weak in the glycogen body cells as compared with oligodendroglia, while the malate dehydrogenase reaction is about equal in both. Cryostat section, 12 μ; 200 ×

ependymal cells showed very high AChE activity and marked activity of NAD-diaphorase, LDH, MDH and AcP (Fig. 3). They showed weak activity of SDH, CYO, ChE and AlkP.

The meninges covering the dorsal surface of the glycogen body were thicker than meninges covering the cord. For all the enzymes except SDH and CYO, the reaction was more intense in this thickened layer of leptomeningeal tissue than in the meninges covering the cord; SDH and CYO were rather weak throughout the meninges.

Table. *Assays of Homogenates of Glycogen Body and Whole Cord (micrograms formazan/mg wet weight tissue/20 min/38° C)*

		I	II	III	Average Ratio
LDH	glycogen body	54.2	41.7	47.9	1:3.5
	cord	161.8	147.9	172.2	
SDH	glycogen body	0.7	0.7	1.4	1:76.8
	cord	75.0	62.5	72.2	

Biochemical assays. Biochemical assays of LDH and SDH activity in homogenates of the glycogen body and cord were done to substantiate the differences observed for these enzymes in the histochemical studies. The ratio of SDH activity in the glycogen body to that in the cord was 1:76.8, while for LDH it was only 1:3.5. This was in agreement with the histochemical observations.

Discussion

The histochemical demonstration of marked activity of LDH and NAD-diaphorase, and of minimal activity of SDH and CYO in glycogen body cells indicated that the glycogen body depends on glycolytic metabolism rather than on tissue respiration via the citric acid cycle. This was strongly supported by the biochemical data. The intense G-6-PDH staining indicated that the cells of the glycogen body were also able to utilize the hexosemonophosphate shunt. The high MDH level would seem to be an exception to this pattern. It has been observed, however, both biochemically (ROBINS and SMITH, 1953) and histochemically (FRIEDE, FLEMING and KNOLLER, 1963a), that the regional gradations of MDH activity often deviate from those of other citric acid cycle enzymes. Conceivably, this reflects the position of MDH at a "branch point" of the cycle; it is known that malate may be converted to oxaloacetate via the citric acid cycle or may yield pyruvate via the "malic enzyme" system.

The lack of cholinesterase in the glycogen body suggests that there is no cholinergic control of the glycogen-storing tissue. The weak AlkP activity could be considered an indication that the glycogen body tissue is not of meningeal derivation (HANSEN-PRUSS, 1923) since meninges of vertebrates, as well as meningeomas (FEIGIN and WOLF, 1959; FRIEDE, 1959; PEPLER, 1960), show intense alkaline phosphatase reaction.

Summary

Enzyme histochemical studies of the glycogen body of the turkey showed very little activity of succinic dehydrogenase and cytochrome oxidase in the glycogen body cells, and marked activity of lactic dehydrogenase, NAD-diaphorase and the hexosemonophosphate shunt enzymes. Gradients of histochemical staining intensity for lactic and succinic dehydrogenase in the glycogen body and spinal cord were confirmed by biochemical assays of homogenates of these tissues. It was concluded that glycogen body metabolism is predominantly glycolytic. Alkaline phosphatase activity was weak; acid phosphatase activity was moderate. There was no acetyl cholinesterase or nonspecific cholinesterase activity in the glycogen body.

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