μmole/liter protected against heat inactivation; adenine, adenosine-5′-triphosphate, and several other compounds had no effect. We have also observed that PRPP prevents loss of activity of purified APRT stored at −10°C.

Although the mechanism whereby PRPP stabilizes the adenine phosphoribosyltransferase molecule has not yet been determined, the situation may be analogous to the stabilization of rat liver tryptophan pyrrolase by its substrate, tryptophan (*8*). 2,3-Diphosphoglycerate, which alters the affinity of oxygen for deoxyhemoglobin, is present in increased amounts in erythrocytes from hypoxic individuals (*9*). Our findings provide a second example of a small, phosphorylated molecule effecting a change in the properties of an erythrocyte protein. In addition, we believe that the data are consistent with the concept that genetic abnormalities affecting one enzyme may, through accumulation of substrate, result in stabilization of a different enzyme. Although such a mechanism apparently influences the activity of APRT enzyme in mammalian cells, this conclusion does not imply that it is the only mechanism operable.

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Glucose Production by Lamprey Meninges

Abstract. *Meningeal tissue of the lamprey larva produces glucose during incubation and contains glucose-6-phosphatase and large amounts of glycogen. Little glucose is liberated by brains from which meninges have been removed. This primitive meningeal tissue may produce glucose for use by the brain during periods of metabolic stress.*

The arachnoid portion of lamprey meninges appears to be more active metabolically than its counterpart in higher vertebrates. It is supplied with capillaries and includes columns of round cells which contain lipid droplets and considerable glycogen (*1*, *2*). Lamprey brain with intact meninges produces free glucose both *in situ* and in vitro through the breakdown of glycogen and the probable action of glucose-6-phosphatase (E.C. 3.1.3.9) (*3*). This report shows that the meninges rather than the nervous tissue is the major site of glucose production.

Larval sea lampreys (*Petromyzon marinus* L.) were anesthetized in tricane (*4*) and decapitated, and the dorsal portion of the braincase was removed under cooled Ringer fluid. The meningeal (*5*) tissue exposed at this point has a soft consistency and contains many melanocytes. It adheres to most of the external surface of the brain but is thickest lateral to the midbrain. This meningeal tissue was teased from the brain with fine forceps and incubated (*6*) or prepared for chemical analysis separately from the remaining nervous tissue.

Tissues were either directly homogenized in iced 0.03*N* HCl or frozen, weighed, and extracted by the procedure used previously (*3*). Glycogen (*7*), glucose, and glucose-6-phosphate (*8*) were measured fluorometrically through enzymatic production of reduced nicotinamide-adenine dinucleotide phosphate. Protein was measured spectrophotometrically and was used to calculate weights of directly homogenized tissues (*9*). Glucose-6-phosphatase activity is expressed as the amount of inorganic phosphate liberated by crude tissue homogenates incubated in 10 m*M* glucose-6-phosphatase at *p*H 6.9 (*10*); while maltase (α-1,4-glucosidase) (E.C. 3.2.1.20) activity was measured as the amount of glucose produced from 2 m*M* maltose in modified enzymatic glucose reagent, *p*H 7 (*11*).

Figure 1 illustrates the relative distribution of glycogen in brain and meninges as revealed by PAS (periodic acid–Schiff) staining (*12*). Meningeal cells stained dark maroon, indicating dense glycogen deposits; nervous tissue stained pink except for large neurons, which stained red. The results of the histochemical staining were confirmed by chemical measurement of glycogen in isolated meninges and the brains from which they were removed (Table 1). Values for cleaned brains approximate the highest concentrations found in brains of other cold-blooded vertebrates (*13*), while meningeal values were fourfold higher, glycogen constituting about 2 percent of the wet weight of the tissue. Glycogen contents might be higher under more favorable experimental conditions in view of the occasional samples of whole brain that showed values greater than 100 mmole of glycosyl units per kilogram (1.6 percent of wet weight). In another species of lamprey (*Lampetra fluviatilis* L.) a maximum content of glycogen in the brain of 3.7 percent has been found (*13*).

About 90 percent of the glucose produced by lamprey brain can be accounted for by the quantity released by meningeal tissue during incubation. The small amounts released by cleaned brain can be attributed to the few remaining meningeal cells and the glucose normally liberated at glycogen branch points by debranching enzyme.

Table 1. Glycogen concentration, glucose production (19° and 26°C), and glucose-6-phosphatase activity (21°C) in lamprey brain and meninges. The results are expressed as the mean ± S.D.; the number of determinations is in parentheses.

Wet weight (mg)	Glycogen (mmole of glycosyl units/kg)	Glucose production (mmole kg^{-1} hr^{-1})	Glucose-6-phosphatase (mmole kg^{-1} hr^{-1})
		Whole brain	
4.7 ± 1.0(38)	81 ± 27(24)	20 ± 2*(5)	50*
		Cleaned brain	
3.4 ± 0.7(17)	27 ± 8(11)	1.8 ± 0.7(4)	14, 17 (2)
		Meninges	
0.8 ± 0.4(18)	137 ± 33(10)	107 ± 26(4)	204 ± 56(3)

* Values previously reported (*3*).

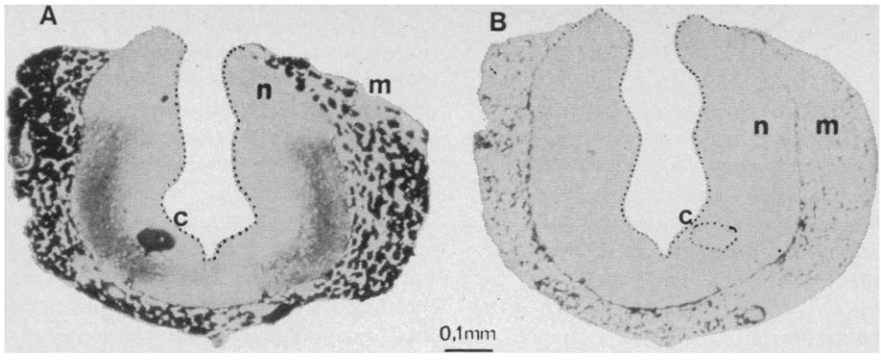

Fig. 1. Transverse sections through the midbrain of a larval lamphrey stained for glycogen by the periodic acid-Schiff procedure. Fixation was in alcohol formalin. Section (B) was preincubated in 0.1 percent malt diastase for 1 hour at 37°C, while (A) was incubated in buffer alone; *n*, nervous tissue of the brain; *m*, meningeal tissue; *c*, a large reticulospinal neuron. The difference between (A) and (B) is even greater than the figure suggests; (B) was overexposed relative to (A) to bring out structures that would otherwise have been invisible. Gage (*1*) has reported a similar staining pattern. The margins of the third ventricle and the border of the cell in (B) have been retouched.

Meninges produced glucose at a rate 50 times that of nervous tissue on the basis of weight (Table 1). Meningeal tissue also showed more glucose-6-phosphatase; meningeal samples had 13 times the activity of nervous tissue on a weight basis. In two additional experiments at higher temperatures, the ratios were 14 and 11. Glucose-6-phosphate is abundant in incubated meninges, averaging 0.51 mmole/kg (wet weight).

Although the major route of glucose production by meningeal tissue appears to be the breakdown of glycogen to glucose-1-phosphate and glucose-6-phosphate, which is acted on by the phosphatase, other routes are also feasible. For instance, gluconeogenesis from lactate or glycerol might contribute to the pool of glucose-6-phosphate. Another route, which bypasses the phosphatase, is the direct liberation of glucose from glycogen branch points by debranching enzyme (*14*). However, this mechanism would normally convert only about 8 percent of the glycogen to glucose unless the tissue had a substantial resynthesis and turnover of glycogen. One other route might be the action of maltase on glycogen. Activities of maltase were somewhat higher in meningeal than nervous tissue: 17 versus 13 mmole/kg per hour in the imidazole buffer system and 12 versus 6 mmole/kg in the phosphate system, both at 23°C.

Although direct evidence is lacking, it is likely that the glucose produced by lamprey meninges enters the nearby nervous tissue. Lamprey brain has no internal blood vessels and is thought to absorb its nutrients from the rich capillary net over its surface (*2*); the meningeal cells share the extracellular space of these capillaries and may contribute to the normal flux of metabolites. Furthermore, glucose appears to enter the isolated brain readily from the surrounding medium, as indicated by the equilibration of glucose in tissue water and medium over the range 1 to 10 mmole/liter at 4°C (*3*). In addition, lamprey brain (with intact meninges) satisfies a conventional criterion for glycolytic metabolism, the conversion of glycogen to lactate under anaerobic conditions, and contains hexokinase, which enables glucose to enter the pathway (*3*). However, it remains to be demonstrated that glucose originating in the meninges is metabolized by the brain.

The function of lamprey meningeal tissue may be the production of glucose for the brain during periods of metabolic stress. One possible situation may arise when the animal attempts to escape by burrowing in the sand or mud in which it normally lives; glucose delivery by the circulatory system may be insufficient to meet the metabolic demands of the nervous system under conditions of maintained activity and low oxygen tension. The brains of free-swimming adult lampreys are covered with meningeal tissue like that of the larvae; adult meninges may act as a glycogen storage site between parasitic feedings.

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4. Larval lampreys were obtained through the courtesy of Mr. E. Louis King, Jr., U.S. Bureau of Commercial Fisheries, Hammond Bay Biological Station, Millersburg, Michigan 49759. They were either kept in open containers of fresh water at 4°C or maintained on a diet of yeast, algae, and microorganisms in an aquarium at room temperature. Tricaine is *m*-aminobenzoic acid ethyl ester, methanesulfonate. It was used at 2×10^{-4} g/ml.
5. The description of this tissue as meningeal rests on its classification as subarachnoid by Hibbard (*2*). Alternatively, it should be described as perimeningeal if the thin layer of connective tissue immediately adjacent to the brain is classified as meninx primitiva, as by C. U. Ariëns Kappers, G. C. Huber, E. C. Crosby [*The Comparative Anatomy of the Nervous System of Vertebrates, Including Man* (Hafner, New York, 1960), vol. I, pp. 56 and 61].
6. Cleaned brains were split along the midline. Separated meningeal and nervous tissues were washed in fresh, iced Ringer fluid (*3*), then placed in 0.5 ml of Ringer fluid in small covered beakers at 19°C (one experiment at 26°C). Fifteen minutes later half the tissue and 0.05 ml of medium from each beaker were frozen for subsequent analysis. At 75 minutes the remaining tissue and another portion of medium were frozen. The amount of glucose liberated during the last hour was referred to the weight of the incubated tissue. Adenosine triphosphate (ATP) and phosphocreatine (PC) were measured (*8*) in all incubated tissues. Their concentrations in meninges were 1.3 ± 0.3 mmole/kg (mean $\pm$ S.E.M.) and 5.7 ± 1.4, and in cleaned brain 1.0 ± 0.1 and 5.7 ± 0.7. These values vary insignificantly from those measured in whole brain incubated with oxygen at room temperature (ATP, 1.0; PC, 6.4) and whole brain frozen *in situ* (ATP, 0.9; PC, 6.6) (*3*).
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10. For measurement of glucose-6-phosphatase cleaned brains and meninges were homogenized in iced 10m*M* imidazole buffer and incubated at 21° to 26°C in 10 m*M* glucose-6-phosphate in 0.1*M* imidazole-HCl buffer *p*H 6.9. Reactions in duplicate tubes were stopped at 0 and 30 minutes with trichloroacetic acid on ice. Because glucose-1-phosphate is unstable when heated in strong acid, assays for the enzyme including the strong acid development of phosphomolybdate may also include a contribution from phosphoglucomutase. Phosphomolybdate was developed in acetate buffer (*8*). Solutions were made up from distilled rather than deionized water.
11. Crude homogenates for measurement of maltase activity were made up in distilled water on ice and added to glucose reagent within 15 minutes. Activity was taken as the difference in glucose production between reagents with and without 2 m*M* maltose. Maltase activity of the reagent was negligible. Glucose reagent was the same as that used for fluorometric analysis (*8*) aside from increased concentrations of glucose-6-phosphate dehydrogenase and the substitution of 20 m*M* imidazole-HCl buffer *p*H 6.9 or 5 m*M* phosphate buffer *p*H 7 for tris(hydroxymethyl)-aminomethane HCl.
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