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Biochemical and Ultrastructural Study of Glycogen in Jejunal Mucosa of Diabetic Rats (37790)

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Diabetes mellitus is accompanied by significant increases in glycolytic rates (1) and glycolytic enzyme activities (2, 3) and by reductions in gluconeogenic enzyme activities (4) in rat jejunal mucosa. Glycogen metabolism, however, has not been studied in the gut of diabetic rats; the concentration and ultrastructural characteristics of glycogen have not, in fact, been established in jejunal mucosa of normal rats. To extend our understanding of the metabolic alterations in the gut of diabetic rats we have performed biochemical and electron microscopic studies of glycogen in jejunal mucosa. The studies demonstrate that there is a significant increase in the glycogen content of jejunal mucosa of diabetic rats and that this glycogen is present in the β conformation.

To further assess the adaptive nature of gut glycogen we have compared glycogen and glycolytic enzyme values in jejunal mucosa and liver of fasted and of glucose-refed rats. While adaptive changes in liver and gut were qualitatively similar, the responses of liver to dietary alterations were consistently greater in magnitude.

Materials and Methods. Animals. Male Sprague–Dawley rats (175–250 g) were fed Purina Lab Chow *ad libitum* except for one group which was starved for 72 hr and a second group which was starved for 72 hr and then refed a diet containing 75% of calories as glucose (5). Diabetes was produced by the intravenous injection of 75 mg streptozotocin/kg body wt as previously described (4) and rats were killed 21–27 days later.

Tissue Preparation. Rats were killed by cervical fracture at approximately 9 am and jejunal mucosa, liver, and renal cortex removed (4). Jejunal mucosa from 15 to 30 cm beyond the pylorus was used for enzyme assays and from 30 to 45 cm for glycogen measurements.

Ultrastructural Studies. Sections of gut removed from the midjejunum were cut into small segments and fixed in half-strength Karnovsky's glutaraldehyde–paraformaldehyde mixture (6) in phosphate buffer and postfixed in phosphate-buffered osmium tetroxide solution. The specimens were embedded in Epon 812 by the method of Luft (7), and thin sections were stained with lead and examined in a Phillips EM-300 electron microscope.

Measurements. Blood glucose measurements were performed in duplicate by the glucose oxidase method (Dextrostix® and reflectance meter, Ames Co., Elkhart, Ind., Ref. 4). Enzyme measurements (3, 5) and glycogen estimations (4) were made as described previously.

Results. Glycogen Content of Jejunal Mucosa. The glycogen concentration was approximately 0.04% of the wet weight of jejunal mucosa of normal rats compared to the liver content of approximately 6% of wet weight (Table I). Moderately diabetic rats had a slight increase in the weight of jejunal mucosa but the glycogen content was unchanged. Severely diabetic rats had a 50% increase in mucosal weight, as previously noted (3), and the glycogen content was twofold higher than in control rats. Fasting was associated with a 46% reduction in jejunal glycogen.

Glycogen Content in Liver and Renal Cortex. For comparison, glycogen was measured in liver and renal cortex in most of these

TABLE I. Glycogen Content of Jejunal Mucosa, Liver, and Renal Cortex of Control, Diabetic, and Fasted Rats.

	Controls	Diabetic		72-hr fasted
		Moderate	Severe	
Rat weight, g	308 ± 10(36)	260 ± 22(9)	227 ± 7(38)[a]	242 ± 6(20)[a]
Jejunal mucosa weight mg/cm	34 ± 1(36)	42 ± 4(8)[b]	51 ± 1(38)[a]	19 ± 1(20)[a]
Blood glucose, mg/100 ml	103 ± 3(33)	352 ± 21(9)[a]	500 ± 10(38)[a]	73 ± 3(12)[a]
Glycogen content				
Jejunal mucosa, μg/g	423 ± 30(36)	446 ± 46(8)	849 ± 72(38)[a]	229 ± 12(20)[a]
Liver, mg/g	59.1 ± 3.4(22)	26.2 ± 9.5(9)	16.5 ± 2.3(29)[a]	0.2 ± 002(12)[a]
Renal Cortex, μg/g	47.6 ± 2.4(22)	570 ± 241(9)	1752 ± 226(30)[a]	—

Moderately diabetic rats had blood glucose values of 201–400 mg/100 ml and severely diabetic rats had values above 400. Glycogen content is expressed as μg or mg/g wet wt. Values are expressed as mean ± SEM with number of animals in parentheses. Significant differences from control values are expressed as [a], $p < 0.001$ and [b], $p < 0.01$.

same animals. Diabetes was accompanied by a 72% reduction in liver glycogen and glycogen was virtually absent from the liver of fasted rats. Renal glycogen was 12-fold higher in moderately diabetic and 38-fold higher in severely diabetic rats. Whereas the glycogen content of jejunal mucosa and liver was not linearly correlated with the blood glucose concentration an excellent linear correlation ($r = +0.89$, $p < 0.001$) was noted between renal glycogen and the degree of hyperglycemia.

Ultrastructural Studies of Jejunal Glycogen. Smooth surfaced spherical particles consistent in size (250–300 Å) and staining properties with β glycogen (8) were found in goblet cells, submucosal cells and smooth muscle cells, but were rarely observed in absorptive cells of nondiabetic rats. However in the diabetic animal β glycogen particles were observed with considerable frequency interspersed among the organelles throughout the absorptive cells and were particularly noticeable in the region of the terminal web (see Fig. 1). The epithelial cells nearer the top of the villus appeared to consistently contain more glycogen than the absorptive cells nearer the crypt mouths. Although difficult to quantify, glycogen did not appear to be more prevalent in nonabsorptive intestinal cells of the diabetic animals than that seen in nondiabetic animals.

Adaptive Responses of Glycogen and Glycolytic Enzymes. Starvation produced a gradual reduction in glycogen content of jejunal mucosa to approximately 50% of control values and similar reductions were observed in the activities of three rate-limiting enzymes of glycolysis (Fig. 2). In the liver, reductions in glycolytic enzyme activities during starvation were similar to those observed in gut but the decrease in liver glycogen content, to less than 0.5% of control values, was more precipitous and more marked than observed for gut glycogen.

When these rats were refed a 75% glucose diet, distinct patterns of response were observed for each parameter studied. The increase in liver glycogen was prompt and exaggerated while changes in gut glycogen were small. Hexokinase activity increased in both tissues to values greater than control values but the percentage increase was much greater in liver. Likewise, the increases of pyruvate kinase and glucose-6-phosphate dehydrogenase activities were much greater in liver than in gut, where the increases were transient.

Discussion. Diabetes is accompanied by significant physiological and biochemical perturbations in the gut which include: increased absorption of glucose (9), increased disaccharidase activities (10), increased glycolytic rates (1), increased glycolytic enzyme activities (2, 3) and reduced gluconeogenic enzyme activities (4); our current studies indicate, in addition, a twofold increase in glycogen content. Some of these changes are

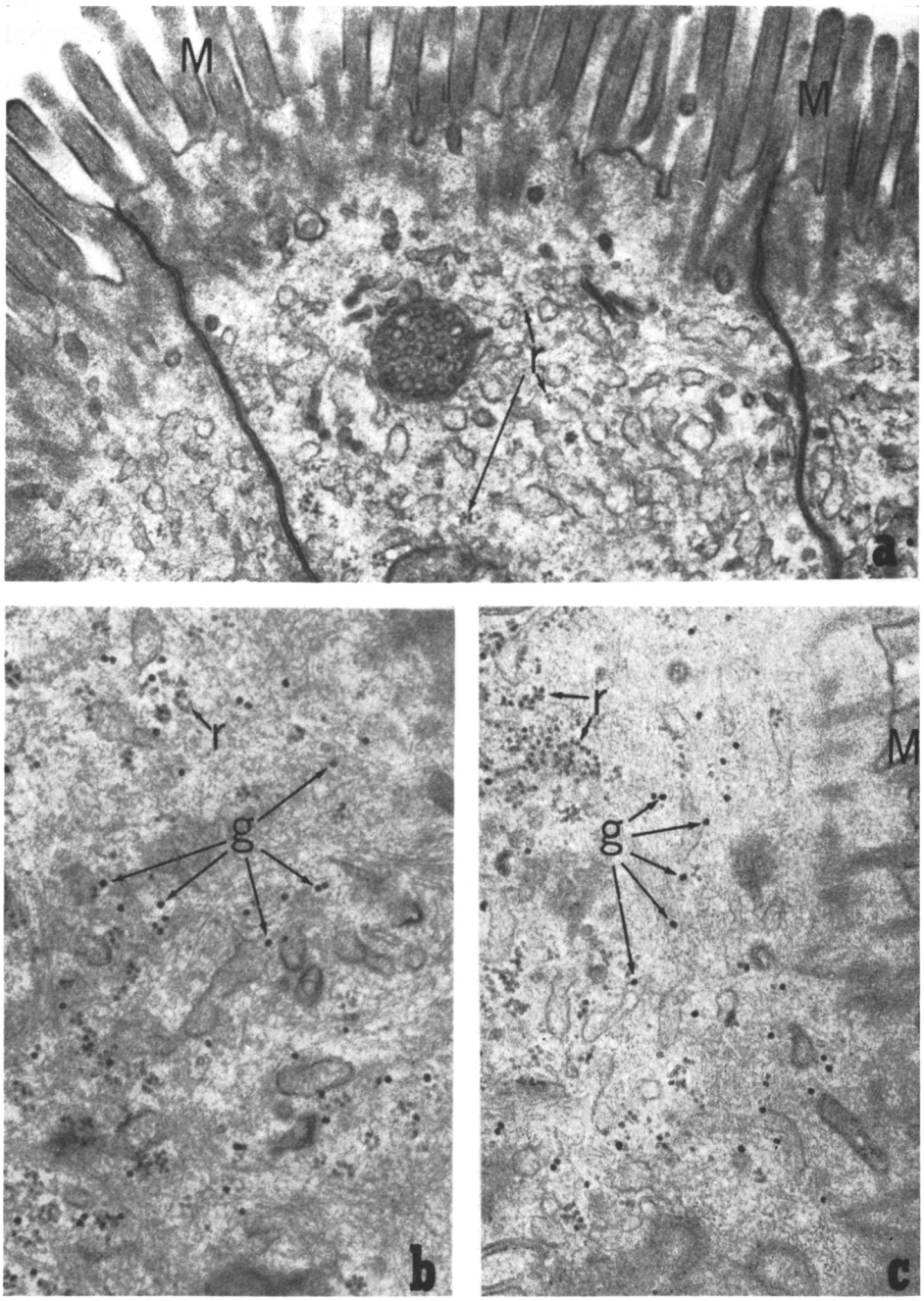

FIG. 1. Electron micrographs of apical region of absorptive cells from midjejunum. (a) *Chow-fed rats.* Portions of three cells show microvilli (M) with filamentous cores blending into the apical region (terminal web). A few free ribosomes (r) and profiles of endoplasmic reticulum are observed in this region. × 30,000. (b and c) *Diabetic rats.* The terminal web region of these two absorptive cells contain many particles characteristic of β glycogen (g). These particles were observed throughout the absorptive cell of diabetics but were particularly well seen in the apical region. × 45,000.

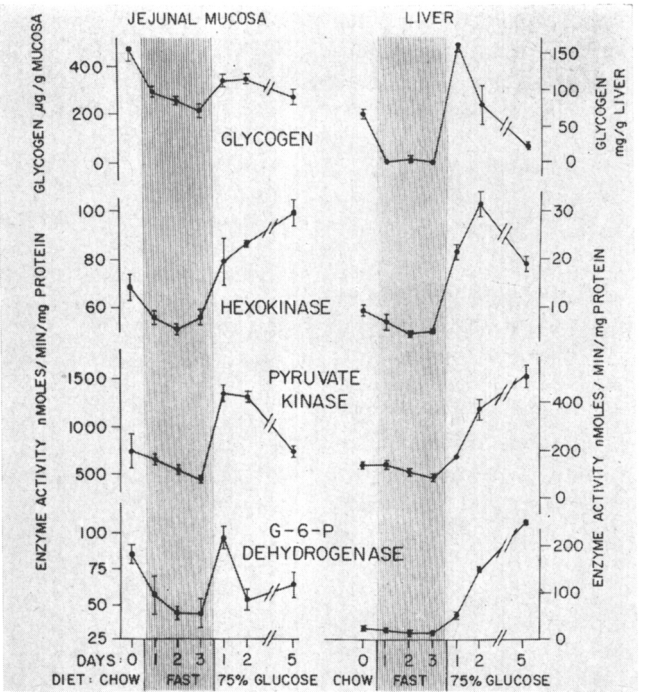

FIG. 2. Effect of fasting and refeeding on glycogen and glycolytic enzyme values of jejunal mucosa and liver. Jejunal mucosal hexokinase was measured in the homogenate while liver hexokinase (representing glucokinase) and other enzymes were measured in the 105,000*g* supernatant fraction. Values represent the means of seven rats at day 0 and four rats at other days and the bars represent one SEM.

related in part to the hyperphagia and mucosal hypertrophy of diabetic animals but the increased glycolytic enzyme activities (3) and increased glucose transport (11) are observed in pair-fed animals with normal mucosal weights. The role that hyperphagia and increased glucose transport plays in the glycogen accumulation we observed is unclear but the observations (4) that renal cortical glycogen is correlated with the degree of hyperglycemia and presumably with increased glucose transport suggest that the increased glucose transport may contribute to glycogen accumulation in the gut of diabetics.

The glycogen content of intestinal mucosa for the rat or other mammals is not well defined although careful studies have been performed in the chick embryo (12). Field *et al.* (13) reported values of 800 ± 80 (mean ± SEM) μg/g jejunal mucosa for four adult humans which is only slightly higher than we observed for the rat. However, Öckerman (14), without providing details of his methods, reported values of 4300 ± 386 μg/g jejunal mucosa for five adult humans and a value of 4300 μg/g jejunal mucosa for a single rat. Since we used (4) a specific glucose oxidase assay for the hydrolyzed glucose and were able to recover 96 ± 5% of the glycogen added to tissue samples (4), we believe the values of 423 ± 30 μg/g jejunal mucosa that we report for 36 normal rats accurately reflects the glycogen content in these animals. We have observed identical values in the jejunal mucosa of normal guinea pigs (15). In addition, the values we report for liver glycogen are in the range reported by many other workers (16–18).

The ultrastructural characteristics of glycogen in the gut of the normal rat or other mammals also have not been described. Browning and Trier (19) have noted an abnormal accumulation of β glycogen in the cytoplasm of undifferentiated jejunal mucosal cells after 24 hr in tissue culture. Our studies demonstrate that a significant quanti-

ty of β glycogen accumulates in the terminal web region of absorptive cells of jejunal mucosa in diabetic animals whereas only small amounts of glycogen, if any, can be detected in this region in normal rats.

While the twofold increase in jejunal mucosal glycogen was clearly different from the decrease in liver glycogen in diabetic rats, the adaptive responses of jejunal and hepatic glycogen to dietary alterations were similar in direction. The magnitude of reductions in glycogen content during starvation were greater in the liver, and the adaptive increase in hepatic glycolytic enzymes were also greater during the refeeding period. Thus these studies demonstrate that the adaptive response of liver to diabetes and to dietary alterations are greater in magnitude than those observed for jejunal mucosa.

Summary. The glycogen concentration of jejunal mucosa was 423 μg/g or 0.04% of wet weight in normal rats. Diabetes was accompanied by a twofold increase in glycogen content whereas a 72-hr fast produced a 50% reduction of gut glycogen. In absorptive cells of diabetic rats β glycogen was observed throughout the cytoplasm but appeared particularly abundant in the terminal web region. The adaptive responses of gut glycogen levels to fasting and refeeding were similar to changes in the activities of gut glycolytic enzymes but glycogen and enzyme changes were less marked than those ob served for liver of the same animals.

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