enzyme activity, was obtained in Fractions 5 to 10 from the column. These fractions contain 6% of the protein in the column load (2.5% of the tissue protein). There was no further separation of inactive protein when the fractions were pooled and passed through Sephadex again, nor on fractionation with ethanol. Material put through the whole of the original purification procedure[1] underwent no change on Sephadex. The purified enzyme could not be induced to crystallize and the activity in solution was destroyed by freezing. At the peak in Fig. 1, the specific activity was 620 000, and we consider this to be a limiting value for the enzyme in preputial gland.

The revised procedure for female-rat preputial gland affords a simple and convenient source of pure β-glucuronidase for use as a hydrolytic agent in the steroid and other fields, with a remarkably high recovery of the enzyme.

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Hepatic glycolytic and gluconeogenic enzymes of the obese-hyperglycemic mouse

The obese-hyperglycemic mouse (Jackson Laboratory C57BL/6J-ob) exhibits certain features of maturity-onset diabetes. These include significant hyperglycemia[1], greater than normal levels of plasma insulin during normal alimentation[2], relative insensitivity of adipose tissue to insulin[3], development of nodular glomerular renal lesions[4] and greater than normal susceptibility to skin infections. The fat metabolism of these mice has been studied extensively, but relatively little work has been done on their hepatic carbohydrate metabolism.

During alloxan diabetes in rats, hepatic glucokinase (ATP:D-glucose 6-phosphotransferase, EC 2.7.1.2), phosphofructokinase (ATP:D-fructose-6-phosphate 1-phosphotransferase, EC 2.7.1.11) and pyruvate kinase (ATP:pyruvate phosphotransferase, EC 2.7.1.40) activities fall significantly and insulin administration restores these activities to normal[5–7]. Conversely, an elevation of the hepatic gluconeogenic enzymes pyruvate carboxylase (pyruvate:CO_2 ligase (ADP), EC 6.4.1.1), phosphoenolpyruvate carboxykinase (GTP:oxaloacetate carboxy-lyase (transphosphorylating), EC 4.1.1.32), fructose-1,6-diphosphatase (D-fructose-1,6-diphosphate 1-phosphohydrolase, EC 3.1.3.11) and glucose-6-phosphatase (D-glucose-6-phosphate phos-

phohydrolase, EC 3.1.3.9) occurs during alloxan diabetes and the administration of insulin results in their return to normal[8]. The activities of the bifunctional enzymes catalyzing the glycolytic–gluconeogenic pathway are not significantly altered during alloxan diabetes or insulin administration[9]. As a result of these findings, WEBER postulated that these three "key" glycolytic and four "key" gluconeogenic enzymes may function as genomic units, insulin acting as an inducer of the glycolytic enzymes and as a suppressor of the gluconeogenic unit[9].

Because of the reciprocal behavior of these "key" glycolytic and gluconeogenic enzymes during alloxan diabetes and insulin administration, it seemed of interest to determine the activities of these enzymes in the liver of the hyperinsulinemic, obese-hyperglycemic mouse. All of the "key" glycolytic enzymes were assayed; the activities of glucose-6-phosphatase and fructose-1,6-diphosphatase were determined as being representative of the "key" gluconeogenic group. Lactate dehydrogenase (L-lactate:NAD oxidoreductase, EC 1.1.1.27) and phosphoglycerate kinase (ATP:D-3-phosphoglycerate 1-phosphotransferase, EC 2.7.2.3) were also assayed as examples of bifunctional enzymes whose activities were reported to be unaltered during alloxan diabetes or insulin administration.

Male, obese-hyperglycemic mice, 2.5–5 months of age, were purchased from The Jackson Memorial Laboratory, Bar Harbor, Me. They were maintained in air-conditioned quarters and were fed Wayne Lab Blox (Allied Foods) and water *ad libitum*. Lean litter mates were treated similarly and served as controls. The mice were killed between 9 a.m. and 10 a.m. by cervical dislocation, and the livers were rapidly removed, weighed, rinsed in ice-cold 0.25 M sucrose and homogenized. Enzyme activities were then assayed using the methods indicated: glucokinase[10]; phosphofructokinase[11]; pyruvate kinase[12]; lactate dehydrogenase and phosphoglycerate kinase[13]; glucose-6-phosphatase[14]; fructose-1,6-diphosphatase[15]. Enzyme activities were expressed as μmoles of product formed per min per mg DNA. DNA was determined by the BURTON modification of the diphenylamine reaction[16].

TABLE I

ACTIVITIES OF HEPATIC GLYCOLYTIC, BIFUNCTIONAL AND GLUCONEOGENIC ENZYMES OF OBESE MICE AND LEAN LITTER MATES

Values represent the mean of between 5 and 10 mice ±S.E.

Enzymes	*Product formed (μmoles product per min per mg DNA)*	
	Lean	*Obese*
Glycolytic enzymes		
Glucokinase	0.4 ± 0.04	1.5 ± 0.06
Phosphofructokinase	1.0 ± 0.1	1.6 ± 0.01
Pyruvate kinase	9.1 ± 0.8	28.6 ± 1.6
Bifunctional enzymes		
Phosphoglycerate kinase	74.0 ± 3.2	82.0 ± 1.8
Lactate dehydrogenase	85.2 ± 6.4	136.0 ± 5.0
Gluconeogenic enzymes		
Fructose-1,6-diphosphatase	2.4 ± 0.2	5.1 ± 0.2
Glucose-6-phosphatase	3.5 ± 0.3	5.7 ± 0.3

The results of this study are summarized in Table I. The activities of glucokinase, phosphofructokinase and pyruvate kinase in the obese-hyperglycemic mice were significantly elevated above those of the lean litter mates. Both glucose-6-phosphatase and fructose-1,6-diphosphatase activities were likewise elevated in the obese mice. Lactate dehydrogenase is elevated in the obese mice, while phosphoglycerate kinase is similar in both groups.

This study demonstrates that even though relative insulin resistance with respect to glucose uptake in the adipose tissue of the obese-hyperglycemic mouse has been demonstrated, the hepatic insulin-responsive glycolytic enzymes appear to reflect the high level of plasma insulin in these animals. The elevation of all three of the "key" glycolytic enzymes is in keeping with WEBER's functional genome concept. However, some doubt has been cast upon this hypothesis by recent work which showed that glycerol feeding to alloxan-diabetic rats results in an increase to normal levels of pyruvate kinase while glucokinase activity remained low[17]. These results suggest that glucokinase alone of the "key" glycolytic enzymes may be regulated by insulin and that pyruvate kinase may be secondarily regulated by the rate of metabolic flow through the glycolytic pathway.

The finding of elevated activities of glucose-6-phosphatase and fructose-1,6-diphosphatase activities in the face of hyperinsulinemia appears to contradict the view that insulin is a suppressor of these enzymes. It may be that the suppressive action of insulin is deficient in this animal. On the other hand, the adrenal glands of the obese-hyperglycemic mouse have been reported to be hyperplastic[18], and since corticosteroids are known to increase the levels of glucose-6-phosphatase and fructose-1,6-diphosphatase[19], no definite conclusion can be made about the above results until the functional adrenal status of these animals is ascertained.

It is generally held that the activities of enzymes regulating a given metabolic pathway reflect the functional activity of that pathway. Thus, it appears that both the glycolytic and gluconeogenic pathways are accelerated in the liver of the obese-hyperglycemic mouse. This finding differs from the reciprocal behavior of these pathways usually observed. The altered dynamics of glucose catabolism and formation observed in the liver of the obese-hyperglycemic mouse may play a role in the pathogenesis of the hyperglycemia in this animal.

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Enlargement of amylopectin by ADP-D-glucose:α-1,4-glucan α-4-glucosyltransferase of spinach

During the purification of ADP-D-glucose:α 1,4-glucan α-4-glucosyltransferase of spinach it was found that the enzyme catalyzes the extensive elongation of outer chains of a primer, potato amylopectin.

The partially purified enzyme with a specific activity[1] of 12 units/mg protein was passed through a column consisting of cellulose impregnated with potato amylopectin. The elution pattern of the enzymatic activity was illustrated in Fig. 1. The pooled eluate usually had a specific activity of 300–500 units/mg protein and was essentially free from amylase(s) (EC 3.2.1.1 or EC 3.2.1.2). By use of this preparation an attempt was made to enlarge potato amylopectin with ADP-D-glucose as glucose donor.

The time course of reaction in the presence of varying concentrations of the primer was shown in Fig. 2. Only with a low concentration of the primer was formation of a flocculent precipitate observed, which indicated the formation of a polymeric product distinguishable from the primer, amylopectin. After collection and washing by centrifugation the precipitate was examined for its properties. As shown in Table I, the amount of the precipitate completely corresponded to that expected from the amount of liberated ADP. Because crystalline sweet potato β-amylase (EC 3.2.1.2) degraded the polymeric product to nearly the theoretical limit (Table II), it was concluded that the newly synthesized portions of the product consisted almost exclusively of linear chains of α-1,4-glucose units. The absorption characteristics of its iodine complex were rather similar to those of potato amylose (Fig. 3). Assuming a relation between DP and the intensity of colour of the iodine complex of linear α-1,4-glucan[6], it was expected that the newly synthesized linear chain portions of the

Abbreviation: DP, degree of polymerization.