The Effect of Fructose Feeding on Glycolytic Enzyme Activities of the Normal Rat Liver*

WALTER M. FITCH, R. HILL, AND I. L. CHAIKOFF

From the Department of Physiology, University of California, Berkeley, California

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A number of studies have brought to light the fact that substitution of fructose for glucose as the sole carbohydrate in a diet results in a profound change in the metabolic behavior of an animal. Thus, the ability of the liver of a glucose-fed, diabetic rat to convert acetate carbon to fatty acids is considerably impaired (1), but when the glucose of the animal's diet is replaced by fructose, hepatic lipogenesis from acetate is restored to normal (2). Later studies of Hill et al. (3, 4) revealed that normal rats and dogs fed a diet containing 58 per cent fructose as sole carbohydrate develop an impaired capacity for utilizing glucose. In order to localize the site of defective glucose utilization in the fructose-fed rat, Hill et al. (3) compared glucose-fed and fructose-fed rats with respect to (a) oxidation of C14-glucose to CO2 by kidney and brain slices; (b) glucose uptake by the diaphragm; and (c) conversion of Cl*-glucose and W-fructose to CO2, fatty acids, and glycogen by slices prepared from the livers of these rats. Fructose feeding did not alter the oxidation of glucose to CO2 by kidney and brain, nor the uptake of glucose by the diaphragm. The liver was the only tissue in which an alteration in metabolic pattern was found after fructose feeding. Impaired glucose utilization has also been observed in the livers of human subjects fed fructose (5).

The mechanism by which fructose feeding induces a metabolic change in the liver is not known. The loss in the liver's capacity to utilize glucose, observed in normal rats fed fructose, is not the result of an insulin lack, for the injection of insulin subcutaneously for 3 days failed to increase that capacity (3). To further our understanding of the effects of fructose feeding we have studied glycolytic enzyme activities of livers of normal rats and dogs fed fructose or glucose as sole carbohydrate in the diet.

EXPERIMENTAL

Treatment of Animals

Male Long-Evans rats were raised and maintained on an adequate stock diet (Labrations, Diablo Animal Laboratories, Berkeley) which contained about 25 per cent protein, 5 per cent fat, and 46 per cent carbohydrate. Ground whole wheat provided the chief source of this carbohydrate. Two synthetic diets, one containing 60 per cent glucose and the other 60 per cent fructose, were fed for exactly 3 days before the rats were killed. The composition of the hexose diets is given elsewhere (6).

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** The terms, "glycolytic" and "glycolysis," are used in this report to refer to glucose catabolism regardless of pathways involved.

Enzyme Assays

The rats were killed by a blow on the head. Their livers were rapidly excised and weighed, and 1-gm. portions were transferred to a cold Potter-Elvehjem homogenizing tube. Three ml. of an ice-cold 0.25 M sucrose solution were added, and the mixture was homogenized for 1 minute by 12 excursions of a loosely-fitting pestle rotated at 350 r.p.m. The resulting homogenate was centrifuged at 100 × g for 10 minutes to remove nuclei and cellular debris. The cell-free, cytoplasmic suspension forming the supernatant layer was used for enzyme assays throughout. Except for weighing and homogenization, all procedures preceding incubation of the enzyme preparation were performed at 0°.

Phosphoglucomutase—This enzyme was assayed by a modification of the method of Najjar (7) in which the rate of disappearance of the easily hydrolyzable glucose 1-phosphate is determined. Of the tissue suspension 0.1 ml. was added to a test tube containing 1 ml. of 0.02 M glucose 1-phosphate in saturated (at 0°) Veronal buffer (pH 7.8) and 0.1 ml. of freshly prepared 0.2 M solution of cysteine. The reaction was carried out at 37°. Two 0.1-ml. aliquots of the reaction mixture were removed immediately after the addition of the tissue suspension and at intervals of 4 or 5 minutes thereafter.

One 0.1-ml. aliquot was mixed with 0.9 ml. of H2SO4 and, after centrifugation of the mixture a 0.5-ml. portion of the supernatant was heated for 1 minute at 100° to hydrolyze remaining glucose 1-phosphate. Its inorganic phosphate content was then determined.

The other 0.1-ml. aliquot was added to 0.9 ml. of ice-cold 4 N H2SO4 and after centrifugation at 0° inorganic phosphate of the mixture was determined on a 0.5-ml. sample of the supernatant layer.

A 50-μl. aliquot was taken from each incubation mixture for protein determination.

Dilution of the sulfuric acid below 4 N fails to deproteinize the samples sufficiently to prevent interference in the subsequent phosphate analysis. An increase in either the normality of the acid or the temperature of the mixture causes considerable hydrolysis of glucose 1-phosphate. At room temperature, 4 N H2SO4 was found to hydrolyze 30 per cent of the glucose 1-phosphate in 1 hour; 8 N acid at 0° hydrolyzed 6 per cent in 1 hour. When the precaution of using 4 N H2SO4 at 0° was taken, no measurable increase in inorganic phosphate was found during incubation, and glucose 1-phosphate disappeared at a constant rate for not less than 10 minutes.

A 4-fold increase in the concentration of the enzyme preparation gave the same initial rate of disappearance of glucose 1-phos-
phate per mg. of liver protein, but the rate did not remain linear for the entire 10 minutes.

There was no change in glucose 1-phosphate concentration when heat-denatured enzyme was used.

It should be noted that the glucose 1,6-diphosphate cofactor was not added to the mutase assay system. Bodansky (8) recently reported that this cofactor is not present in liver in sufficient concentration to insure maximal enzyme activity.

Glucose 6-Phosphatase—The method used was a modification of that described by Langdon and Weakley (9). The reaction was run at 37°C. Of the cytoplasmic suspension 0.1 ml. was transferred to a tube containing 1.0 ml. of 0.02 M glucose 6-phosphate made up in 0.05 M maleate buffer of pH 6.8. Immediately thereafter and at intervals of 4 or 5 minutes, 0.2 ml. of the incubation mixture was withdrawn and added to 0.8 ml. of 8 N H2SO4. After centrifugation of the mixture, a 0.5-ml. aliquot was taken for inorganic phosphate determination.

A 50-µl. aliquot was removed from each incubation mixture for protein determination.

It was found that a 2.4-fold increase in enzyme concentration did not alter the linearity of the reaction over a 10-minute interval. No measurable loss in enzyme activity occurred during storage of the tissue fraction at 0°C for 8 hours.

6-Phosphoglucone Isomerase—This enzyme was assayed by a modification of the method of Bruns and Hinsberg (10), in which the rate of disappearance of fructose 6-phosphate is determined. To a tube containing 0.2 ml. of 0.04 M fructose 6-phosphate and 2 ml. of saturated (at 0°C) Veronal buffer (pH 7.8) were added 10 µl. of the tissue fraction. The reaction was carried out at 37°C. Immediately after the addition of the cytoplasmic suspension, and at intervals of 4 or 5 minutes thereafter, 0.1-ml. aliquots of the incubation mixture were withdrawn and added to 3 ml. of 30 per cent HCl, for fructose determination. An aliquot of 1.0 ml. was also taken from each incubation tube for protein determination.

Removal of fructose 6-phosphate was constant for a period of not less than 10 minutes. Less than 3 per cent loss in enzymatic activity was observed when the tissue preparation was stored for 5 days.

6-Phosphogluconate Dehydrogenase—This enzyme was assayed by a modification of the method of Glock and McLean (11), in which the rate of TPNH formation is measured. Twenty µl. of the liver preparation were added to a cuvette containing 3 ml. of 0.25 M glycylglycine buffer (pH 7.6), 0.1 ml. of 0.1 M MgCl2, and 25 µl. of 0.2 M 6-phosphogluconic acid. The reaction was started by the addition of 100 µl. of a 1.0 per cent TPN solution. A blank, run simultaneously, was identical in composition to the above sample except for the omission of the 6-phosphogluconic acid. The rate of reduction of TPN was followed at 340 mµ in the Beckman model DU spectrophotometer. The difference between blank and sample was linear, with respect to time, for at least 20 minutes.

The TPN concentration was 5 times that used by Glock and McLean (11) because we found that, at their recommended level, the rate of TPN reduction was dependent upon the TPN concentration.

6-Phosphogluconate Dehydrogenase—The assay (11) for this enzyme was exactly the same as that for phosphogluconate dehydrogenase except for the addition of 25 µl. of 0.2 M glucose 6-phosphate to the incubation mixture. The resulting rate of TPN production is therefore the sum of the activities of phosphogluconate dehydrogenase and phosphogluconate dehydrogenase. Upon subtracting from this sum the activity previously found for the phosphogluconate dehydrogenase alone, the activity of phosphogluconate dehydrogenase is obtained. No change occurred in TPNH concentration when heat-denatured enzyme was used, nor did optical density change when either TPN or glucose 6-phosphate plus 6-phosphogluconic acid was omitted.

Chemical Analyses

Phosphate—Phosphate was determined by the method of Fiske and SubbaRow (12). Because this procedure calls for sulfuric acid in the color development, this acid was also used for deproteinization. Sulfuric acid, 8 N, was used except when it was important to prevent the hydrolysis of glucose 1-phosphate, in which case 4 N sulfuric acid at 0°C was used for protein denaturation.

Fructose—Fructose was measured by Roe's method (13). It was found, as stated by Stein (14), that the samples do not need deproteination for this determination. Fructose 6-phosphate, as obtained from Schwartz Manufacturing Company, gave a value 0.68 times that of fructose on a molar basis. Umbreit (15) gives 0.61 as the conversion factor.

Glucose—Heparinized blood samples were deproteinated with 10 per cent trichloroacetic acid; the mixture was then centrifuged, and aliquots of the supernatant layer were neutralized with NaOH. The glucose was determined by use of a notatin preparation supplied by Worthington Biochemical Corporation. This procedure is based upon the methods of Keston (16) and Teller (17).

Protein—Protein was determined by a modified tyrosine assay based on the procedure of Lowry et al. (18).

Nucleotide Reduction—Assays of triphosphopyridine nucleotides were determined by following the optical density at 340 mµ in the Beckman model DU spectrophotometer. Herecker and Kornberg's (19) extinction coefficient of 6.22 × 10⁴ cm² per mole was used in the calculations.

RESULTS

Enzyme activity was calculated as micromoles of substrate utilized or as micromoles of a product formed per minute per gm. of liver protein (specific activity), per unit weight of liver, and per unit weight of the animal. The results are expressed as specific activities in Table I.

The significance of the differences among the various diet groups was measured by the rank-sum test (20). This test is valid under more general conditions than is the usual significance test, and has practically the same discriminatory power. A difference is said to be statistically significant if it occurs less than 5 per cent of the time by chance alone.

Phosphoglucomutase—The feeding of the 60 per cent glucose diet did not significantly change the level of activity of this enzyme (Table I). The results of feeding the 60 per cent fructose diet varied, depending upon which measure of enzyme activity was used. The measurement per unit weight of liver showed a significant decrease. The specific activity measure showed a significant decrease from the level observed in the glucose group, but not from the level observed in the "Labration" group.

Glucose 6-Phosphatase—The chief source of carbohydrate in the Labration diet is whole ground wheat, and this diet contains
Glucose diet for the Labration diet resulted in a small increase in the corresponding average for the Labration-fed rats.

Phosphogluconate dehydrogenase and 6-phosphogluconate dehydrogenase are increased by hexose feeding. In the case of the glucose-fed rat, a 6-fold increase in phosphoglucone dehydrogenase activity and a 2-fold increase in phosphogluconate dehydrogenase activity were noted here. The response of these two enzymes to fructose was far greater than that which followed glucose feeding, a 10-fold increase for phosphoglucone dehydrogenase and a 3-fold increase for phosphogluconate dehydrogenase.

A more pronounced response to fructose- than to glucose-feeding was observed, not only in the case of the dehydrogenases but also in two other enzyme systems, i.e. glucose 6-phosphatase and isomerase. The liver removes a major part of absorbed fructose from portal blood (26) and converts it to glucose. The observed increases in isomerase and phosphatase activities in the fructose-fed rat may therefore represent an adaptive response to an augmented burden on the system converting fructose to glucose. At this time we have no explanation for the greater activity levels of the dehydrogenases in the livers of the fructose-fed rat.

**DISCUSSION**

In order to throw light on the mechanism responsible for the changed metabolic behavior of the liver of the fructose-fed animal, we compared the levels of five glycolytic enzymes namely, phosphatase, mutase, isomerase, and the dehydrogenases of glucose 6-phosphate and 6-phosphogluconic acid, in the livers of glucose-fed and fructose-fed rats. No evidence was found for a decline in activity of any of these five enzymes in the liver of the fructose-fed rat. Indeed, in the case of four of the enzymes, the levels of activities in the livers of the fructose-fed rats exceeded those in the glucose-fed rat.

1. The activities of phosphoglucomutase, glucose 6-phosphatase, phosphoglucone isomerase, and the dehydrogenases of 6-phosphogluconate and glucose 6-phosphate were measured in the livers of normal rats that were (a) reared and maintained on an adequate stock diet which contained no free hexoses and in which whole ground wheat was the principal source of carbohydrate, and (b) transferred for 3 days to two synthetic diets in which 60 per cent glucose or fructose constituted the sole carbohydrate source. The values for enzyme activity observed with the stock diet served as a base line for comparison of the glucose and fructose effects.

2. When the hexose-containing diets were substituted for the stock diet, the activities of isomerase and of both dehydrogenases were augmented, but the response to fructose feeding exceeded by far that observed with glucose. Only the fructose diet augmented the phosphatase activity, whereas neither diet affected the mutase activity.

3. An adaptive response in the hepatic system converting fructose to glucose is suggested to explain the increased phosphoglucone isomerase and glucose 6-phosphatase activities in the fructose-fed rats.

4. Since the feeding of the 60 per cent fructose diet failed to bring about a decrease in the activities of any of the five enzymes studied here, the conclusion is drawn that the loss in capacity to utilize glucose observed in the fructose-fed rat results from a decrease in the activity of the hepatic glucokinase system.
from either a decrease in the glucokinase activity of the liver or an interference in the mechanism responsible for transporting glucose to the site of glucokinase activity in the cell.

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REFERENCES

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