Rate of Glycogenesis in Liver of Depancreatized Rat after Parenteral Administration of Glucose and Fructose.* (22869)

ROBERT W. LONGLEY, RONALD J. BORTNICK, AND JOSEPH H. ROE
Department of Biochemistry, School of Medicine, George Washington University, Washington, D.C.

Previous work(1) has shown that fructose forms glycogen in the liver faster, and to a greater degree, than does glucose when fructose and glucose are injected intraperitoneally into normal fasted rats. These findings suggested that it would be of interest to extend the same method of studying glycogenesis to totally depancreatized rat following administration of these sugars. Treadwell and Roe, Jr., published a procedure for total pancreatectomy of the rat(2). When evaluated by histological studies, determination of amylase content of areas which the pancreas normally occupies, and glucose tolerance tests, it was found that this operation removes essentially all of the pancreas. This operative procedure was used for the studies reported. Since it was desired to study the effect of absence of insulin on glycogenesis in a large number of rats, it was important to establish criteria of the presence of marked diabetes. Only animals conforming to the following criteria were placed in the experiment: (1) Polyuria (20 to 50 cc of urine/day) in fed rats; (2) Glucosuria in fed rats, as indicated by a 3 or 4 plus Benedict's qualitative test; (3) animals receiving fructose were given a glucose tolerance test previous to glycogenesis experiment, and sugar tolerance of animals receiving glucose was tested during the glycogenesis experiment by determining a control and a one-hour post-injection blood sugar value. Only animals were accepted for the experiment which showed a one-hour post-injection blood sugar level above 200 mg% following injection of 2 g of glucose/kg body weight. Fasting blood sugar level could not be used as a criterion of the presence of experimental diabetes. This is indicated by the data of Table I, in which are shown blood sugar levels of depancreatized rats at different intervals during a 30-hour fast. Within 18 hours after withholding food, 4 of the 6 animals had a normal blood sugar level. Animal No. 5 on other occasions showed blood sugar levels of 80 and 89 mg% after a 24-hour fast. The blood sugar of all animals returned to hyperglycemic levels in 12 hours after food was allowed again. It appears that normal blood sugar level of fasted depancreatized rat corresponds to the presence of a low liver glycogen value. In 27 depancreatized rats that had a normal blood sugar level after a 24-hour fasting period and were given saline injections, the average liver glycogen was 83 mg per 100 g of liver. Data in Table II show a lower glycogen level in depancreatized rats than in normal rats upon a Purina chow diet. In these studies the liver glycogen content was about one-fourth that in normal animals. However, some glycogen was formed in the liver in absence of the pancreas. It is suggested that these results are probably due to absence from the alimentary tract of pancreatic digestive enzymes that split carbohydrates and proteins.

Methods. White rats from the same stock as used in previous studies(1), weighing 150 to 200 g, were depancreatized. Two to 4 weeks after the operation, the animals were fasted for 24 hours. At the end of the fast they received an intraperitoneal injection of

TABLE I. Blood Sugar Level over a 30-Hour Fasting Period. Depancreatized rats were removed from Purina chow. Values expressed as mg %.

Hr of fasting	Animal No. 1	2	3	4	5	6
0	450.0	193.5	228.0	453.3	413.1	331.8
6.25	399.0	103.5	234.6	339.3	330.6	190.5
14.75	390.3	80.2	95.6	106.5	235.0	75.5
18.0	356.7	72.7	95.2	63.0	338.5	69.0
21.0	360.9	62.8	106.4	65.5	354.2	76.9
24.0	317.4	55.3	112.2	56.1	348.2	82.6
30.0	176.9	60.9	114.6	65.4	302.8	71.2
Returned to food 12.0	485.0	215.7	344.1	463.2	444.9	343.8

* Supported by a grant from N.I.H., U.S. Public Health Service.

TABLE II. Effect of *Ad Libitum* Feeding on Liver Glycogen. Animals were removed from an ample supply of Purina chow checkers, anesthetized, and whole liver removed for analysis.

	No. of animals	Avg wt, g	Avg liver wt, g	Avg glycogen value, mg %	Avg blood sugar, mg %
Normal	5	270	11.11	3454.3	130.9
Depancreatized	5	180	8.10	895.8	392.0

10% solution of glucose or fructose at dose level of 2 g/kg body weight. At different intervals after administration, the animals were anaesthetized with nembutal and the whole liver was quickly removed and homogenized in 5% trichloroacetic acid. The glycogen was determined by the anthrone method of Carroll, Longley and Roe(3). Control animals received saline of a comparable volume and treated the same as sugar injected animals. The average liver glycogen value of saline injected controls was subtracted from average liver glycogen value of the sugar injected animals.

Results. Liver glycogen values following administration of glucose and fructose, plotted against time, are shown in Fig. 1. It can be seen that fructose forms liver glycogen in the depancreatized rat more rapidly, and to a greater extent, than does glucose under the same conditions. However, glucose

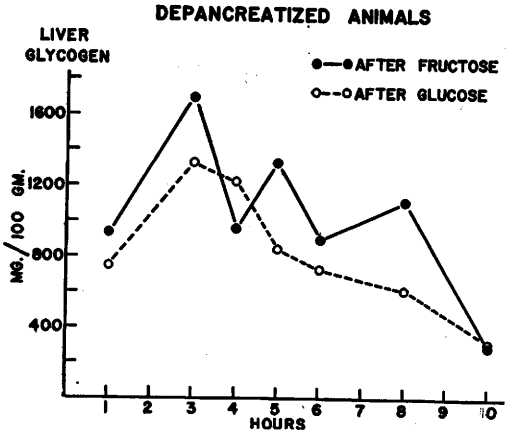

FIG. 1. Liver glycogen formation in depancreatized rats following intraper. inj. of fructose and glucose. Curves represent values from a total No. of animals as follows: fructose, 30 rats; glucose, 33 rats.

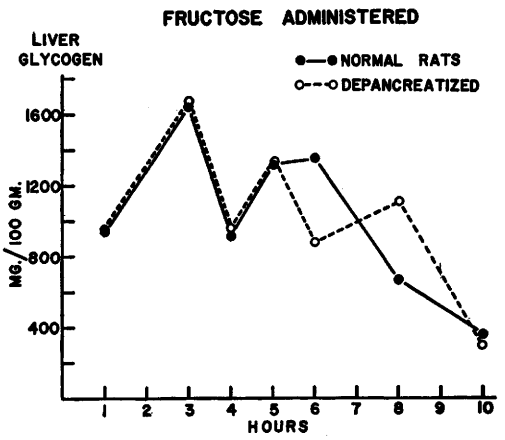

FIG. 2. Comparison of rates of glycogenesis in normal and depancreatized rats following intraper. inj. of fructose. Curves represent values from a total No. of animals as follows: normal rats, 66; depancreatized rats, 30.

does form liver glycogen in the depancreatized rat at a considerable rate.

Fig. 2 shows a comparison of response to fructose administration in the normal and the depancreatized rat. The response is strikingly similar during the first 5 hours and is essentially the same during later hours after administration. It is interesting to note that the drop in liver glycogen curve at the 4-hour point, as observed with normal rats, was repeated in the case of depancreatized animals.

Fig. 3 shows a comparison of response to glucose injection in the normal and the depancreatized rat. It can be seen that the depancreatized animals formed liver glycogen at essentially the same rate as that observed with the normal animals, when glucose was administered intraperitoneally.

Discussion. The results obtained with fructose in the depancreatized animal were not surprising in view of considerable evidence indicating that fructose utilization is not impaired in the absence of insulin(4-7).

It is generally agreed that insulin is essential for the formation of liver glycogen from glucose. It has also been claimed that insulin promotes hexokinase activity by opposing inhibition of this enzyme by the anterior pituitary(8). Our results with experimentally diabetic rats indicate that the metabolic processes in the liver leading to formation of

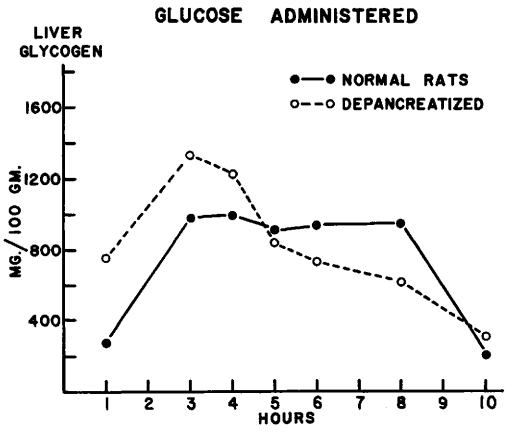

FIG. 3. Comparison of rates of glycogenesis in normal and depancreatized rats following intraper. inj. of glucose. Curves represent values from a total No. of animals as follows: normal rats, 46; depancreatized rats, 33.

glycogen are not directly influenced by insulin. In fact, our studies lead to the suggestion that insulin may have an effect on glycogenesis which is contrary to the generally accepted view, namely, it may decrease the rate of glycogenesis in the liver by diminishing the level of hyperglycemia that results from administered glucose. This interpretation seems applicable since an increase in blood sugar to hyperglycemic levels is a stimulus to glycogenesis, though the blood sugar level is probably not the only determining factor. This view is supported by the work of Cori, who found that injection of insulin into normal rats markedly diminished formation of glycogen in the liver following oral administration of glucose and fructose(9).

Summary. 1. Following intraperitoneal injection, fructose formed liver glycogen more rapidly, and to a greater extent, than glucose in depancreatized rats. 2. When injected intraperitoneally, glucose formed liver glycogen as rapidly in depancreatized rats as in normal rats. 3. Absence of the pancreas from the otherwise intact rat showed no apparent effect upon activity of any of the enzymes involved in formation of glycogen from fructose or glucose in the liver. 4. The data obtained suggest that the metabolic processes in the liver involved in formation of glycogen from fructose or glucose are not directly influenced by insulin.

1. Longley, R. W., Shirkey, A. L., Alvig, O. H., and Roe, J. H., PROC. SOC. EXP. BIOL. AND MED., 1955, v90, 510.

2. Treadwell, C. R., and Roe, J. H., Jr., *ibid.*, 1954, v86, 878.

3. Carroll, N. V., Longley, R. W., and Roe, J. H., *J. Biol. Chem.*, 1956, v220, 583.

4. Chernick, S. S., and Chaikoff, I. L., *ibid.*, 1951, v188, 389.

5. Miller, M., Drucker, W. R., Owens, J. E., Craig, J. W., and Woodward, H., Jr., *J. Clin. Invest.*, 1952, v31, 115.

6. Smith, L. H., Ettinger, R. H., and Seligson, D., *ibid.*, 1953, v32, 273.

7. Dolger, H., Kupfer, S., Bookman, J. J., and Carr, J., *ibid.*, 1953, v32, 562.

8. Price, W. H., Cori, C. F., and Colowick, S. P., *J. Biol. Chem.*, 1945, v160, 633.

9. Cori, C. F., *ibid.*, 1926, v70, 582.

Received November 1, 1956. P.S.E.B.M., 1957, v94.