tissues. Palmitic acid incorporated the highest amount of acetate-C^{14} in all the phospholipid classes. Smaller, but significant amounts of this precursor were also utilized in the synthesis of 8,11,14-eicosatrienoic, arachidonic and docosapentaenoic acids by the testes.

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Autoregulation of Glucose Metabolism in the Isolated Perfused Rat Liver.* (32410)

EDWARD F. MCCRAW,† MICHAEL J. PETERSON, AND JAMES ASHMORE
Department of Pharmacology, Indiana University School of Medicine, Indianapolis

Between 1850 and 1853, Claude Bernard described glycogen as a temporary storage form of carbohydrate, and the ability of the liver to maintain blood glucose levels in the absence of carbohydrate in the diet(1). Soskin in 1938 refined these observations and demonstrated that the dog liver would take up glucose when perfused with hyperglycemic blood, and would release glucose when the blood sugar was low(2). Searle and Chaikoff, using the technique of measuring glucose concentration and specific activity of C^{14} glucose in the blood(3), found that hyperglycemia inhibited glucose production. These observations have been reconfirmed in both animals and man(4-7).

In the present study autoregulation of glucose production and utilization was investigated using the isolated perfused rat liver. Livers from normal fasted, alloxan diabetic, and adrenalectomized rats were perfused with media containing glucose concentrations varying from 0 to 1000 mg/100 ml. Both glucose production and utilization were determined.

Materials and methods. Albino, male rats of the Wistar strain, obtained from Holtzman Co., weighed from 200-250 g. Animals were maintained on lab chow (Ralston Purina Company, St. Louis, Mo.) and water *ad lib.* Normal rats were fasted 24 hours prior to use.

Rats were made diabetic by the injection of alloxan, (Eastman Organic Chemicals, Rochester, N. Y.), 40 mg/kg I. V.; and maintained on 4 units of protamine zinc insulin (Eli Lilly and Co., Indianapolis, Ind.) for 2 weeks prior to use. Insulin was with-

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† Present address: Dept. of Medicine, Univ. of Alabama Med. Center, Birmingham.

drawn 72 hours prior to removal of the liver. All blood sugars were above 300 mg/100 ml in the diabetic rats used. They were not fasted.

Rats were adrenalectomized using pentobarbital anesthesia, and were maintained on 0.9% saline as drinking water and regular lab chow for 3-5 days prior to sacrifice. Food was withdrawn 12 hours prior to removing the liver.

Livers were removed using pentobarbital anesthesia and placed in a Miller perfusing apparatus (Metaloglass Inc., Boston, Mass.). The perfusing fluid was an Umbreit-Ringer medium containing KCl 5 mM, $CaCl_2$ 1 mM, $MgCl_2$ 0.5 mM, NaCl 121 mM and $NaHCO_3$ 25 mM. This medium also contained 4 μc glucose-U-C_{14} (specific activity 15.5 mc/mM), sulfobromophthalein 15 mg, Albumin 3 g (Fraction V, Sigma Chemical Co., St. Louis, Mo.) and heparin 0.8 mg/100 cc of medium. The medium was continuously gassed with oxygen 95%, carbon dioxide 5%.

Perfusion for 30 minutes was allowed for the liver to stabilize. Sodium lactate 20 mM was then added to the medium, and the perfusion continued for 90 minutes. Results were discarded if the flow rate of medium was slow or sulfobromophthalein clearance was below 0.25 mg/g liver/90 min. Samples of medium were taken at time zero, and each 30 minutes and analyzed for glucose, specific activity of glucose, sulfobromophthalein, and lactate.

Glucose was measured using glucose oxidase (8). Glucose C^{14} was measured by converting glucose to the phenylosazome, placed on a planchet and counted on a gas flow counter (9). Lactic acid was determined by the enzymatic method of Hohorst(10) using lactic dehydrogenase and DPN. Subfobromophthalein concentration was determined on an

TABLE I. Total Glucose Production, Net Glucose Production (Output) and Glucose Utilization (Uptake) by Isolated Perfused Rat Livers. (Values are recorded as the mean ($\bar{x}$) and standard error of the mean (SEM) and are expressed as μmoles/g wet liver/90 min).

State of animal	No. of livers perfused	Initial glucose conc, mg/100 ml		Total glucose production, μm/g/90 min	Net glucose production, μm/g/90 min	Glucose utilization, μm/g/90 min
Normal fasted	3	0	$\bar{x}$	41.6	38.8	2.8
			SEM	7.9	7.2	.8
	4	50	$\bar{x}$	27.0	21.5	5.5
			SEM	4.0	4.9	1.1
	4	100	$\bar{x}$	26.2	13.2	13.0
			SEM	4.1	4.3	3.9
	3	180	$\bar{x}$	23.1	8.6	14.5
			SEM	6.2	4.4	1.8
	4	300	$\bar{x}$	21.3	(—)11.9	33.2
			SEM	2.7	4.6	7.1
Diabetic not fasted	4	0	$\bar{x}$	58.5	52.7	5.8
			SEM	17.3	16.4	1.1
	3	100	$\bar{x}$	71.2	57.1	14.1
			SEM	11.7	10.6	7.0
	4	300	$\bar{x}$	21.4	7.7	13.7
			SEM	6.6	5.8	5.5
	4	600	$\bar{x}$	25.8	(—)37.2	63.0
			SEM	9.1	15.7	10.9
	4	1000	$\bar{x}$	27.0	(—)43.2	70.2
			SEM	12.0	5.2	6.8
Adrenalectomized fasted	3	0	$\bar{x}$	16.7	15.9	.8
			SEM	5.6	5.6	.1
	3	50	$\bar{x}$	1.6	(—)8.5	10.1
			SEM	3.5	1.8	5.7
	2	100	$\bar{x}$	8.0	(—)9.6	17.6
			SEM	2.2	6.1	3.5

aliquot of medium which was diluted, alkalinized, and absorption determined on a Beckman DU at 585 mμ.

Results of glucose metabolism were calculated using the following formulae:

(1) Total glucose production = net production + utilization

(2) Net production =

$$\frac{\mu\text{moles glucose 120 min} - \mu\text{moles glucose 30 min}}{\text{Wt of liver}}$$

(3) Utilization =

$$\frac{\text{Total CPM } C^{14} \text{ 30 min} - \text{total CPM } C^{14} \text{ 120 min}}{\text{Specific activity } C^{14} \text{ glucose at 30 min}} \times \frac{1}{\text{Wt of liver}}$$

Initial and final samples of liver tissue were analyzed for glycogen by the method of Good *et al*(11), and the sugars were measured by the method of Nelson as modified by Somogyi(12,13). Net glucose production was corrected for the change in glycogen.

Results. The results expressed as μ moles/g wet liver/90 min. are summarized in Table I. In the normal fasted rats glucose production varied from 41.6 ± 7.9 μ moles/g/90 min using an initial glucose concentration of zero mg/100 ml to 21.3 ± 2.7 μ moles/g/90 min at 300 mg/100 ml initial glucose concentration. Utilization increased from 2.8 ± 0.8 μ moles/g/90 min to 33.2 ± 7.1 μ moles/g/90 min between 0-300 mg/100 ml initial glucose concentration. The point at which total glucose production equalled glucose utilization was at an initial glucose concentration of 200 mg/100 ml.

The rate of glucose utilization in the adrenalectomized rats (Table I) is almost the same as that seen in the normal fasted rat. The most striking change observed in the adrenalectomized rats was the decrease in total glucose production. The point at which glucose production equalled utilization was at an initial glucose concentration of 60 mg/100 ml.

Livers from alloxan diabetic rats exhibited a very high rate of glucose production (58-71 μ moles/g/90 min.) with low initial glucose concentration in the medium (Table I). This is almost twice that found in the normal fasted rats. At an initial glucose concentration of 300 mg/100 ml glucose production in livers of alloxan diabetic rats fell to normal values and remained unchanged with further increases in medium glucose concentration. Glucose utilization in the alloxan diabetic liver was lower than normal with medium glucose concentration below 300 mg/100 ml, and then rose rapidly to very high values at the glucose concentration in the medium was increased. The point at which glucose production equals glucose utilization in the diabetic livers was 340 mg/100 ml.

Discussion. The initial glucose concentration at which glucose production equalled glucose utilization in the normal fasted rats was 200 mg/100 ml. This figure is in the same range as that observed by other investigators. Cahill *et al*(14) found a value of 150 mg/100 ml in the perfused dog liver, and Miller(15) reported a value of 200 mg/100 ml in the isolated perfused rat liver. The plasma glucose in normal rats is maintained between 140-160 mg/100 ml. Thus, under basal conditions it might be expected that glucose production would equal glucose utilization by the liver when the perfusion medium glucose was between 140-160 mg/100 ml. In rats starved 24 hours, gluconeogenesis should be stimulated, and the activity of glucokinase decreased between 18-55%(16,17). Both factors would be expected to contribute to the higher cross-over value of 200 mg/100 ml observed in our experiments.

The utilization of glucose observed in adrenalectomized rat livers was almost the same as that of the normal fasted rat. This would agree with the observations of Ashmore *et al*(18) that there is no decrease in the utilization of glucose and in the glucokinase activity(14) in adrenalectomized rats. The primary defect observed in hepatic metabolism following adrenalectomy was a marked decrease in gluconeogenesis from lactate. Exton and Park(19) using the isolated perfused liver, have shown a decreased gluconeogenesis from lactate in adrenalectomized rats. This metabolic block was overcome if fructose was used as the substrate. They concluded that in adrenalectomized rats, there was a metabolic block between lactate and the triose phosphate. Lewis *et al*(20) demon-

strated a decreased ability to form glucose from lactate in adrenalectomized phloridzinnized rats. This decrease was overcome if the animals were treated with cortisone. Landau(21) has reported an increased gluconeogenesis from lactate in liver slices taken from rats treated with cortisone. However, Winternitz *et al*(22) found no difference in metabolism of infused lactate in the adrenalectomized rat and the adrenalectomized rat treated with cortisone. Eisenstein *et al*(23) have reported normal gluconeogenesis from lactate and pyruvate in the isolated perfused liver from adrenalectomized rats. The present study would support the conclusion that adrenalectomy results in a decrease in gluconeogenesis from lactate.

In livers of diabetic rats there is a rapid rise in utilization of glucose between 300-600 mg/100 ml initial glucose concentrations. This rise in utilization of glucose was not accompanied by a change in the glycogen content of liver, and as mentioned above, all results were corrected for changes in hepatic glycogen content. It is unlikely that the change in glucose utilization could be due to the distribution of glucose, since in the rat (24) glucose distribution is almost complete in 8-10 minutes after injection. The first sample used in these calculations was taken 30 minutes after the perfusion was started, and the final sample 120 minutes after the perfusion was started. Furthermore, the liver is freely permeable to glucose(25). Therefore, changes in glucose utilization in these alloxan diabetic rat livers could not be due to changes in glucose penetration into the cell.

The rate of glucose utilization by the liver then must depend on its rate of phosphorylation. The major enzymes normally considered responsible for glucose phosphorylation to glucose-6-phosphate (G-6-P) have been called hexokinases. These have been recently classified by Katzen(26) as type 1 through type 4. The Km (glucose) for types 1, 2 and 3 is 10^{-5}, 10^{-4} and 10^{-6} respectively, which means that these enzymes should be maximally saturated at very low glucose concentrations in the medium (below 18 mg/100 cc). The increased utilization in these diabetic livers could not then be explained by a change in hexokinase activity. Glucokinase (Katzen type 4, Km [glucose] 10^{-2} M) has been shown to be markedly reduced in activity in diabetic livers(27-30). Increased glucose utilization at high glucose concentrations in these diabetic livers cannot be explained on the basis of glucokinase activity.

Rafter(31) reported that glucose could be phoshorylated with inorganic pyrophosphate (PPi) if liver microsomes were added to the incubation medium. Hass *et al*(32) reported that glucose-6-phosphatase (G-6-Pase) could phosphorylate glucose. Nordlie (33-35) has described a microsomal enzyme he called pyrophosphate glucose phosphotransferase which converts glucose to G-6-P in the presence of PPi. He found this enzyme's activity to be increased in diabetic rat livers; this has been confirmed by Fisher and Stetten(38). Nordlie stated (personal communication) that the Km (glucose) for this enzyme was 110-120 mM in untreated alloxan diabetic rats and 90-100 mM in treated rats. Since this Km corresponds to a blood sugar of approximately 1800 mg/100 ml it appeared unlikely that the present observations could be explained on the basis of this enzyme. However, Stetten has recently published data(36,37) which indicate that inorganic pyrophosphatase, pyrophosphate glucose phosphotransferase and G-6-Pase are all the same enzyme. She had incubated PPi and glucose with microsomes varying the glucose concentration and measured the amount of inorganic phosphate (Pi) and G-6-P formed. At a glucose concentration of 0.02 M (corresponds to 360 mg/100 ml) 27% of PPi used goes to form G-6-P. Increased glucose utilization in the diabetic rat livers between 300-600 mg/100 ml could then be explained on the basis of this reaction.

Stetten also has shown(36) that PPi (the substrate) and G-6-P (the product) compete for the same enzyme. At a glucose concentration of 0.05 M (900 mg/100 ml) Stetten found 45% of the PPi used goes to form G-6-P. This might explain our observation of a plateau in glucose utilization between 600-1000 mg/100 ml. At this glucose concentra-

tion glucose and G-6-Pase could be in equilibrium. With our methods we would record no change in utilization when glucose is in equilibrium with G-6-P.

Summary. Rat livers perfused with 20 mM sodium lactate and increasing glucose concentrations from 0 to 1000 mg/100 ml come to a point of equilibrium at which glucose production equals glucose utilization. This occurs with an initial glucose concentration of about 200 mg/100 ml in the normal fasted rat, 60 mg/100 ml for the adrenalectomized rat and 340 mg/100 ml for the alloxan diabetic rat. An increase in glucose utilization was observed in diabetic livers between 300-600 mg/100 ml. This is thought to be due to the action of pyrophosphate phosphotransferase. There was a plateau in glucose utilization in diabetic livers between 600-1000 mg/100 ml initial glucose concentration in the medium, and it is thought this represents an equilibrium between glucose and glucose-6-phosphate.

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