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Carbohydrate Supply as a Regulator of Rat Liver Phosphoenolpyruvate Carboxykinase Activity

Abstract. *Administration of glucose, fructose, and glycerol to fasted rats produced a significant depression of liver phosphoenolpyruvate carboxykinase activity within 4 to 8 hours; galactose and ribose were much less effective. All the compounds yielded appreciable quantities of liver glycogen. The depression of phosphoenolpyruvate carboxykinase activity by glucose and glycerol was diminished by the concomitant administration of 2-deoxyglucose. The latter depressed glycogen formation from administered carbohydrate in muscle but not in liver. In rats made diabetic by alloxan, depression of elevated phosphoenolpyruvate carboxykinase activity by insulin was dependent upon a dietary source of carbohydrate. These results were interpreted to indicate that depression of certain gluconeogenic enzymes after carbohydrate ingestion is initiated by the metabolism of carbohydrate in some extrahepatic site.*

Homeostatic mechanisms in animals maintain blood sugar within a narrow range except when assaulted by massive carbohydrate intake or when endocrine balance is disturbed. It has frequently been proposed that part of the regulation may be mediated by induction and repression of enzyme formation (*1*) like that so well documented for microorganisms (*2*). In the case of the enzymes involved in gluconeogenesis by rat liver, the "adaptive" changes of enzyme activity are readily demonstrable, but whether these changes are the cause or result of altered rates of carbohydrate synthesis is not yet established (*3, 4*).

Table 1. Effect of various hexoses, glycerol, and ribose on liver phosphoenolpyruvate carboxykinase activity and glycogen in rats fasted 24 hours. At completion of the fasting time, a solution containing 0.5 g of the compound was administered orally every 2 hours. After 8 hours, the animals were killed, and liver samples were assayed for enzyme activity and glycogen. Numbers in parentheses represent number of rats. Means and their standard deviation are given in columns 2 and 3.

Compound administered	PEP carboxykinase (nmole • min^{-1} • mg of $protein^{-1}$)	Glycogen (%)
None (9)	94 ± 5.6	0.012 ± 0.02
Glucose (5)	52 ± 8.3	5.7 ± .02
Fructose (4)	53 ± 13.6	
Glycerol (4)	37 ± 7.6	4.9 ± .10
Galactose (7)	73 ± 9.7	3.7 ± .07
Ribose (4)	87 ± 2.4	1.1 ± .05

This is an extension of previous reports (*5, 6*) on the influence of dietary or parenterally administered carbohydrate on rat liver phosphoenolpyruvate (PEP) carboxykinase activity. Male rats (*7*), some of which were made diabetic by intravenous injection of alloxan (50 mg/kg), were used in all experiments. Methods for preparing the supernatant fraction resulting from high-speed centrifugation of rat liver homogenates and for assay of the PEP carboxykinase have been described (*8*). Glycogen was determined by the anthrone procedure after the tissue was digested with hot potassium hydroxide and the glycogen was precipitated with ethanol (*9*). Protein was determined by the biuret method (*10*).

Glucose, fructose, and glycerol decreased the PEP carboxykinase activity in fasting rats, whereas galactose was less effective (Table 1). Extending the experiments to 12 hours or more brought out an increased effect of galactose which may have resulted from its gradual conversion to glucose in the liver. Although ribose yielded appreciable quantities of glycogen in the liver, it did not depress enzyme activity. Thus, there appears to be no inverse relationship between liver glycogen concentration and the activity of this gluconeogenic enzyme (*6, 11*).

The results showing little depression of PEP carboxykinase activity with galactose and ribose indicated that suppression of gluconeogenesis may possibly be initiated in peripheral tissue such as muscle, because neither of these compounds is metabolized there to any extent, if at all. As a test of this hypothesis, glucose and glycerol (the latter is metabolized primarily in the liver) were used in conjunction with 2-deoxy-D-glucose (2-DOG), a potent inhibitor of carbohydrate metabolism (*12*). The effectiveness of glucose and glycerol in depressing PEP carboxykinase activity is diminished by the concomitant administration of 2-DOG (Table 2). In that large amounts of liver glycogen were found in all experiments, including glycerol plus 2-DOG, there is apparently no impairment of carbohydrate formation or utilization in this organ. In the presence of 2-DOG, carbohydrate formed from glycerol in the liver was probably not effective in depressing hepatic PEP carboxykinase activity. Somewhat surprising were the amount of liver glycogen found when 2-DOG was administered alone and the increment brought about by 2-DOG in rats given glucose. Although it appeared unlikely that 2-DOG could be incorporated into glycogen, this possibility was checked directly by hydrolyzing the glycogen to glucosyl units with Diazyme (*13*) and assaying for 2-DOG with the quinaldine reagent (*14*). These analyses indicated that less than 0.1 percent of the hexose units in the glycogen could have been 2-DOG.

From Table 3, it is apparent that 2-DOG does not diminish glycogen accumulation in the liver, but it tends

Table 2. Relation of liver phosphoenolpyruvate carboxykinase to carbohydrate supply, studied with 2-deoxyglucose in animals fasted for 24 hours. The procedure was the same as in Table 1, except that 0.5 g of 2-DOG was given to alternate lots of rats 30 minutes prior to the glucose or glycerol, and the animals were killed 4 hours later. Numbers in parentheses represents number of rats.

Compound administered	PEP carboxykinase (nmole • min^{-1} • mg of protein)	Glycogen (%)
None (6)	108 ± 8.7	0.02 ± 0.01
2-DOG (6)	101 ± 13	1.6 ± .01
Glucose (11)	71 ± 13*	3.2 ± .03
Glucose plus 2-DOG (11)	84 ± 11 †	4.5 ± .08
Glycerol (11)	78 ± 12*	2.8 ± .06
Glycerol plus 2-DOG (11)	97 ± 11‡	2.7 ± .07

* Significantly different from lot 1 ($P < .001$). † Different from lot 3 ($P < .05$) and significantly different from lot 1 ($P < .001$). ‡ Significantly different from lot 4 ($P < .01$) and from lot 5 ($P < .001$).

Table 3. Effect of 2-DOG administration on amounts of glycogen in liver and muscle. Two animals were used for each treatment, which was the same as that described in Table 2.

Compound administered	Glycogen	
	Liver (%)	Muscle (%)
None	0.003, 0.001	0.23, 0.25
2-DOG	1.57, 1.48	0.23, 0.18
Glucose	3.24, 3.05	0.35, 0.41
Glucose plus 2-DOG	3.98, 5.60	0.14, 0.26
Glycerol	1.94, 1.94	0.32, 0.20
Glycerol plus 2-DOG	4.17, 3.51	0.08, 0.23

Table 4. Effect of insulin and actinomycin on the depression of liver phosphoenolpyruvate carboxykinase in rats made diabetic by alloxan. A single subcutaneous injection of 20 units of protamine zinc insulin was given in the late afternoon. At 0 and 6 hours, actinomycin (175 units) was administered intraperitoneally. The animals were killed after 12 hours. The initial glucose concentration in the blood of all diabetic rats exceeded 440 mg/100 ml. Numbers in parentheses represent number of rats.

Compound administered	PEP carboxykinase (nmole · min^{-1} · mg of $protein^{-1}$)
None (5)	217 ± 21
Insulin (5)	61 ± 15
Insulin plus fasting for 12 hours (2)	208
Actinomycin (5)	144 ± 12
Actinomycin plus insulin (5)	90 ± 18

to inhibit the deposition in muscle. These findings can probably be explained best by (i) the inhibition by 2-DOG of glucose transport in muscle (*15*) (by contrast, glucose penetration into liver cells is not dependent on a transport mechanism); and (ii) inhibition by 2-DOG-6-phosphate of hepatic glucose 6-phosphatase (*12*) with consequent diversion of glucose monophosphate from degradation to glycogen formation and accumulation. Two explanations may be offered for the observation that 2-DOG did not inhibit the (presumed) conversion of glycerol to liver glycogen. Liver phosphohexoisomerase may be less susceptible to inhibition by 2-DOG-6-phosphate than the corresponding enzyme in other tissues (*12*), or the concentration of the inhibitory 2-DOG-6-phosphate may be kept low by the activity of glucose 6-phosphatase. We have interpreted these results to indicate that the influence of administered carbohydrate on hepatic PEP carboxykinase may be exerted in tissues other than liver.

Insulin is effective in depressing enzymes related to gluconeogenesis, but the question of its primary action at the gene level remains controversial (*16*). Table 4 presents data which show that insulin depresses PEP carboxykinase activity only when a dietary source of carbohydrate is available. Insulin did not depress the enzyme activity in fasting, diabetic rats. An attempt was made to determine whether insulin could depress PEP carboxykinase activity in the face of blocked RNA and protein synthesis. In some experiments (Table 4), insulin and actinomycin were given alone, and in combination, to diabetic rats with increased PEP carboxykinase activity. There is some indication that actinomycin plus insulin produced less depression than insulin alone, but the fact that actinomycin alone caused significant depression renders this type of experiment inconclusive.

Our studies suggest the possibility that some site other than liver may initiate the effect of glucose on PEP carboxykinase. A humoral factor or metabolite picked up by the liver from the circulation could, in turn, suppress gluconeogenesis. Even more likely, the concentration of circulating amino acids could determine the activity of PEP carboxykinase. Lack of circulating glucose or lack of glucose utilization (in diabetes or when 2-DOG was given) would cause amino acids to be liberated from peripheral tissues when adrenals are intact (*17*); administered gluconeogenic amino acids cause increased activity of PEP carboxykinase even in fasted rats (*4*). Conversely, available glucose would diminish this stimulus to enzyme formation. While our studies do not contradict the many diverse effects produced by insulin, they are consistent with the unitary hypothesis that this hormone works at the cell membrane of responsive tissues to regulate glucose utilization (*18*).

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A Requirement for Two Cell Types for Antibody Formation in vitro

Abstract. *A suspension of mouse spleen cells can be separated into two populations on the basis of their ability or inability to adhere to plastic dishes. It was found that both adherent and nonadherent cells were necessary for the induction of antibody formation to sheep red blood cells in vitro. Exposure of adherent cells to antigen for brief periods of time was sufficient to initiate a maximal in vitro response.*

Antibody synthesis may result from the interaction of two functionally different cell types: one which phagocytizes and "processes" the antigen to provide the stimulus for a second type, the lymphoid cells which synthesize specific antibody (*1*). Unequivocal support for this suggestion has not been obtained from in vivo experiments. The following studies, however, in which mouse spleen cells are cultured in vitro with sheep erythrocytes as antigen after the method of Mishell and Dutton (*2*), provide provisional support for the hypothesis that two functionally different cell types indeed are required for the induction of antibody synthesis to the antigen.

Spleen cell suspensions were prepared by gently teasing apart the spleens of unimmunized DBA/2 Jax mice in cold