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The Effect of Food Deprivation on In Vivo Gluconeogenesis in the Suckling Rat

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Summary

Radioactive pyruvate was administered in trace or substrate concentrations to fed or 24 hr food deprived male and female Wistar rats of 5, 10, 20 and 30 days of age and adults. The formation of glucose-^{14}C 30 min after the administration of pyruvate was higher in fed suckling rats than in adults. Food deprivation increased the rate of glucose-^{14}C synthesis in all groups given a trace concentration of pyruvate and in the adults and 30 days old rats given a substrate concentration of pyruvate. Food deprivation failed to significantly elevate glucose formation in 5, 10 and 20 days old rats given substrate concentrations of pyruvate. The formation of glycogen-^{14}C in the liver was slightly reduced in 5 and 10 days old rats and augmented in 20 days old rats compared to adults. Food deprivation increased liver glycogen formation in 20 and 30 days old rats and in adults, but not in younger animals. The relationship between the rate of gluconeogenesis and the availability of lipids in the rats is discussed. An explanation for the lack of a gluconeogenic response to food deprivation in suckling rats is given based on the level of citrate synthesis from oxalacetate.

Ley-Words: *Suckling-Food Deprivation – Gluconeogenesis – Liver Glycogen*

Introduction

The capacity of rat liver and kidney for gluconeogenesis is acquired during the first few days of extrauterine life (*Ballard and Oliver* 1963, *Ballard and Oliver* 1965, *Yeung and Oliver* 1967, *Zorzoli, Turkenkopf and Mueller* 1969). The *in vivo* rate of gluconeogenesis is extremely low in foetal rats, increases during the first hours after birth (*Ballard* 1971) reaches a maximum between the second and tenth day of life (*Vernon and Walker* 1972b) and decreases thereafter. We observed that the steady state concentration of key metabolites in rat liver and blood changes as the animals grow older (*Aranda, Blázquez and Herrera* 1973). We were surprised to find that fasting increases the liver concentration of citrate in rats 5 and 10 days old, but not in adults (*Aranda, Blázquez and Herrera* 1973, *Herrera and Freinkel* 1968, *Herrera, Knopp and Freinkel* 1969, *Aranda, Montoya and Herrera* 1972). If this increase in citrate concentration was due to an increased coupling of acetyl-CoA with oxaloacetate, then a decrease in availability of oxaloacetate for the reaction catalyzed by pyruvate carboxykinase would be expected to occur. This, in turn, would be expected to retard the increase in gluconeogenesis during fasting in young rats. In order to examine this hypothesis, we studied the effect of fasting on the rate of gluconeogenesis in suckling rats. Both "trace" and "substrate" concentrations of pyruvate were used in order to determine the role of availability of precursor on the rate of gluconeogenesis in rats.

Methods

Male and female rats of 5, 10, 20 and 30 days of age and adult (160-190 gm) female virgin Wistar rats were used. They were housed and killed as described previously (*Aranda, Blázquez and Herrera* 1973). The rats were either given free access to food or deprived of food for 24 hr prior to sacrifice. Pyruvate-3-^{14}C (specific activity 21 mC/mmol) was administered 10, 30 or 60 min prior to sacrifice. Rats of 5, 10 and 20 days of age were injected under the skin on the back while 30 days old rats and adults were injected intraperitoneally. 5 days old rats received pyruvate in either a trace concentration of 1 μC labelled pyruvate or a substrate concentration of 1 μC labelled pyruvate plus 30 μmoles of cold pyruvate in 0.1 ml of saline. 10 days old rats received a trace dose of 1.5 μC labelled pyruvate in 0.1 ml saline, with or without 45 μmoles of cold pyruvate (substrate concentration). 20 and 30 days old rats received a trace dose of 3 μC labelled pyruvate in 0.1 ml saline, with or without 90 μmoles of cold pyruvate (substrate concentration). Adults received a trace dose of 5 μC labelled pyruvate, with or without 500 μmoles of cold pyruvate (substrate concentration).

Glucose-^{14}C levels in the blood and in liver glycogen was analyzed using methods described previously (*Herrera, Knopp and Freinkel* 1969). The specific activity of glucose in blood samples deproteinized with $Ba(OH)_2$-$ZnSO_4$ (*Somogyi* 1945, *Huggett and Nixon* 1957) and in glycogen in aliquots of liver frozen in liquid N_2 (*Good, Kramer and Somogyi* 1933) was determined. The "glucose space" in 5, 10, 20, and 30 days old rats and in adults was taken to be 60%, 45%, 40%, 37% and 30% of body weight, respectively (*Vernon and Walker* 1972a, *Friedmann, Goodman and Weinhouse* 1967).

Results

The incorporation of ^{14}C into glucose was highest in all groups 30 min after administration of pyruvate-3-^{14}C. Furthermore, the greatest differences in ^{14}C incorporation were observed between the groups at 30 min after injection. Therefore, the results obtained 10 min and 60 min after pyruvate administration are

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of limited (little) importance for the present report and will not be considered further.

The level of glucose formation and the specific activity of glucose observed in the blood 30 min after administration of trace or substrate concentrations of pyruvate-3-^{14}C appears in Table 1. The formation of glucose-^{14}C after administration of trace concentrations of pyruvate was significantly higher in the fed 5, 10 and 20 days old rats than in the fed adults ($p < 0.001$). 24 hr of food deprivation significantly elevated the formation of glucose-^{14}C in the 5, 10, and 30 days old rats and adults given trace concentrations of pyruvate, but not in the 20 days old rats.

The formation of glucose-^{14}C after the administration of substrate concentrations of pyruvate was significantly greater in the fed 5, 10 and 20 days old rats than in the fed adults ($p < 0.01$-0.001). 24 hr of food deprivation significantly elevated the formation of glucose-^{14}C in adults given substrate concentrations of pyruvate. As a result, there was no longer a significant difference between 5, 10 and 20 days old rats and the adults given substrate concentrations of pyruvate after 24 hr of food deprivation. Glucose-^{14}C formation was significantly greater in the 30 days old rats given substrate concentrations of pyruvate than in the adults ($p < 0.05$) but the magnitude of the difference was less than that observed between younger rats and adults. In contrast to the effect of food deprivation in younger rats, a significant increase in glucose-^{14}C formation was observed after food deprivation in 30 days old rats given either trace or substrate concentrations of pyruvate.

The specific radioactivity of glucose in the blood of young rats was significantly greater than in adults under all experimental conditions ($p < 0.05$-0.001). The magnitude of specific radioactivity of blood glucose was directly related to the age of the animals; the highest specific radioactivity was observed in the youngest (5 days old) rats. 24 hr of food deprivation lead to a relatively greater rise in the specific radioactivity of blood glucose in adult rats than in younger animals.

The level of ^{14}C incorporation into liver glycogen 30 min after administration of trace or substrate concentrations of pyruvate in the rats appears in Table 2. Compared to fed adults, the incorporation of ^{14}C into liver glycogen was significantly elevated only in fed 20 days old rats given the trace dose of labelled pyruvate. In contrast to the significant increase in ^{14}C incorporation into liver glycogen observed after 24 hr of food deprivation in 20 and 30 days old rats and adults, food deprivation failed to significantly alter the level of ^{14}C incorporation into liver glycogen in 5 and 10 days old rats.

The specific radioactivity of glycogen in the liver of young rats was significantly greater than in adults under all experimental conditions ($p < 0.05$-0.001) except in 20 and 30 days old rats given substrate concentrations of pyruvate. A significant increase in

Table 1. Incorporation of ^{14}C into blood glucose 30 min after administration of trace or substrate concentrations of pyruvate-3-^{14}C to fed or fasted suckling or adult rats

Age	Dietary status	Glucose ^{14}C (% administered counts)		Glucose specific radioactivity (cpm/mg)	
		trace[a] conc.	substrate conc.	trace conc.	substrate conc.
5 days	Fed	16.77 ± 1.15***	15.74 ± 0.91***	30700 ± 3015***	24790 ± 1733***
	24 h-starved	27.05 ± 1.26***	13.22 ± 0.84	69464 ± 5926***	42763 ± 2304***
	P[b]	< 0.001	N.S.	< 0.001	< 0.001
10 days	Fed	11.63 ± 0.72***	12.37 ± 0.30***	16336 ± 1461***	12044 ± 1543***
	24 h-starved	18.53 ± 1.28	13.31 ± 2.64	36252 ± 5530***	25557 ± 6235**
	P	< 0.01	N.S.	< 0.01	N.S.
20 days	Fed	16.03 ± 1.84***	13.03 ± 1.97**	11961 ± 1667***	10753 ± 860***
	24 h-starved	17.11 ± 0.80*	18.15 ± 4.43	18764 ± 1324***	18881 ± 5434*
	P	N.S.	N.S.	< 0.01	N.S.
30 days	Fed	9.00 ± 1.56*	8.10 ± 0.96*	4200 ± 1245*	4501 ± 1046**
	24 h-starved	15.08 ± 0.76	15.26 ± 0.83	12191 ± 1708**	11885 ± 1035***
	P	< 0.01	< 0.001	< 0.01	< 0.01
Adults	Fed	4.87 ± 0.27	5.31 ± 0.46	997 ± 67	860 ± 153
	24 h-starved	12.82 ± 1.16	16.05 ± 1.50	3437 ± 452	3492 ± 137
	P	< 0.001	< 0.001	< 0.001	< 0.001

[a] Mean ± S.E. of 5-6 rats/group

[b] P refers to the differences between fed and fasted groups and astherisks to the difference between each group and the adults under the same dietary status (*** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$)
No astherisk or N.S. = not significant ($P > 0.05$)

liver glycogen specific activity occurred in all groups after 24 hr of food deprivation ($p < 0.05$-0.001). Nevertheless, the highly significant difference in liver glycogen specific activity was maintained between adults and 5 and 10 days old rats after 24 hr of food deprivation ($p < 0.01$-0.001).

Figure 1 shows the total *in vivo* gluconeogenic efficiency in rats. Gluconeogenic efficiency was computed by adding the percent of radioactivity converted to blood glucose and that recovered as liver glycogen 30 min after administration of substrate concentration of pyruvate. In fed rats, gluconeogenic efficiency was highest in 5 days old rats and lowest in adults. 24 hr of food deprivation lead to a significant rise in gluconeogenic efficiency in 20 and 30 days old rats and in adults ($p < 0.05$-0.001 and 0.001, respectively) but no significant change occurred after food deprivation in 5 and 10 days old rats.

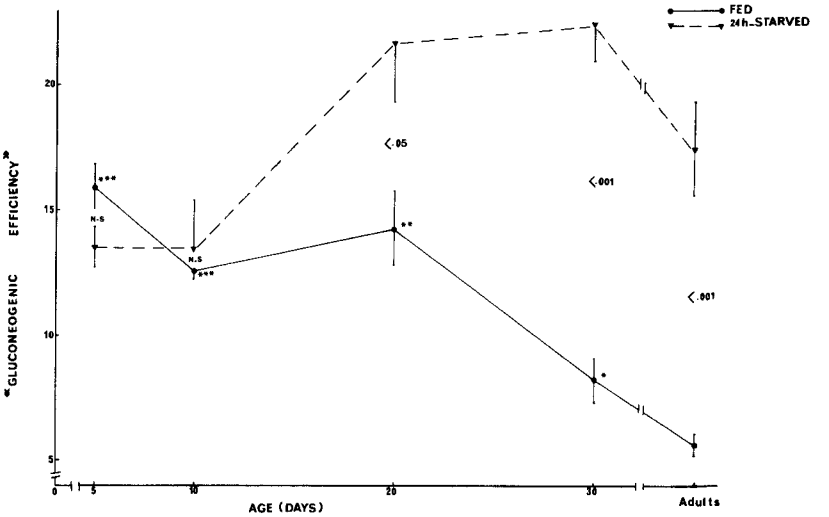

Fig. 1. The gluconeogenic efficiency (defined in text) 30 min after administration of substrate concentrations of pyruvate in fed (circles) or 24 hr food deprived (triangles) 5, 10, 20 and 30 days old rats and in adults. P values between the points refer to the differences between fed and fasted groups and astherisks to the differences between each group and the adults under the same dietary condition: *** = $p<0.001$, ** = $p<0.01$, * = $p<0.05$

Table 2. The incorporation of ^{14}C into liver glycogen 30 min after administration of trace or substrate concentrations of pyruvate-3-^{14}C to fed or fasted suckling or adult rats

Age	Dietary status	Glycogen ^{14}C (% administered counts)		Glycogen specific radioactivity (cpm/mg)	
		trace[a] conc.	substrate conc.	trace conc.	substrate conc.
5 days	Fed	0.279 ± 0.070	0.195 ± 0.027	997 ± 166***	916 ± 191**
	24 h-starved	0.300 ± 0.017***	0.199 ± 0.030*	35184 ± 6869**	22045 ± 4304**
	P[b]	N.S.	N.S.	<0.01	<0.001
10 days	Fed	0.192 ± 0.021	0.182 ± 0.022	232 ± 51**	252 ± 57**
	24 h-starved	0.299 ± 0.049**	0.150 ± 0.013*	31002 ± 3340***	14935 ± 1713***
	P	N.S.	N.S.	<0.001	<0.001
20 days	Fed	1.082 ± 0.279*	1.211 ± 0.485	392 ± 127*	496 ± 214
	24 h-starved	5.910 ± 0.732*	3.650 ± 0.721	9391 ± 481***	4833 ± 1647
	P	<0.001	<0.05	<0.001	<0.05
30 days	Fed	0.412 ± 0.152	0.187 ± 0.042	75 ± 24*	133 ± 214
	24 h-starved	8.314 ± 1.738*	7.293 ± 1.327**	9681 ± 725***	7549 ± 2545
	P	<0.01	<0.01	<0.001	<0.05
Adults	Fed	0.275 ± 0.089	0.341 ± 0.085	11 ± 3	11 ± 2
	24 h-starved	2.580 ± 0.570	1.490 ± 0.471	1625 ± 631	2775 ± 633
	P	<0.01	<0.05	<0.05	<0.01

[a] Mean ± S.E. of 5-6 rats/group.

[b] P refers to the differences between fed and fasted groups and astherisks to the differences between each group and the adults under the same dietary status (*** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$). No astherisk or N.S. = not significant ($p > 0.05$).

Discussion

The present results agree with others showing an enhanced *in vivo* and *in vitro* production of glucose in suckling rats compared to adults (*Ballard and Oliver* 1962, *Ballard and Oliver* 1963, *Ballard and Oliver* 1965, *Yeung and Oliver* 1967, *Ballard* 1971, *Vernon and Walker* 1972). The fact that enhancement of glucose production occurred in suckling rats given substrate concentrations of pyruvate, which "load" gluconeogenic mechanisms and offset variations in availability of endogenous precursor, indicates that the findings are not simply due to differences in the dilution of metabolites. The fact that the difference in glucose production in 5 and 10 days old rats and in adults was observed as soon as 10 min after the administration of trace concentration of pyruvate-3-^{14}C (10.62 ± 1.11, 9.84 ± 0.96 and 5.50 ± 0.76, respectively) suggests that the findings are not simply due to decreased utilization of glucose in suckling rats (*Hahn and Koldvsky* 1966, *Vernon and Walker* 1972a). That the findings are not simply due to differences in glycogen formation in the liver is supported by the fact that 5 and 10 days old rats failed to show an enhanced formation of liver glycogen compared to adults.

It is interesting to note the level of gluconeogenic efficiency in 5, 10 and 20 days old rats. Compared to 5 days old rats, a decrease in gluconeogenic efficiency was observed in rats of 10 days of age followed by an increase in 20 days old rats. These changes parallel the liver fatty acid concentration and blood ketone body level found in 5, 10 and 20 days old rats (*Aranda, Blázquez and Herrera* 1973). This suggest that the rate of gluconeogenesis in rats of these ages is related to the amount of lipids available. Such a relationship between the rate of gluconeogenesis and availability of lipids obtains in rats on a high fat diet (*Blázquez, Castro and Herrera* 1970). Suckling rats may be another example of an enhanced rate of gluconeogenesis in presence of normal or low levels of liver acetyl-CoA (*Aranda, Blázquez and Herrera* 1973). Evidently, once gluconeogenesis is activated, it does not need to be sustained by elevated levels of acetyl-CoA (*Herrera and Freinkel* 1968).

In contrast to older rats, food deprivation failed to increase glucose-^{14}C production in 5 and 10 days old animals. Here again, 5 and 10 days old rats resemble rats on a high fat diet in which food deprivation does not increase gluconeogenesis (*Blázquez, Castro and Herrera* 1970). In contrast to adults (*Herrera and Freinkel* 1968, *Herrera, Knopp and Freinkel* 1969, *Aranda, Montoya and Herrera* 1972), there is a rise in utilization of fatty acids for the formation of ketone bodies and liver citrate in 5 and 10 days old rats (*Aranda, Blázquez and Herrera* 1973). The elevated formation of citric acid in 5 and 10 days old rats may prevent the preferential use of oxalacetate for gluconeogenesis. This, in turn, may prevent the rise in the rate of glucose synthesis from pyruvate in food deprived 5 and 10 days old rats. The inverse relationship observed between the change in the citrate concentration in the liver and the rate of gluconeogenesis in the food deprived young rats is another exception for the concept of citrate driving gluconeogenesis by inhibition of the phosphofructokinase reaction (for a recent review see *Newsholme and Start* 1973).

It is evident that other factors such as resistance to hormonal drive (*Avdalovic, Rukavina and Eberhardt* 1970) and hormonal changes occurring during the suckling period and transition to a solid diet (*Blázquez, Montoya and López-Quijada* 1970, *Blázquez, Sugase, Blázquez and Foà* 1972) may also contribute to the failure of food deprivation to enhance gluconeogenesis in suckling rats.

It is noteworthy that food deprivation enhanced gluconeogenesis in 20 days old rats. Carbohydrate intake in these animals is typically elevated compared to younger rats due to their intake of some solid diet, but their liver is still loaded with fatty acids from the mothers' milk (*Aranda, Blázquez and Herrera* 1973). In spite of this "mixed" situation, 20 days old rats showed an augmented glucose synthesis in the fed state compared to adults. On the other hand, the liver glycogen formation was elevated in 20 days old rats compared to younger animals, probably due to higher plasma insulin levels in older rats (*Blázquez, Montoya and López-Quijada* 1970). The metabolic benefit of the "mixed" situation in 20 days old rats is most evident during food deprivation: both blood glucose and liver glycogen concentrations are maximally preserved (*Aranda, Blázquez and Herrera* 1973) thanks to the enhanced level of gluconeogenesis in presence of an augmented handling of lipids.

Formation of liver glycogen-^{14}C from labelled pyruvate increased in the food deprived 20 and 30 days old rats and in adults. This agrees with our previous observation (*Herrera, Knopp and Freinkel* 1969). Although the mechanism underlying the increased glycogenesis with fasting after pyruvate administration is still unclear, the fact that it is found using both "substrate" and "trace" concentrations suggest that liver glycogenesis proceeds at a higher rate in the fasted state than in the fed state. However, an elevated glycogen concentration is not preserved in the fasted state, due to a concurrent increase in glycogenolysis.

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