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# Effects of Suckling and Food Deprivation on the Activity of Citrate Enzymes in the Liver of the Rat

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Summary

The activity of citrate enzymes in the liver of fed and 24 hr food deprived rats of 5, 10, 20 and 30 days of age was measured. Citrate cleavage enzyme activity was highest in the rats of 5 days of age and lowest in 10 days-old rats. After 24 hr food deprivation, the activity of this enzyme fell in the rats of 5 and 30 days of age, rose in the 10 days-old rats and remained unchanged in 20 days-old animals. Citrate synthase activity was also highest in the 5 days-old rats. It was lowest in the 20 days-old rats. The activity of this enzyme fell in 5 days-old rats, rose in 20 days-old rats and remained unchanged in 10 and 30 days-old animals after 24 hr food deprivation. NADH-linked isocitrate dehydrogenase activity was also highest in the 5 days-old rats. It was lowest in the 30 days-old animals. The activity of this enzyme rose in the 10, 20 and 30 days-old rats and fell in the 5 days-old animals after food deprivation. NADPH-linked isocitrate dehydrogenase activity was highest in the 30 days-old rats and lowest in the 20 days-old animals. Food deprivation lead to a rise in the activity of this enzyme in 20 days-old rats, a fall in 10 days-old rats and no change in the other two age groups. The levels of enzyme activity observed in the rats have been related to changes in the steady state concentration of liver citrate that occur during development in fed and food deprived rats.

**Key-Words:** *Suckling – Food Deprivation – Liver Metabolism – Citrate Enzymes*

## Introduction

We have recently shown that the steady state concentration of liver citrate does not differ between suckling rats and adults (*Aranda, Blázquez and Herrera* 1973). However, food deprivation has different effects on liver citrate concentration in young versus old rats. Liver citrate concentration falls in food deprived normal adults (*Start and Newsholme* 1968, *Herrera and Freinkel* 1968, *Herrera, Knopp and Freinkel* 1969, *Aranda, Montoya and Herrera* 1972, *Aranda, Blázques and Herrera* 1973), but rises in food deprived rats of

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5 and 10 days-old rats (*Aranda, Blázques and Herrera* 1973). The present study of the activity of enzymes intimately related to the metabolism of citrate in the liver of suckling rats was done to investigate the mechanisms responsible for the differences in the effect of food deprivation on liver citrate levels in rats during development.

## Methods

Newborn male and female Wistar rats were maintained with their mothers prior to 24 hr food deprivation or sacrifice as described previously (*Aranda* et al. 1973). The rats were killed by decapitation without anesthesia at 5, 10, 20 or 30 days of age. The liver was removed immediately, weighed, placed in chilled 0.25 M sucrose containing 0.1% Triton X-100 and homogenized in a glass homogenizer with teflon pestle in 99 volumes of the sucrose-Triton medium. The homogenate was centrifuged at 4°C for 10 min at 1500 x gravity. A 1:10 dilution of the supernatant was used as the enzyme source.

Citrate cleavage enzyme (EC 4.1.3.8) was assayed as described by *Takeda, Suzuki and Inoue* (1969) with 5.4 mM ATP, 0.3 mM NADH, 12.2 mM $MgSO_4$, 2.0 mM $MnCl_2$, 2.0 mM $ZnSO_4$, and 0.02 mg malate dehydrogenase in the medium. The reaction was started by the gradual addition of citrate (final concentration (f.c.) 10.7 mM), coenzyme A (f.c. 0.1 mM) and 2-mercaptoethanol (f.c. 5.4 mM). Citrate synthase (E.C. 4.1.3.7.) was assayed as described by *Srere* (1969a) with 0.4 mM aspartate, 0.4 mM alpha-ketoglutarate, 0.4 mM 5.5'-dithiobis-(2-dinitrobenzoic acid) and 0.04 mg glutamic oxalacetic transaminase in the medium. The reaction was started with acetyl-CoA (f.c. 0.5 mM). NADH-linked isocitrate dehydrogenase (EC 1.1.1.41) was determined according to *Plaut* (1969) with 2.1 mM $NAD^+$, 11.2 mM $MgSO_4$, 2.0 mM $MnCl_2$ and 2.0 mM$ZnSO_4$ in the medium. The reaction was started by adding ADP (f.C. 5.4 mM) and isocitrate (f.c. 2.9 mM). NADPH-linked isocitrate dehydrogenase (EC 1.1.1. 42) was assayed by substituting 1.8 mM $NADP^+$ for the 2.1 mM $NAD^+$ used in the NADH-linked isocitrate dehydrogenase reaction mixture.

All assays were performed within 6 hr after removal of the liver. A final volume of 0.7 ml at 25°C and pH 7.5 was used in all assays with 50 mM imidazole and 5 mM EDTA in the medium. An Eppendorf 1101 M spectrophotometer with a multiple recorder was employed to measure absorbence in the enzyme assays. Proteins were determined in the enzyme sources by the method of *Lowry, Rosebrough, Farr and Randall* (1951). Enzyme activity was expressed as nanomoles of substrate converted per min per mg protein at 25°C.

## Results

Table 1 shows the level of activity of citrate enzymes in fed and food deprived rats of 5, 10, 20 and 30 days of age. The highest activity of citrate synthase, citrate cleavage enzyme, and NADH-linked isocitrate dehydrogenase in the liver of the fed animals was observed in the 5 days-old rats. A statistically significant decrease in the activity of these three enzymes occurred after 24 hr food deprivation in the 5 days-old rats. The activity of NADPH-linked isocitrate dehydrogenase was not significantly influenced by food deprivation in the 5 days-old rats.

The lowest activity of citrate cleavage enzyme was observed in the liver of the fed rats at 10 days of age. Food deprivation significantly increased the activity of this enzyme in the 10 days-old rats. Food deprivation also significantly increased the activity of NADH-linked isocitrate dehydrogenase in the 10 days-old rats. The activity of NADPH-linked isocitrate dehydrogenase was significantly decreased by food deprivation, while the activity of citrate synthase was not significantly altered.

The lowest activity of citrate synthase and NADPH-linked isocitrate dehydrogenase was observed in the liver of 20 days-old rats. A significant increase in the activity of these two enzymes followed 24 hr food deprivation in these rats. Food deprivation also significantly increased the activity of NADH-linked isocitrate dehydrogenase in the 20 days-old rats, while the activity of citrate cleavage enzyme remained unchanged.

The lowest activity of NADH-linked isocitrate dehydrogenase was observed in the fed 30 days-old rats. Food deprivation significantly increased the activity of this enzyme in these rats. The highest activity of NADPH-linked isocitrate dehydrogenase was observed

Table 1 Effects of Food Deprivation on Citrate Related Enzymes in the Liver of Suckling Rats

| Age of the rats | Dietary status | nmoles of substrate converted/min/mg protein | | | |
|---|---|---|---|---|---|
| | | citrate cleavage* enzyme | citrate synthetase | NADPH-isocitrate dehydrogenase | NADH-isocitrate dehydrogenase |
| 5 days | Fed | 44.1 ± 3.9 (4) | 61.5 ± 8.7 (4) | 11.4 ± 0.8 (4) | 4.99 ± 0.57 (4) |
| | P** | < .05 | < .05 | N.S. | < .01 |
| | 24 hr food deprived | 24.2 ± 4.3 (4) | 35.7 ± 4.2 (4) | 15.2 ± 1.4 (4) | 2.20 ± 23 (4) |
| 10 days | Fed | 10.9 ± 0.7 (4) | 15.8 ± 1.1 (4) | 24.4 ± 2.8 (4) | 1.09 ± 0.41 (4) |
| | P | < .05 | N.S. | < .05 | < .05 |
| | 24 hr food deprived | 15.8 ± 1.5 (4) | 14.9 ± 1.3 (4) | 12.5 ± 4.9 (4) | 2.32 ± 0.24 (4) |
| 20 days | Fed | 16.1 ± 1.0 (6) | 6.3 ± 1.1 (6) | 1.7 ± 0.4 (6) | 0.63 ± 0.18 (6) |
| | P | N.S. | < .001 | < .001 | < .001 |
| | 24 hr food deprived | 15.5 ± 2.2 (5) | 16.3 ± 1.9 (6) | 33.1 ± 1.7 (6) | 5.4 ± 0.3 (6) |
| 30 days | Fed | 30.9 ± 2.2 (6) | 13.0 ± 1.6 (6) | 34.6 ± 2.4 (6) | 0.6 ± 0.1 (5) |
| | P | < .001 | N.S. | N.S. | < .001 |
| | 24 hr food deprived | 15.4 ± 1.0 (6) | 14.3 ± 2.3 (6) | 37.3 ± 1.6 (6) | 3.2 ± 0.6 (6) |

*Mean ± S.E.: ( ) = number of animals / group; ** P refers to the differences between fed and fasted groups; N.S. = not significant, i.e. $p > 0.05$

in the 30 days-old rats and food deprivation failed to significantly alter the activity of this enzyme in these rats. A significant decrease in the activity of citrate cleavage enzyme followed food deprivation while the activity of citrate synthase remained unchanged in the 30 days-old rats.

## Discussion

It is evident that the activity of citrate enzymes *in vitro* cannot be directly extrapolated to *in vivo* conditions. Nevertheless, the present findings can be related to the changes in the steady state concentration of citrate that occur in the liver in rats during development. The metabolic changes during development are associated with the change in the rat's diet from mother's milk, a high-fat, high-protein and low-carbohydrate food (*Dymsza, Czajka and Miller* 1964), to laboratory chow, a high-carbohydrate, low-fat diet.

The citrate concentration in the liver of 5 days-old rats is lower than that found in animals of 10, 20 and 30 days of age (*Aranda, Blázquez and Herrera* 1973). The low level does not appear to be due to a decreased capacity for citrate synthesis in 5 days-old rats, since their liver citrate synthase activity is higher than in older animals. It does appear, however, to be related to an augmented utilization of citrate due to the elevated activity of citrate cleavage enzyme and NADH-linked isocitrate dehydrogenase. On the other hand, the elevated activity of citrate synthase in the 5 days-old rats suggest that their tricarboxylic acid cycle turnover rate may be increased (*Srere* 1969b).

A balance between citrate synthesis and utilization in the 10 and 20 days-old rats can account for the maintenance of their liver steady state citrate concentration at the same level as in 30 days-old rats. For example, the activity of both citrate synthase and NADPH-linked isocitrate dehydrogenase is low in the liver of the 20 days-old rats. Thus, the low activity of the enzyme responsible for the synthesis of citrate is compensated by low activity of an enzyme involved in citrate utilization in the 20 days-old rats. This may lead to a steady state citrate concentration in the 20 days-old rats comparable to that in the 30 days-old rats.

Food deprivation increases the liver citrate concentration in 5 days-old rats (*Aranda, Blázquez and Herrera* 1973). This can be accounted for by the marked decrease in citrate cleavage enyzme and NADH-linked isocitrate dehydrogenase that accompanies and compensates for the decrease in citrate synthase activity in the food-deprived 5 days-old rats. Food deprivation also increases the liver citrate concentration in 10 days-old rats (*Aranda, Blázquez and Herrera* 1973). In contrast to the 5 days-old rats, the activity of both citrate cleavage enzyme and NADH-linked isocitrate dehydrogenase increase in 10 days-old food deprived animals. However, this appears to be compensated by a decrease in the activity of NADPH-linked isocitrate dehydrogenase so that the liver concentration of citrate rises in the 10 days-old rats. It is interesting to note that other metabolic parameters such as gluconeogenesis (*Yeung and Oliver* 1967), pyruvate kinase activity (*Marshall, Middleton and Walker* 1972), and hormonal sensitivity (*Avdalovic, Rukavina and Eberhardt* 1970) differ markedly between 5 and 10 days-old rats.

Food deprivation fails to alter the liver citrate concentration in 20 days-old rats (*Aranda, Blázquez and Herrera* 1973). In these rats, the increased activity of citrate synthase is compensated by an increase in the activity of the two isocitrate dehydrogenase enzymes. This appears to maintain the liver citrate concentration at the same level after food deprivation as in the fed state. This unique situation may be related to the fact that 20 days-old rats consume both mother's milk and laboratory chow. It is interesting to note that plasma insulin levels rise (*Blázquez, Montoya and Lopez-Quijada* 1970), food deprivation increases the rate of gluconeogenesis (*Aranda and Herrera* 1973) and lipid handling is still augmented in rats of 20 days of age. The relationship of these changes to the activity of citrate enzymes in the liver of 20 days-old rats remains to be elucidated.

Food deprivation decreases the liver citrate concentration in 30 days-old rats. It has a similar effect in adult rats (*Aranda, Blázquez and Herrera* 1973, *Aranda, Montoya and Herrera* 1972, *Herrera and Freinkel* 1968). This is to be expected because at 30 days of age the suckling period is over and the rats consume the same diet as adults. The effect of food deprivation on the activity of citrate enzymes is similar in 30 days-old rats and adults (*Srere* 1972).

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# Intracellular Transport and Storage of Newly Synthesised Proteins in the Guinea Pig Pancreatic A Cell

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Summary

The pathway and time course of the intracellular transport of new synthesised proteins in the guinea pig pancreatic A cell has been determined by electron microscopic radioautography. Protein biosynthesis occurs in the elements of the rough surfaced endoplasmic reticulum, the newly synthesised proteins being transferred via transitional areas to the Golgi complex where they may be present from 20-50 minutes after labelling. Storage granules containing newly synthesised proteins are found at 50 or 120 minutes after the end of pulse-labelling. The cellular mechanisms for the biosynthesis, intracellular transport and storage of proteins in the A cell apparently closely resemble those previously described in the B cells of rats and guinea pigs.

**Key-Words:** *Protein Synthesis – Pancreatic A Cell – Guinea Pig – Intracellular Transport*

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## Introduction

The pathways and time course of the intracellular transport of newly synthesised proinsulin and insulin in the pancreatic B cell have been determined by radioautography in the adult rat ( *Howell, Kostianovsky and Lacy* 1969), the newborn rat in tissue culture (*Orci, Lambert, Kanazawa, Amherdt, Rouiller and Renold* 1971); and in the adult guinea pig (*Howell* 1973). The results obtained in these experiments have been correlated with biochemical studies of the subcellular distribution of newly synthesised proteins and of the conversion of proinsulin to insulin in the B cells (*Howell and Lacy* 1971, *Kemmler and Steiner* 1971, *Orci* et al. 1971, *Sorenson, Steffes and Lindall* 1971).

Until recently, however, little attention has been devoted to protein synthesis in the pancreatic A ($A_2$ or $\alpha_2$) cell. As part of a systematic study of the