

## Metabolites in the Liver, Brain and Placenta of Fed or Fasted Mothers and Fetal Rats\*

E. Herrera and N. Freinkel

Cátedra de Fisiología General, Facultad de Biología, Universidad de Barcelona, Spain and Center for Endocrinology, Metabolism and Nutrition and the Departments of Medicine and Biochemistry, Northwestern University Medical School, Chicago, Illinois, USA

### Summary

Steady state concentrations of acetyl-CoA and citrate were determined on the 19th day of gestation in liver, brain and placenta from fed or 48 h food deprived pregnant rats and fetuses. Acetyl-CoA concentration in the liver of the pregnant rat increased with fasting while it did not change in fetal liver or the placenta. Citrate content in the liver of the mother decreased with starvation while it did not change in fetal liver. Citrate concentration increased in placenta and fetal brain, but not in maternal brain. The lack of changes in the concentration of acetyl-CoA and citrate in fetal liver may be related to the limited diversion of this structure to fat metabolism during dietary deprivation. Contrariwise, the increased concentration of citrate in the placentas and the brains of the fetuses from fasted mothers may reflect their capacities to utilize lipid products as oxidative fuels during maternal starvation.

**Key-Words:** *Starvation – Food Deprivation – Fat Metabolism – Acetyl-CoA – Citrate – Fetus – Placenta – Liver – Brain – Ketone Bodies*

### Introduction

During late pregnancy, the continued growth of the fetus necessitates an uninterrupted extraction of nutrients from the mother. Maternal oxidative requirements are met by an enhanced rate of fat metabolism during pregnancy (Knopp, Herrera and Freinkel 1970). Fuel regulation may become particularly precarious in the fasted state. Maternal catabolism becomes exaggerated so that there is a supranormal rise of circulating free fatty acids (FFA), ketones and gluconeogenic activation coincident with a reduction in plasma glucose to hypoglycemic levels (Herrera, Knopp and Freinkel 1969) and a manifest hypoaminoacidemia (Metzger, Hare and Freinkel 1971). We have designated this metabolic response of the mother to dietary deprivation late in pregnancy as "accelerated starvation" (Freinkel 1965).

\*Supported in part by research grant AM 10699 and training grant AM 05071 from the National Institute of Arthritis and Metabolic Diseases, Bethesda, Maryland, U.S.A., and by a grant from the Kroc Foundation.

The present study was carried out to evaluate whether such "accelerated starvation" and altered availability of fuels is attended by changes in the steady-state levels of key metabolites within certain maternal and fetal tissues. Acetyl-CoA and citrate, key metabolites in the interactions of carbohydrate and lipids metabolism, were selected for study because their concentrations in liver are known to change very dramatically with fasting in different experimental conditions (Herrera and Freinkel 1968, Herrera, Knopp and Freinkel 1969, Aranda, Montoya and Herrera 1972, Aranda Blazquez and Herrera 1973, Montoya and Herrera 1974).

### Materials and Methods

Female Wistar rats were mated at two months of age and sacrificed by decapitation on day 19 of gestation. Prior to sacrifice, they were given either continuous access to standard food pellets or deprived of all food but not drinking water for the preceding 48 h. Placentas, livers and brains were excised from the mother and fetus and frozen within 12 seconds after sacrifice by placing them between aluminium clamps precooled with liquid N<sub>2</sub>. The frozen tissues were powdered and extracted with 6% HClO<sub>4</sub> (Herrera and Freinkel 1968) for the estimation of acetyl-CoA (Herrera and Freinkel 1967) and citrate (Moellering and Gruber 1966). Aliquots of the frozen tissues were also analyzed for DNA-phosphorus (Schmidt and Thannhauser 1945, Fiske and Subbarow 1925), phospholipid-phosphorus (Folch, Lees and Sloane Stanley 1957, Fiske and Subbarow 1925) and neutral lipids (NL) esterified fatty acids (Duncombe 1964) as described elsewhere (Herrera and Freinkel 1968, Aranda et al. 1972). In view of the effects of pregnancy and starvation on cell size (Herrera et al. 1969, Herrera and Freinkel 1968) the data were expressed as concentration per micromole DNA-phosphorus.

### Results

The data appear in Table 1. Forty-eight hours of food deprivation in the 19 day gravid mother rats caused the conceptus free body weight to fall and the liver DNA-phosphorus concentration to rise significantly. Food deprivation led to a rise in the content of acetyl-CoA in the maternal liver and a fall in the concentration of citrate, as we have reported previously (Herrera et al. 1969). Contrary to this picture, neither the fetus body weight and liver DNA-phosphorus concentration nor the placenta weight and DNA-phosphorus concentration were altered by

Table 1. Effect of 48 h of food deprivation on metabolism in the mother, fetus and placenta in the 19 day pregnant rat. Values shown are means  $\pm$  S.E.M. There were 8-14 animals/group.  
\* =  $p < 0.01$ , \*\* =  $p < 0.001$  (food deprived vs. fed groups).

	Body Weight gm	Liver DNA phosphorus $\mu$ moles/gm	Liver Ace- tyl-CoA nmoles/ $\mu$ mol DNA-P	Liver Citrate nmoles/ $\mu$ mol DNA-P	Brain DNA phosphorus $\mu$ moles/gm	Brain Citrate $\mu$ moles/ $\mu$ mol DNA-P
Mother Fed	281 $\pm$ 4	6.0 $\pm$ 0.1	7.5 $\pm$ 0.5	100.1 $\pm$ 16.3	1.8 $\pm$ 0.2	74.6 $\pm$ 12.1
Mother Food deprived	221 $\pm$ 6**	8.2 $\pm$ 0.2**	11.0 $\pm$ 0.5**	21.9 $\pm$ 3.3**	1.7 $\pm$ 0.2	77.9 $\pm$ 24.1
Fetus Fed	2.1 $\pm$ 0.1	25.1 $\pm$ 0.9	0.66 $\pm$ 0.08	17.1 $\pm$ 2.4	16.9 $\pm$ 1.0	12.0 $\pm$ 1.4
Fetus Food deprived	1.9 $\pm$ 0.1	28.0 $\pm$ 1.4	0.78 $\pm$ 0.08	18.3 $\pm$ 2.4	16.4 $\pm$ 1.8	28.5 $\pm$ 1.8**
	Weight gm	DNA-phos- phorus $\mu$ moles/gm	Acetyl-DoA nmoles/ $\mu$ mol DNA-P	Citrate nmoles/ $\mu$ mol DNA/P	NL esterified fatty acid $\mu$ moles/ $\mu$ mol DNA-P	Phospholipid-P $\mu$ moles/ $\mu$ mol DNA-P
Placenta Fed State	0.47 $\pm$ 0.02	4.68 $\pm$ 0.74	1.60 $\pm$ 0.44	40.4 $\pm$ 6.8	2.24 $\pm$ 0.10	3.90 $\pm$ 0.09
Placenta Food deprived State	0.47 $\pm$ 0.01	5.22 $\pm$ 0.29	1.48 $\pm$ 0.28	61.6 $\pm$ 4.2*	3.88 $\pm$ 0.29**	3.55 $\pm$ 0.10

fasting of the mother. In addition, concentrations of acetyl-CoA per  $\mu$ mole DNA-P in fetal liver and placenta were much lower than in maternal liver and were not altered after fasting the mother for 48 hours. Such fasting failed to produce a significant change in the concentration of citrate in the fetal liver while the citrate level rose significantly in the placenta, instead of falling as in the mother's liver.

Previous investigators noted a rise in muscle citrate levels under conditions that lead to a heightened diversion to products of fat metabolism (*Parmegiani and Bowman* 1963, *Angielski and Szutowicz* 1967). This introduced the possibility that the rise in citrate in the placenta of fasted rats might also be associated with an enhanced diversion to products of fat metabolism. To investigate this, the concentration of NL esterified fatty acids and phospholipids were evaluated in the placenta (Table 1).

A highly significant increase in the neutral fat content of the placenta was observed whereas the concentration of phospholipids did not disclose any significant change.

In view of the presence of enzymes for ketones utilization in brain (*Williamson, Bates, Pages and Krebs* 1971) and the increased availability of ketones in the fetal circulation during maternal dietary deprivation (*Scow, Chernick and Smith* 1958) the concentration of citric acid in the fetal brain was examined. Citric acid levels within fetal brain increased

significantly with fasting while that in the brain of the mother failed to be significantly affected by food deprivation (Table 1).

## Discussion

We have previously shown that glucose oxidation by human placental slices diminishes in the presence of extracellular FFA and that an increased proportion of assimilated glucose is used for the formation of glyceride-glycerol under such circumstances (*Freinkel* 1965). The glyceride-glycerol is retained as neutral lipids rather than phospholipids. In the present studies, we have documented that placental steatosis can also be elicited *in vivo* by increasing extracellular FFA via maternal fasting during late pregnancy.

We have shown that this increase in placental esterified fatty acids is confined to neutral lipids and that it is attended by a concurrent increase in the placental content of citric acid. The citric acid changes in the placenta differ from the concurrent alterations in the maternal liver. Herein as we have reported previously (*Herrera et al.* 1969) and confirmed in the present studies, the increased accumulation of neutral fat that occurs after 48 hours of maternal dietary deprivation during late pregnancy, is accompanied by a fall in citric acid. In the maternal liver, the diminished concentration of citric acid has been ascribed to an obtuned Krebs cycle and an heightened ketogenesis. By analogy

to other structures (*Parmeggiani and Bowman* 1963, *Angielska and Szutowicz* 1967) one may suggest that the concurrent increased content of citric acid in the placenta reflects an enhanced oxidation of FFA, and perhaps ketones, especially since the capacity of the placenta to oxidize FFA has been well documented (*Robertson, Sprecher and Karp* 1971). Thus citric acid metabolism may be viewed as yet another parameter by which the placenta does not conform to *Claude Bernard's* early designation of it as the "fetal liver" (*Hagerman* 1964, *Villee* 1960). What about the concurrent changes in the fetus? Starvation of the mother did not appear to modify metabolites (and by inference, pathways of metabolism) in the fetal liver. This is what one would expect since the liver in the fetus presumably does not contain mechanisms for the utilization of large amounts of ketones as fuel (*Lookwood and Bailey* 1971). However, the ketones could subservise important fuel functions in other parts of the fetus. The elevation of citrate concentration found in the fetal brain from fasted rats suggests that some such utilization may indeed have supervened. This agrees with the finding of an increase in the enzymes for the utilization of ketone bodies in the fetus when the mother's ketone bodies concentration is elevated (*Dierks-Ventling* 1971, *Thaler* 1972). The precise portions of the fetal brain in which this occurs and whether it correlates with focal accumulations of citrate remain to be elucidated.

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Requests for reprints should be addressed to: Prof. E. Herrera, Cátedra de Fisiología General, Facultad de Biología, Universidad de Barcelona, Barcelona-7 (Spain)