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

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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).

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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 preceeding 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_2. The frozen tissues were powdered and extracted with 6% $HClO_4$ (*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 ± 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 μmoles/gm	Liver Acetyl-CoA nmoles/μmol DNA-P	Liver Citrate nmoles/μmolμ DNA-P	Brain DNA phosphorus μmoles/gm	Brain Citrate μmoles/μmol DNA-P
Mother Fed	281 ± 4	6.0 ± 0.1	7.5 ± 0.5	100.1 ± 16.3	1.8 ± 0.2	74.6 ± 12.1
Mother Food deprived	221 ± 6**	8.2 ± 0.2**	11.0 ± 0.5**	21.9 ± 3.3**	1.7 ± 0.2	77.9 ± 24.1
Fetus Fed	2.1 ± 0.1	25.1 ± 0.9	0.66 ± 0.08	17.1 ± 2.4	16.9 ± 1.0	12.0 ± 1.4
Fetus Food deprived	1.9 ± 0.1	28.0 ± 1.4	0.78 ± 0.08	18.3 ± 2.4	16.4 ± 1.8	28.5 ± 1.8**
	Weight gm	DNA-phosphorus μmoles/gm	Acetyl-DoA nmoles/μmol DNA-P	Citrate nmoles/μmol DNA/P	NL esterified fatty acid μmoles/μmol DNA-P	Phospholipid-P μmoles/μmol DNA-P
Placenta Fed State	0.47 ± 0.02	4.68 ± 0.74	1.60 ± 0.44	40.4 ± 6.8	2.24 ± 0.10	3.90 ± 0.09
Placenta Food deprived State	0.47 ± 0.01	5.22 ± 0.29	1.48 ± 0.28	61.6 ± 4.2*	3.88 ± 0.29**	3.55 ± 0.10

fasting of the mother. In addition, concentrations of acetyl-CoA per μmole 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 (*Parmeggiani 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 subserve 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.

References

Angielski, S., A. Szutowicz: Tissue content of citrate and citate-cleavage enzyme activity during starvation and refeeding. Nature (London) 213: 1252-1253 (1967)

Aranda, A., E. Blázquez, E. Herrera: Liver components, blood glucose and ketone bodies in fed and starved suckling rats. Horm.Metab.Res. 5: 350-355 (1973)

Aranda, A., E. Montoya, E. Herrera: Effects of hypo- and hyperthyroidism on liver composition, blood glucose, ketone bodies and insulin in the male rat. Biochem.J. 128: 597-604 (1972)

Dierks-Ventling, C.: Prenatal induction of ketone-body enzyme in the rat. Biol.Neonate 19: 426-433 (1971)

Duncombe, W.G.: The colorimetric micro-determination of long-chain fatty acids. Biochem.J. 88: 7-10 (1963)

Fiske, D., Y. Subbarow: The colorimetric determination of phosphorus. J.Biol.Chem. 66: 375-400 (1925)

Folch, J.M. Lees, G.H. Sloane Stanley: A simple method for the isolation and purification of total lipids from animal tissues. J.Biol.Chem. 226: 497-509 (1957)

Freinkel, N.: Effects of the conceptus on maternal metabolism during pregnancy. In: On the Nature and Treatment of Diabetes. B.S. Leibel and G.A. Wrenshall, editors. Excerpta Medica Foundation, Publishers, Amsterdam 1965, 679-691

Hagerman, D.D.: Enzymatic capabilities of the placenta. Fed.Proc. 23: 785-790 (1964)

Herrera, E., R.H. Knopp, N. Freinkel: Carbohydrate metabolism in pregnancy. VI. Plasma fuels, insulin, liver composition, gluconeogenesis and nitrogen metabolism during late gestation in the fed and fasted rat. J.Clin.Invest. 48: 2260-2272 (1969)

Herrera, E., N. Freinkel: Interrelationships between liver composition plasma glucose and ketones and hepatic acetyl-CoA and citric acid during prolonged starvation in the male rat. Biochim.Biophys.Acta 170: 244-253 (1968)

Herrera, E., N. Freinkel: Internal standards in the stimulation of acetyl-CoA in liver extracts. J.Lipid Res. 8: 515-518 (1967)

Knopp, R.H., E. Herrera, N. Freinkel: Carbohydrate metabolism in pregnancy. VIII. Metabolism of adipose tissue isolated from fed and fasted pregnant rats during late gestation. J.Clin.Invest. 49: 1438-1446 (1970)

Lockwood, E.A., E. Bailey: The course of ketosis and the activity of key enzymes of ketogenesis and ketone-body utilization during development of the postnatal rat. Biochem.J. 124: 249-254 (1971)

Metzger, B., J.W. Hare, N. Freinkel: Carbohydrate metabolism in pregnancy. IX: Plasma levels of gluconeogenic fuels during fasting in the rat. J.Clin.Endocr. 33: 869-872 (1971)

Mollering, E.A., W. Gruber: Determination of citrate with citrate lyase. Anal.Biochem. 17: 369-376 (1966)

Montoya, E., E. Herrera: Effect of streptozotocin on liver composition and blood glucose, ketone bodies and insulin in the fed and fasted male rats. Hormone Res. 5: 29-38 (1974)

Parmeggiani, A., R.H. Bowman: Regulation of phosphofructokinase activity by citrate in normal and diabetic muscle. Biochem.Biophys.Res.Comm. 12: 268-273

Robertson, A., H. Sprecher, W. Karp: Oxidation of palmitate by human placental tissue slices. Physiol.Chem. and Physics 3: 293-301 (1971)

Schmidt, G., S.J. Thannhauser: A method for determination of deoxyribonucleic acid, ribonucleid acid and phosphoproteins in animal tissues. J.Biol.Chem. 160: 83-89 (1945)

Scow, R.O., S.S. Chernick, B.B. Smith: Ketosis in the rat fetus. Proc.Soc.Exp.Biol.Med. 98: 833-835 (1958)

Thaler, M.M.: Effects of starvation on normal development of β-hydroxybutyrate dehydrogenase activity in foetal and newborn rat brain. Nature (London) 236: 140-141 (1972)

Villee, C.A.: In the placenta and fetal membranes, p. 100, ed. by Villee, C.A., Baltimore, Md.: Williams and Wilkins 1960

Williamson, D.H., M.W. Bates, M.A. Page, H.A. Krebs: Activities of enzymes involved in acetoacetate utilization in adult mammalian tissues. Biochem.J. 121: 41-47 (1971)

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