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Spontaneous Development of Gluconeogenesis in Fetal Rat Livers during Incubation in Organ Culture (39449)

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Gluconeogenesis, one of the most important functions of the rat liver, develops rapidly after birth but remains dormant in the fetus (1-3). It is not clear whether this is because the enzymatic machinery, though present, is suppressed or because *de novo* synthesis of gluconeogenic enzymes is initiated only after birth. Much of the available information has been accumulated with fetuses manipulated while being retained in the uterus (4, 5). Investigations with fetal rat liver *in situ* are, however, subject to many uncontrollable variables which make interpretation of some of the data uncertain. Because of the inherent limitations of working with intact fetuses, we have chosen to study fetal rat liver in organ culture in the expectation that this system, being more rigorously defined and easier to control, would provide a model for studies in the development of perinatal gluconeogenesis (6).

In earlier experiments with fetal rat liver explants, we found that gluconeogenesis from alanine spontaneously developed with time during incubation. Our results correlated well with the observation of Mandelli *et al.* (7) and Dodek *et al.* (8) that phosphoenolpyruvate carboxykinase (EC 4.1.1.32, PEPCK) of liver spontaneously increased during organ culture. These findings are consistent with the conclusion that PEPCK is a limiting enzyme in the regulation of gluconeogenesis in the perinatal rat (9). Since the energy needs of the newborn animal are probably met largely by lipid, the development in the liver of alternate gluconeogenic pathways may be expected. In this paper we present evidence that the livers of 20-day-old fetal rats utilize glycerol and serine but not alanine and lactate as substrates for gluconeogenesis. Fetal livers in organ culture convert all substrates to glucose or glycogen.

Materials and methods. Fetuses from anesthetized CFE rats (Carworth Farms, New City, N.Y.) were taken surgically under sterile conditions at 19 to 21 days of gestational age as determined from their weight and crown-rump length (10). Liver fragments were established in culture as described by Wicks (11). The medium was Eagle's minimum essential medium with Hanks' balanced salt solution containing 0.11 *M* glucose and 0.008 *M* sodium bicarbonate (12). Incubation was at 37° under a mixture of 98% air-2% carbon dioxide. Measurement of incorporation of radioactive precursors into glucose and glycogen are as described in a previous paper (13). Explants directly incubated with the radioactive compounds are called "fresh" livers; those incubated in medium for 42 hr, then transferred to fresh medium containing radioactive precursors are called "aged" livers.

Results. In Table I, the incorporation of radiotracer from L-[U-^{14}C]-alanine, L-[U-^{14}C]serine, L-[U-^{14}C]lactate or D-[U-^{14}C]glycerol into glucose and glycogen of freshly isolated and "aged" fetal liver are compared. In "fresh" livers there was almost no incorporation of label from [U-^{14}C]alanine or [U-^{14}C]lactate into glucose or glycogen. Incorporation of label into carbohydrate residues increased significantly after 42 hr of preincubation, in confirmation of our previous results. [^{14}C]alanine and [^{14}C]lactate were about equally incorporated into free glucose. Although [^{14}C]alanine was a somewhat better precursor of glycogen than was [^{14}C]lactate, their overall utilization for gluconeogenesis was not significantly different because glycogen comprised only 5 to 10% of the carbohydrate formed.

[^{14}C]Serine was incorporated into both

TABLE I. INCORPORATION OF RADIOACTIVE PRECURSORS INTO GLUCOSE AND GLYCOGEN BY FETAL RAT LIVER EXPLANTS IN ORGAN CULTURE.[a]

Substrate	Replicates	Preincubation (hr)	Glycogen (mg/g wet weight)	^{14}C in glycogen (dpm/dish)	^{14}C in glucose (dpm/dish)	^{14}C in total carbohydrate (% of dose)
L-[U-^{14}C]alanine	4	0	13.81 ± 4.25	43 ± 3	0	0.004 ± 0.0005*
	7	42	0.79 ± 0.42	250 ± 153	3890 ± 1630	0.36 ± 0.16
L-[U-^{14}C]lactate	4	0	16.1 ± 5.7	104 ± 9	142 ± 115	0.019 ± 0.008*
	4	42	0.93 ± 0.10	101 ± 27	3100 ± 520	0.25 ± 0.14
L-[U-^{14}C]serine	5	0	23.8 ± 1.4	228 ± 104	3249 ± 239	0.16 ± 0.001*
	6	42	0.39 ± 0.07	1060 ± 204	11060 ± 1042	0.62 ± 0.05
D-[U-^{14}C]glycerol	4	0	17.6 ± 5.1	3470 ± 975	21825 ± 805	1.08 ± 0.08
	4	42	0.50 ± 0.12	1887 ± 628	40490 ± 7650	1.80 ± 0.35

[a] Explants were from animals 20 to 21 days of gestational age. Activities added to culture dishes were 0.5 μCi [U-^{14}C]alanine (sp act., 140 Ci/mole); 0.5 μCi of [U-^{14}C]glycerol (sp act, 112 Ci/mole); 0.5 μCi of [U-^{14}C]lactate (sp act, 137 Ci/mole); 1 μCi of [U-^{14}C]serine (sp act, 153 Ci/mole). Final concentration of substrate was 3.4 m*M*. Values shown are means ± standard error.

* $P < 0.005$ for comparison of incorporation of label into preincubated and freshly isolated liver.

glycogen and glucose in fresh livers. The total incorporation was increased about fourfold in aged livers. In the case of [U-^{14}C]glycerol, incorporation of label into total carbohydrate was initially high in fresh liver. Preincubation did not significantly increase gluconeogenesis or the relative distribution of ^{14}C from glycerol into glycogen and glucose. Under all conditions, gluconeogenesis from glycerol was higher than from any of the other labeled molecules studied. It will be noted that preincubation resulted in extensive loss of glycogen from the explants. The remaining glycogen was of high specific activity. Thus, while glycolysis greatly exceeded glycogenesis from glucose, gluconeogenesis appeared to be the predominant mode of glycogen synthesis.

Discussion. We have found, in accord with the observations of others (1–3), that gluconeogenesis does not take place in rat liver during fetal development. However, gluconeogenesis from alanine or lactate is initiated after liver explants are maintained in organ culture for an interval of 42 hr. During this preliminary incubation period important changes occur, as indicated by the increased response of the tissue to steroid hormones (11). The nature of these changes is unknown but may involve in part the destruction of inhibitory hormones and other molecules. It is important to note that, even after preliminary incubation, the rate of gluconeogenesis is much slower in explants than in the intact postnatal rat. Where incorporation of 3-carbon precursors is measured in minutes in the latter, significant incorporation requires hours in the former. The machinery of gluconeogenesis is therefore present in the fetal liver but in a state of rudimentary development.

The behavior of serine and glycerol as gluconeogenic substrates in fresh fetal liver is consistent with the conclusion that the route including phosphoenolpyruvate plays a controlling role in the development of gluconeogenesis from pyruvate, lactate, or alanine. Serine is transformed into glucose in two possible ways: through serine dehydratase to pyruvate or through L-serine:pyruvate aminotransferase to glycerate. The latter route bypasses the pyruvate–phosphoenolpyruvate sequence (15–17). In fresh liver, this pathway predominates; in aged liver, the phosphoenolpyruvate carboxykinase-dependent pathway emerges to provide an additional port of entry, and consequently there is a rise in incorporation of serine into carbohydrate. Recent evidence indicates that hydroxyproline, like serine, is an important gluconeogenic precursor after birth (15). Enhanced utilization of hydroxyproline involves conversion of one of its metabolites through serine to phosphoglycerate and thence to glucose.

In contrast with the effects of incubation

on the incorporation of serine into glucose and glycogen, incorporation of glycerol is initially high in fresh liver and is increased by less than twofold after preincubation. This is because glycerol enters the gluconeogenic pathway at the triose phosphate level through glycerol kinase which is known to be active in fetal liver (18–20) and totally bypasses phosphoenolpyruvate carboxykinase.

The ability of the fetal liver to utilize glycerol more efficiently than other potential substrates may reflect the postnatal requirements. The energy needs of the newborn are probably met largely from lipid. Among the physiological changes consistent with increased lipid metabolism after birth are a rise in plasma free fatty acid, a fall in respiratory quotient from 1.0 to 0.7, and an increase in the level of ketone bodies and glycerol in the plasma. Therefore, glycerol, resulting from hydrolysis of storage and dietary lipid, may be an important substrate for gluconeogenesis (21, 22).

The preexisting enzymes allow the animal to utilize lipid rapidly as a valuable alternative source of glucose. The evidence, therefore, suggests that the appearance of gluconeogenesis after birth is dependent on both the net synthesis of enzymes and the initiation of the expression of preexisting enzymes.

Summary. Freshly isolated fetal liver explants in organ culture did not convert L-[^{14}C]alanine or L-[^{14}C]lactate to carbohydrate, but L-[^{14}C]serine and D-[^{14}C]glycerol were both transformed. When explants were subjected to 42 hr of preliminary incubation without supplements, followed by transfer to fresh medium with added precursor, all four substrates underwent gluconeogenic transformation. It was concluded that the ability of fetal rat liver in organ culture to convert alanine and lactate to carbohydrate evolves slowly, but the conversion of glycerol, and to a lesser extent serine, to glucose and glycogen is initiated immediately.

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