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The Response of Glucose-6-Phosphatase in Human and Rat Fetal Liver Cultures to Dibutyryl Cyclic AMP[1] (37920)

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Addition of dibutyryl cyclic AMP (dAMP) to cultures of fetal rat livers leads to an increase in the activities of phosphoenolpyruvate carboxykinase (PEPK) and tyrosine transaminase (TTA) (1, 2). A similar effect was found for human fetal livers (3). Since the best known effect of dAMP is activation of glycogen breakdown (4), and since this can occur in fetal liver cultures (5), one would also expect an increase in glucose formation via activation or induction of glucose-6-phosphatase (G-6-Pase). An increase in the activity of this enzyme after *in vivo* injection of rat fetuses with glucagon, thyroxine, or dAMP has been reported (6). However, *in vitro,* the only reference to an effect of dAMP on liver G-6-Pase is that by Wicks to his own unpublished work (1). Since it is known that G-6-Pase activity in human fetal liver appears much sooner during gestation and is much higher than in rat fetal liver (7, 8), it seemed of particular interest to compare the two species and to examine whether the enzyme responds in a way similar to PEPK and TTA.

Methods. Fetal human livers were collected under sterile conditions from hysterotomy specimens (therapeutic abortions). Fetal age varied between 8.5 and 15 weeks after conception. Liver pieces were incubated for a period of 24 hr in Eagle's minimum essential medium plus Earle's balanced salt solution (2, 3), and dAMP, prednisolone, glucagon, thyroxine, or a combination of these were added for the last 18 hr of incubation. The concentrations were as follows: dAMP 10^{-4} *M*, prednisolone 10^{-5} *M*, glucagon 10^{-4} *M*, thyroxine 10^{-5} *M*. Cycloheximide (2×10^{-5} *M*) or actinomycin D (1 μg/ml) were added for the same 18-hr period. G-6-Pase was assayed according to (7), fructose 1,6-diphosphatase (FDPase) according to (9), and proteins according to (10). Chemicals were obtained from Calbiochem or Sigma Chemical Co. and the culture mediums from Grand Island Biological Co. Rat fetal livers for culturing were obtained as described previously (2). For each agent tested, at least two, usually three, samples of the same liver were cultured separately.

Results. G-6-Pase activity increases with fetal age in both man and rat (Fig. 1). In both species, addition of dAMP, glucagon, or prednisolone + dAMP raises activity. However, in the rat this effect is not observed in very young and very old fetuses, while in man the effect is apparent in all cases. Prednisolone alone has no effect on G-6-Pase in rat liver but does increase its activity significantly in human fetal liver. It does not, however, have more than an additive effect when given together with dAMP (Table I).

The increase due to dAMP or glucagon is less than 100% and it must be enquired whether it is due to activation or new synthesis of the enzyme. Both cycloheximide and actinomycin D inhibit the effect of dAMP and glucagon (Table I), indicating new enzyme synthesis. Addition of thyroxine was without effect.

Since dAMP addition to the medium

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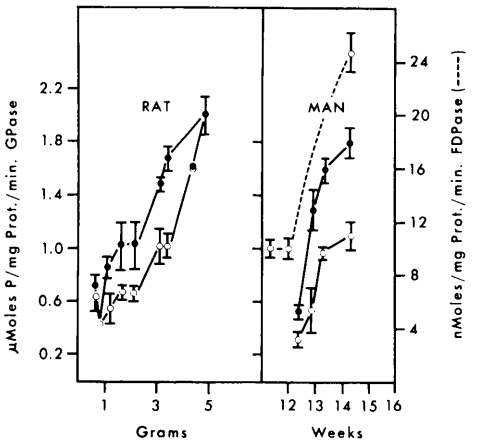

FIG. 1. Developmental changes in glucose-6-phosphatase activity and the effect of dAMP and glucagon in human and rat fetal liver and the changes in fructose 1,6-diphosphatase in human liver.

Each point of the control values (○) is the mean of 3 separate cultures from the same liver. Each point of the values after addition of dAMP and glucagon (●) is the mean of nine separate cultures (3 for dAMP, 3 for glucagon, and 3 for prednisolone + dAMP) from the same liver as the control value for that age or weight. The data for dAMP, glucagon, and prednisolone + dAMP were pooled, since responses to each of these treatments were similar.

raises the activities of several enzymes related to gluconeogenesis (TTA, PEPK, G-6-Pase), its effect on fructose 1,6-diphosphatase, another unidirectional gluconeogenic enzyme, was also examined. No effect of dAMP was noted (Table I).

In contrast to TTA and PEPK, which showed an increase in activity during culturing in human fetal liver (3), culturing for 24 hr had no effect on G-6-Pase and FDPase activities in either human or rat fetal tissues.

Discussion. Our data show that both in the rat and in man G-6-Pase can be induced in fetal liver cultures by dAMP and glucagon and that this effect is probably due to new enzyme synthesis. In the rat, these data are in agreement with those reported by Greengard (6) *in vivo,* except that this author found an effect also in rats 1 day before birth. In addition, in her experiments thyroxine was also effective. Our rat fetal livers, just before term, in fact, behaved like postnatal liver *in vivo,* since 2 days after birth, Greengard found a much higher basal activity, and also no longer found any effect of glucagon or dAMP. Why this difference exists between *in vitro* and *in vivo* effects is difficult to say. Probably, however, in the

TABLE I. Changes in G-6-Pase and FDPase Activities in Human and Rat Liver Cultures.

	Glucose-6-phosphatase (EC 3.1.3.9)		Fructose-1,6-diphosphatase (EC 3.1.3.11)
	Man	Rat[c]	Man
Control	−0.33 ± 9.4 (3)[a]	+5.0 ± 5 (8)	+34.6 ± 29.4 (5)
dAMP	+75.0 ± 10.3 (3)	+39.7 ± 7 (5)	+11.0 ± 18.3 (4)
Glucagon	+39.0 ± 7.0 (2)	+56.2 ± 15 (5)	−12.7 ± 11.4 (3)
Prednisolone	+20.0 ± 1.1 (3)	+3.0	−13.0 ± 12.7 (3)
Prednisolone + dAMP	+81.5 ± 14.1 (4)	+66.5 ± 9.5 (3)	−4.0 ± 18.0 (4)
Thyroxine	−11.0	−1.36 ± 5.8 (3)	
Prednisolone + dAMP + cycloheximide	+10 [66%][b]		
Prednisolone + dAMP + actinomycin D	+26 [59%][b]		
Actinomycin D	+23		

[a] Figures indicate percentage change from values in fresh tissue of the same livers ± SE. Number of fetuses in parenthesis.

[b] Increase in the same fetal liver without cycloheximide or actinomycin D.

[c] The youngest and oldest rat fetuses shown in Fig. 1 have not been included.

in vivo situation other regulatory factors may play a role, e.g., release of norepinephrine in response to injections of thyroxine and perhaps dAMP.

In the human fetus, the situation is somewhat different. In the first place, G-6-Pase is present at adult levels already at midterm (7, 8), i.e., around the 20th week of gestation. Our data were obtained with younger fetuses, in which an age-dependent increase in activity was noted, just as in the fetal rat, and dAMP and glucagon were always effective. No data are available for older fetuses. We are, however, hardly justified in extrapolating from the rat data, since both PEPK and TTA respond differently in fetuses of the two species (2).

Finally, it may be pointed out that in contrast to PEPK and G-6-Pase, FDPase activity in the rat fetal liver is 50% of that in adult animals, i.e., it is relatively much higher than the other two enzymes already in the fetus. Hence, it may be possible that this excess activity is sufficient, even under conditions when gluconeogenesis is accentuated in the fetus, for the extra production of glucose, which probably never attains the high postnatal levels described so often.

Summary. Glucose-6-phosphatase was determined in fetal human and rat livers. It increased in activity with fetal age in both species. Culturing fetal livers for 24 hr in Eagle's essential medium had no effect on the enzyme. Addition of glucagon or dibutyryl cyclic AMP for the last 18 hr of culturing increased enzyme activity significantly in both man and rat. However, in fetal livers from rat fetuses weighing more than 4.6 g, this effect was not observed. Fructose-1,6-diphosphatase in human fetal liver also increased in activity between the 12th and 15th week of gestation, but neither glucagon nor dibutyryl cyclic AMP had any effect in cultures.

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