PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 150, 7-10 (1975)

High Glucose Concentration and Phosphoenolpyruvate Carboxykinase Activity in Human and Rat Fetal Liver Cultures[1] (38962)

PETER DODEK, L. KIRBY, J. FROHLICH, P. HAHN, AND B. HO-YUEN

Departments of Paediatrics, and Obstetrics Gynaecology, University of British Columbia

It has been shown (1) that the rapid postnatal rise in the activity of phosphoenolpyruvate carboxykinase (EC 4.1.1.32 PEPK) in rat liver can be suppressed by insulin, glucose, glycerol, and pyruvate administration. These *in vivo* experiments, however, do not establish whether the effect of glucose is directly on the liver, or whether it is indirect via insulin release and suppression of the release of glucagon. The latter hormone is considered the physiological stimulus for the rapid postnatal synthesis of phosphoenolpyruvate carboxykinase (PEPK) (2).

In *in vitro* cultures of rat fetal livers and hepatoma cells, Wicks (3) showed that insulin inhibited the inductive effect of glucagon or cyclic AMP on PEPK synthesis, while high glucose concentration had no effect. However, the experiments were performed with tissues or cells that had been cultured for at least 24 hr. Mandelli *et al.* (4) and Hahn *et al.* (5) had shown that under appropriate conditions, PEPK activity increases spontaneously during the first 24 hr in culture not only in rat but also in human fetal liver, i.e., without the addition of any "inducer." Hence, it seemed appropriate to examine whether this spontaneous rise in PEPK activity could be inhibited by high glucose concentrations.

Methods. Pregnant Wistar rats (Woodland Farm, Ontario) were sacrificed on the 19th–22nd day of gestation. The fetal livers were removed under sterile conditions, pooled, cut into small pieces, and incubated in Eagle's minimum essential medium with Earl's balanced salt solution under 95% O_2 and 5% CO_2 at 37°, as described previously (6, 7). Human fetal livers were obtained from legal abortions and were treated in the same way. The medium contained 100 mg % glucose. To some flasks, additional glucose was added to a maximum concentration of 918 mg %. Cycloheximide, if added, was present at a final concentration of 3.85×10^{-5} *M*. The final concentration of insulin was 10^{-5} *M*. Part of the fresh liver tissue was immediately homogenized in 0.25 *M* sucrose (1:10 wt/vol) and centrifuged at 100,000*g* at 5° for 30 min in an IEC B50 centrifuge. The supernatant was frozen. After 24 hr of incubation, the rest of the tissue was treated similarly and PEPK activity was determined in the supernatant according to Ballard and Hanson (8). Pyruvatekinase (EC 2.7.1.40) and α-glycerolphosphate dehydrogenase (NADH dependent, EC 1.1.1.8) and citrate cleavage enzyme (EC 4.1.3.8) were also determined according to (9). The method of Lowry (10) was used for protein determination. Cyclic AMP was determined according to (11), by the immunoprecipitation technique. Pregnant rats were killed with a blow on the head. Three fetuses were rapidly removed; their abdomen was opened and the liver rapidly immersed in liquid nitrogen. The livers of the other fetuses were placed into the culture medium incubated for the times indicated and then further treated. The frozen tissue and the incubated tissues were homogenized in absolute ethanol and then treated as described by (11).

Results. First, the effect of a high glucose concentration was examined in 14 litters (Table I) consisting of fetal rats with an average weight of 2 g. As described previously, culturing the liver for 24 hr led to a trebling of PEPK activity and to a decrease in PK and α-GPDH activities. Addition of 918 mg % glucose from the start of culture caused a further increase in PEPK activity in liver from young fetuses but was without effect on the other two enzymes. In human liver cultures, a high glucose concentration had the opposite effect: it decreased PEPK activity by $27 \pm 4.4\%$ ($P < 0.01$). Insulin slightly inhibited the spontaneous rise in

[1] Supported by MRC (Canada).

TABLE I. ENZYME ACTIVITIES IN FETAL RAT LIVER CULTURED FOR 24 HR IN THE PRESENCE OF A HIGH GLUCOSE CONCENTRATION

	PEPK (nmoles)	PK (μmoles)	α-GPDH (μmoles)	CCE (nmoles)
Fresh	1.4 ± 0.24	0.33 ± 0.02	0.17 ± 0.02	16.1 ± 3
Control	4.23 ± 0.43[a]	0.28 ± 0.01[a]	0.09 ± 0.006[a]	3.2 ± 1.2[a]
High glucose (918 mg %)	6.35 ± 0.61[b]	0.27 ± 0.02	0.102 ± 0.01	11.2 ± 2.1[b]

Values are in nmoles or μmoles/mg protein/min at 30° and are the mean of 28 samples obtained from 14 litters, mean body weight 2 g, except for CCE which shows the mean of four samples from two litters, mean weight 4 g.

Fresh: tissue analysed immediately after sacrifice. Control: tissue after 24 hr in 100 mg % glucose.

[a] $P < 0.01$ against fresh.

[b] $P < 0.01$ against control. The pair test was used.

PEPK = phosphoenolpyruvate carboxykinase; PK = pyruvatekinase; α-GPDH = α glycerophosphatedehydrogenase; CCE = citrate cleavage enzyme.

PEPK activity in fetal rat liver cultures and was without effect in human fetal liver (Table II).

It was noted that the effect of glucose depended on the size of the fetus from which the livers were collected. Hence, this was further examined. Figure 1 shows that as the fetus approaches term, PEPK activity after 24 hr in culture is greater. It is also evident that in fetuses smaller than 2.5 g (about the 19th day of gestation) glucose invariably enhances the effect of culturing, while in older fetuses the spontaneous rise in PEPK during culturing is suppressed. Figure 1 also shows that cycloheximide completely inhibits the increase of PEPK activity both in the absence and presence of glucose.

Various concentration of glucose were tested (not shown) and 500 mg % was found to have the same effect as 918 mg %. Lower concentrations were ineffective. In larger fetuses, the high glucose concentration also decreases α-glycerolphosphate dehydrogenase activity and cycloheximide had no further effect. Pyruvatekinase did not react in this way (not shown).

Since it is generally thought that the stimulus for PEPK synthesis is cyclic AMP (2, 3), it must be enquired how far the spontaneous rise in the activity of this enzyme, presumably also due to new synthesis since it is inhibited by cycloheximide, can be accounted for by cyclic AMP formation stimulated by culturing itself or by hormones released during sacrifice of the fetus. It is apparent from Table III that after 10 min in culture, cyclic AMP levels in fetal rat liver have about doubled. After that time, levels decrease again and by 45 min are equal to those found initially. Even 24 hr later, levels do not seem to have changed to any extent. The content of cyclic AMP in the medium after 24 hr of culturing is negligible.

TABLE II. EFFECT OF A HIGH GLUCOSE CONCENTRATION AND INSULIN ON PERCENTAGE CHANGES IN PEPK ACTIVITY IN CULTURED HUMAN AND RAT FETAL LIVER

	n	24 hr Culture	+ Insulin	+ Glucose (750 mg %)
Rat fetus	(6)	100%	72.8 ±6.4%[a]	164 ±20%[a]
(mean wt 2 g)		(6.34 ±1.58)		
Human fetus	(5)	100%	102 ±10%	72.8 ±4.4[a]
(mean age 12 wk)		(5.08 ±0.94)		

n = number of experiments. Figures in parentheses show actual enzyme activity in nmoles/mg prot/min equal to 100%.

[a] = $P < 0.01$ against 100%.

Discussion. Our data show, in agreement with previous work (5), that the spontaneous increase in PEPK activity during culturing of fetal rat liver is at least partly due

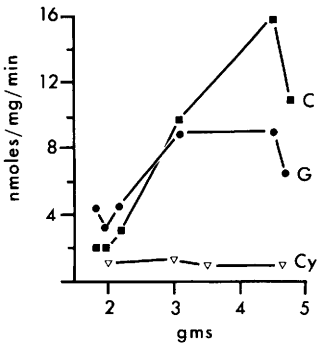

FIG. 1. PEPK activity of rat fetal liver cultured for 24 hr at 37° with 100 mg % (C) or 918 mg % (G) glucose or with 918 mg % glucose and cycloheximide (Cy). Abscissa: weight of fetuses in grams. Each point is the mean of 6–10 experiments. SE not shown (smaller than circles).

TABLE III. CYCLIC AMP CONTENT OF FETAL LIVER TISSUE CULTURED FOR VARIOUS TIME PERIODS *In Vitro*

Mean fetal weight = 3.5 g		
Time in culture	n^a	pmoles/mg wet wt
0	3	0.22 ± 0.04
10 min	3	0.53 ± 0.02
20 min	1	0.19
50 min	3	0.28 ± 0.05
4 hr	3	0.236 ± 0.02
24 hr	3	0.174 ± 0.05

[a] n = number of experiments.

to new protein synthesis since cycloheximide completely inhibits it. It is possible that this rise is initiated by activation of the liver adenylcyclase at the start of the culture, as indicated by the transient rise in cyclic AMP content of incubated liver. High concentrations of glucose in the culture medium increase PEPK activity in the livers of the young rat fetuses. This is somewhat surprising. It must be considered, however, that actual values of PEPK are very low in such livers and that the spontaneous increase during culturing is also lower than in older fetuses. Since liver glycogen concentrations in these younger fetuses are also lower (12), it is possible that supplying extra glucose is equivalent to supplying enough energy to maintain PEPK synthesis and perhaps even increase it slightly. In contrast, the glycogen content of fetal livers from older fetuses is very high (12) and thus tissues do not lack energy during the first 24 hr of culturing.

In older rat fetuses and in human fetuses, glucose had the expected effect. It inhibited the rise in PEPK activity normally observed during culturing. This indicates that glucose or one of its metabolites acts directly on enzyme synthesis by inhibiting it. There is no reason to assume that the effect of glucose is an inhibition of actual enzyme activity. In the same way, it is very unlikely that the stimulating effect of glucose on PEPK activity in young fetal rat livers is an activation of the enzyme. If that were the case, then activity after 24 hr should also be increased in the presence of cycloheximide since this substance, although preventing the increase in activity during culturing does not decrease enzyme activity below the initial level.

Finally, it is of interest to note that cytoplasmic α-glycerolphosphate dehydrogenase activity is inhibited both by high glucose concentration and by cycloheximide in fetal rat liver cultures. In contrast, citrate cleavage enzyme activity is higher if liver is cultured with high glucose concentrations than if it is cultured at the normal glucose concentrations. In this case, however, what is really happening is that the high glucose concentration prevents activity from declining during culturing. All these data indicate that glucose or one or several of its metabolites has direct effects on liver enzymes and that, presumably, *in vivo* this substance does not only act via insulin release but may also act more directly on the liver, as also suggested by others (14).

Summary. In cultures of human and rat fetal liver, phosphoenolpyruvate carboxykinase activity increases during the first 24 hr of culturing. This increase can be suppressed by adding cycloheximide to the culture medium or by adding a high glucose concentration. This, however, applies only to human fetal liver and to fetal liver from rats obtained just before term. In younger rat fetal liver, glucose, on the contrary, increases the activity of phosphoenolpyruvate carboxykinase. A high glucose concentration in the medium also leads to higher

citrate cleavage enzyme activity and to lower α-glycerolphosphate dehydrogenase (cytoplasmic) activity in rat fetal liver cultures.

1. Yeung, D. and Oliver, I. T., Biochem. J. **108,** 325 (1968).
2. Greengard, O., Biochem. J. **115,** 19 (1969).
3. Wicks, W. D., Ann. N.Y. Acad. Sci. **185,** 152 (1971).
4. Mandelli, M., Piceni-Sereni, L., and Sereni, F., Biochim. Biophys. Acta **309,** 519 (1973).
5. Hahn, P., Kirby, L., and Frohlich, J., *in* "Perinatal Pharmacology," Raven Press (in press).
6. Kirby, L. and Hahn, P., Can. J. Biochem. **51,** 476 (1973).
7. Kirby, L. and Hahn, P., Pediat. Res. **7,** 75 (1973).
8. Ballard, F. J. and Hanson, R. W., Biochem. J. **104,** 866 (1967).
9. Bücher, T., Luh, W., and Pette, D. *in* "Handbuch der Physiol. Pathol. Analyse." Springer, Berlin (1964).
10. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R., J. Biol. Chem. **193,** 265 (1951).
11. Tsang, C. P., Lehotay, D. C., and Pearson, Murphy B. E., J. Clin. Endocrinol. Metab. **35,** 809 (1972).
12. Greengard, O., *in* "Biochemical Action of Hormones," Vol. 1, p. 53. Academic Press, New York (1970).
13. Jacquot, R. and Kretchmer, N., J. Biol. Chem. **239,** 1301 (1964).
14. Treadow, B. R. and Khairallah, E. A., Nature New Biol. **239,** 131 (1972).

Received April 7, 1975. P.S.E.B.M., 1975, Vol. 150.