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Changes in hepatic messenger RNA for phospho*enol*pyruvate carboxykinase (GTP) during development

(mRNA translation/wheat germ cell-free system/glucagon/cyclic AMP)

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ABSTRACT **Phospho*enol*pyruvate carboxykinase (GTP) [GTP:oxaloacetate carboxy-lyase(transphosphorylating); EC 4.1.1.32] is absent in rat liver cytosol during fetal life and is synthesized initially at birth. *De novo* synthesis of the enzyme can be induced prematurely by injection of dibutyryl cyclic AMP or glucagon into fetal animals *in utero*. In this study a wheat germ translation assay was used to quantitate the level of total functional mRNA for phospho*enol*pyruvate carboxykinase in the liver of fetal rats at 21 days of pregnancy under different induction situations. The translatable mRNA for the enzyme was marginally detectable in fetal rat liver. Administration of either glucagon or dibutyryl cyclic AMP to fetal rats *in utero* caused a marked induction of functional mRNA for this enzyme. Three hours after administration of dibutyryl cyclic AMP, the level of translatable mRNA increased almost 23-fold, but by 6 hr the level dropped approximately 60%. Administration of actinomycin D prior to dibutyryl cyclic AMP in 21-day fetal rats prevented the appearance of newly synthesized poly(A)-containing RNA in the cytoplasm as well as the induction of translatable mRNA for phospho*enol*pyruvate carboxykinase. In animals delivered prematurely and maintained for varying periods, the translatable mRNA for the enzyme accumulated in the liver at a rate comparable to that observed for enzyme synthesis.**

The mechanisms underlying hormonal regulation of protein synthesis in animal tissues have been studied with various models (1–3). Little is known, however, about how hormones act to control the synthesis of regulatory enzymes. To date, the most extensively studied enzymes in this area are tryptophan oxygenase (4, 5), tyrosine aminotransferase (6, 7), and phospho*enol*pyruvate (*P-enol*pyruvate) carboxykinase [GTP:oxaloacetate carboxy-lyase(transphosphorylating); EC 4.1.1.32] (8–10). All three of these are hepatic enzymes whose levels are controlled by glucocorticoids or cyclic AMP (cAMP). *P-enol*pyruvate carboxykinase develops initially in liver cytosol at birth, completing the enzymes of the gluconeogenic sequence (11). The enzyme can be prematurely induced before birth by injection of glucagon (12) or cAMP (13) to fetal rats *in utero*, suggesting that its appearance at birth is not dependent directly on developmental processes. In a previous study (14), we have shown that the initial appearance of *P-enol*pyruvate carboxykinase in rat liver cytosol at birth is due to a stimulation of enzyme synthesis, which occurs in the absence of detectable rates of enzyme degradation over the first 36 hr after birth. Furthermore, injection of dibutyryl cAMP (Bt_2cAMP) or glucagon into fetal rats *in utero* stimulates *P-enol*pyruvate carboxykinase synthesis (15). Since *P-enol*pyruvate carboxykinase is absent in fetal rat liver and is responsive to premature induction by injection of the animals *in utero* with cAMP, it is one of the best models for determining mechanisms by which the cyclic nucleotide regulates enzyme synthesis. In this study we have determined the levels of mRNA for *P-enol*pyruvate carboxykinase during development and after administration of glucagon and cAMP *in utero*. Our results further strengthen the evidence indicating that cAMP regulates the synthesis of *P-enol*pyruvate carboxykinase by stimulating the initial appearance of mRNA coding for the enzyme in the liver.

MATERIALS AND METHODS

Materials. Reagents were purchased from the same suppliers listed in detail in previous papers (8, 9). Actinomycin D and glucagon were from Sigma Chemical Co., St. Louis, MO. The wheat germ was a generous gift of W. C. Mailhot of General Mills Inc., Minneapolis, MN.

Animals. Pregnant rats at 21 days of gestation and male rats weighing 140 g, from Charles River Breeding Labs. (Wilmington, MA), were fed lab chow (Wayne Lab. Blox, Albred Mills, Inc., Chicago, IL) *ad lib.* Surgical procedures involving pregnant rats (16) were started at 8 a.m. on the 21st day of gestation. The fetuses were injected intraperitoneally through the uterine wall with Bt_2cAMP (1 μmol per fetus) and glucagon (200 μg per fetus) in a total volume of 25 μl. Actinomycin D (100 μg/100 g of body weight) was administered in the same way, but 30 min before Bt_2cAMP injection. For measurements of RNA synthesis, [^{3}H]ortic acid (1.5 mCi/kg of body weight) was injected 60 min before the animals were killed. Control fetuses were injected with 0.154 M NaCl as indicated in the legends to appropriate figures. Prematurely delivered animals were obtained by uterine section by techniques outlined by Yeung and Oliver (17). The average duration of the operation was 5 min. Fetuses or prematurely delivered animals with an average weight of 5 g were killed by cervical fracture. The procedures for treatment of adult, male rats with Bt_2cAMP were as described in detail by Iynedjian and Hanson (8).

Isolation of Poly(A)-RNA and Translation in a Wheat Germ Cell-Free System. Three to 5 g of liver frozen in liquid N_2 immediately after excision was used for the extraction of total RNA. For experiments with fetal animals, 15–24 livers from the litter of two pregnant rats were pooled for each RNA extraction. Prematurely delivered animals were maintained for various times in a humidity-controlled incubator. In total, 10 livers from 8 litters were pooled at various times for a total of 12–15 newly delivered animals for each time point. Total RNA was extracted by phenol/*m*-cresol/water (76:11:13 vol/vol) containing 0.076% (wt/vol) λ-hydroxyquinoline as described by Nienhuis *et al.* (18) and modified by Iynedjian and

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Abbreviations: *P-enol*pyruvate, phospho*enol*pyruvate; cAMP, cyclic AMP; Bt_2cAMP, dibutyryl cAMP; $NaDodSO_4$, sodium dodecyl sulfate.

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Hanson (9). The only exception was that during LiCl precipitation, the duration of stirring was decreased to 1 hr at 4°. The RNA was dissolved in 10 mM Tris·HCl (pH 7.5), heated at 65° for 2 min, adjusted to 0.5 mM NaCl, and rapidly cooled prior to affinity chromatography on oligo(dT)-cellulose (19). The poly(A)-RNA eluted from the column with 10 mM Tris·HCl (pH 7.5) was adjusted to 0.4M NaCl, precipitated at −20° for 18 hr with 2 vol of ethanol, dissolved in water, and stored frozen in liquid N_2. RNA-labeling experiments were carried out as described by Iynedjian and Hanson (8).

The general procedure for the preparation of the wheat germ extract was that of Roberts and Paterson (20) except that, according to Marcus *et al.* (21), centrifugation was at 23,500 × *g* and the preincubation step was omitted. Cell-free protein synthesis was performed in a total incubation volume of 0.61 ml, as described (8, 9). Quantitation of released *P-enol*pyruvate carboxykinase was by specific immunoprecipitation of the high-speed supernatant (105,000 × *g* for 1 hr) followed by sodium dodecyl sulfate ($NaDodSO_4$)/polyacrylamide gel electrophoresis (8–10). Each mRNA translation experiment included (as a reference) a standard poly(A)-RNA isolated from the liver of starved rats. Incorporation of [^{3}H]leucine into *P-enol*pyruvate carboxykinase directed by 20 μg of the reference poly(A)-RNA was 8425 ± 848 dpm, as measured in seven different translation experiments.

RESULTS

Induction of Fetal Rat Liver mRNA Coding for *P-Enol*-pyruvate Carboxykinase by Bt_2cAMP or Glucagon. Since *P-enol*pyruvate carboxykinase synthesis is absent in the livers of fetal rats (14, 15), we posed two initial questions using these animals. First, we determined whether functional mRNA for the enzyme was present in fetal rat liver, and second, whether injection of Bt_2cAMP or glucagon, which prematurely induces *P-enol*pyruvate carboxykinase synthesis *in vivo* (15), can elicit the appearance of template for the enzyme. Previous studies (22) had shown that the stimulatory effect of Bt_2cAMP on *P-enol*pyruvate carboxykinase synthesis in liver cells was not due to the isobutyrate formed upon further metabolism of Bt_2cAMP.

Fetal rats, injected *in utero* with Bt_2cAMP, glucagon, or saline solution, were killed 3 hr later and the poly(A)-RNA was isolated from total liver RNA. The template activity of poly(A)-RNA for *P-enol*pyruvate carboxykinase was determined in the wheat-germ protein-synthesizing system (Table 1). We noted a very low, almost negligible, level of translatable mRNA for *P-enol*pyruvate carboxykinase in the liver of fetal rats. However, mRNA for the enzyme could be induced by *in utero* injection of Bt_2cAMP or glucagon, without changing the total mRNA template activity as measured by [^{3}H]leucine incorporation into both total and released proteins. Bt_2cAMP was more effective than glucagon in inducing the appearance of *P-enol*pyruvate carboxykinase mRNA in fetal rat liver, causing a 23-fold increase as compared to a 10-fold stimulation in the level of enzyme template after glucagon administration. The wheat-germ assay itself was linear with added poly(A)-RNA and would incorporate [^{3}H]leucine into proteins at a linear rate for up to 60 min (data not shown).

Characterization of the products synthesized by the wheat-germ system under the direction of poly(A)-RNA from induced and uninduced animals is shown in Fig. 1, in which the immunoprecipitated enzyme product was separated by $NaDodSO_4$/polyacrylamide gel electrophoresis. The major peak of radioactivity comigrated with *P*-[^{14}C]*enol*pyruvate carboxykinase and was shown previously to copurify with the authentic enzyme (10). The three protein peaks shown in Fig. 1 are, in the order of size starting from the cathode, *P-enol*-pyruvate carboxykinase and the heavy and light chain of the antibody. Template activity for *P-enol*pyruvate carboxykinase of 20 μg of poly(A)-mRNA from the liver of fetal animals was

Table 1. Effect of Bt_2cAMP and glucagon on levels of *P-enol*pyruvate carboxykinase mRNA in fetal rat liver

	Poly(A)-RNA tested, μg	[^{3}H]Leucine incorporated into Total protein*	Released protein*	Enzyme†
Control	10	22.6 ± 0.9	10.9 ± 0.6	0.4 ± 0.1
Bt_2cAMP	10	20.8 ± 1.2	10.8 ± 0.5	8.4 ± 1.0
Glucagon	10	19.4 ± 0.9	11.1 ± 0.7	3.0 ± 0.2
Control	20	36.0 ± 1.2	18.0 ± 1.1	0.7 ± 0.1
Bt_2cAMP	20	35.2 ± 0.8	19.0 ± 1.1	13.5 ± 0.7
Glucagon	20	35.4 ± 1.7	19.1 ± 1.1	4.8 ± 0.2

Poly(A)-RNA was isolated from total tissue RNA extracted 3 hr after injection of Bt_2cAMP (1 μmol/fetus) or glucagon (200 μg/fetus), which were intraperitoneally administered *in utero* to 21-day-old fetuses. Control animals (injected with 0.154 M NaCl) were killed at the same time. Either 10 or 20 μg of poly(A)-RNA was translated in the wheat-germ system (incubation volume, 0.61 ml). Values are dpm in *P-enol*pyruvate carboxykinase or protein for addition of a given amount of poly(A)-RNA after correction for endogenous incorporation. Data are the means ± SEM for six controls and three experiments with either Bt_2cAMP or glucagon. For each RNA extraction, 15–24 fetal livers were used.

* dpm × 10^{-6}.

† dpm × 10^{-3}.

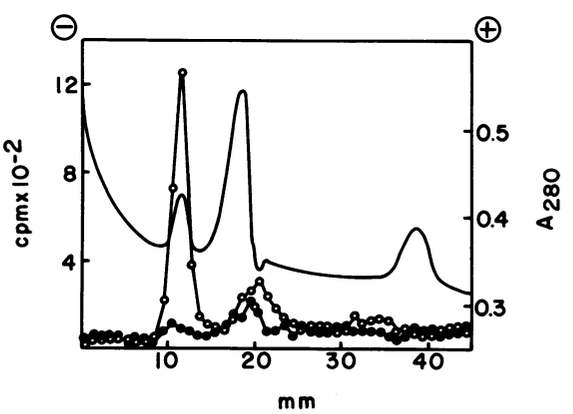

FIG. 1. Isolation of newly synthesized *P-enol*pyruvate carboxykinase by $NaDodSO_4$/polyacrylamide gel electrophoresis. $NaDodSO_4$/polyacrylamide gel electrophoresis of immunoprecipitates obtained after translation of fetal liver poly(A)-RNA in a wheat-germ cell-free system. Poly(A)-RNA, extracted from liver of 21-day-old fetal rats, either controls or treated with Bt_2cAMP, was incubated at 24° in the presence of wheat-germ extract and [^{3}H]leucine as detailed (8). After 90 min of incubation the reaction mixtures were centrifuged at 164,000 × *g* for 1 hr. Supernatants were used for immunoprecipitation of *in vitro*-synthesized *P-enol*pyruvate carboxykinase by a specific antibody with cytosol from the livers of starved rats as a source of carrier enzyme. The immunoprecipitates were washed, dissolved, and analyzed by electrophoresis on cylindrical 11% polyacrylamide gels containing $NaDodSO_4$ as described (8). After electrophoresis, the gels were scanned at 280 nm(−) and extruded in 1-mm fractions into vials containing 3 ml of a liquid scintillation fluid made up of 1000 parts of Formula 949 (New England Nuclear, Boston, MA), 50 parts of NCS tissue solubilizer (Amersham/Searle, Arlington Heights, IL), and 22 parts of 8 M ammonium hydroxide. Radioactivity in immunoprecipitates obtained after translation of 20 μg of fetal rat liver poly(A)-RNA: Bt_2cAMP-induced (○); control (●).

negligible compared with the levels detected 3 hr after administration of Bt_2cAMP. A small second peak, which was precipitated by *P-enol*pyruvate carboxykinase antibody and has a lower molecular weight (about 60,000), was also detected, perhaps due to premature release of peptidyl-tRNA from wheat-germ ribosomes. The quantity of translatable *P-enol*-pyruvate carboxykinase mRNA noted in fetal rat liver is at the lower level of sensitivity of the wheat-germ protein-synthesizing system. Thus it is impossible to state unequivocally that enzyme mRNA is not present in the livers of fetal rats. It is clear, however, that Bt_2cAMP greatly enhanced the translatable mRNA for the enzyme over a very short time period.

Time Course of Induction of *P-Enol*pyruvate Carboxykinase mRNA by Bt_2cAMP: Comparison with Changes in Synthesis Rate of the Enzyme. Administration of Bt_2cAMP to fetal rats *in utero* caused a rapid accumulation of *P-enol*-pyruvate carboxykinase mRNA over the first 3 hr, which paralleled the increase in enzyme synthesis (Fig. 2). Between 3 and 6 hr the level of translatable *P-enol*pyruvate carboxykinase mRNA decreased rapidly, again in parallel with the decrease in the synthesis rate of the enzyme. Thus, whatever the mechanism by which Bt_2cAMP stimulates *P-enol*pyruvate carboxykinase mRNA induction, the effect is transient. Since we observed, in separate experiments (unpublished data), that continued injections of Bt_2cAMP can retard the drop in *P-enol*pyruvate carboxykinase synthesis, it is probable that the continued presence of the cyclic nucleotide is required to ensure the maintenance of high levels of enzyme mRNA.

Development of *P-Enol*pyruvate Carboxykinase in Rat Liver after Birth. In order to test whether the increase in enzyme synthesis observed after birth (14) is related to the appearance of mRNA for *P-enol*pyruvate carboxykinase, we extracted poly(A)-RNA from livers of fetal animals before birth and at several times after birth. This RNA fraction was assayed

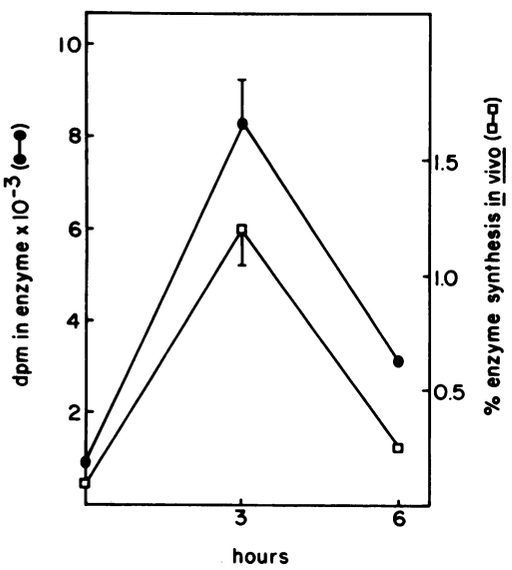

FIG. 2. Effect of Bt_2cAMP on synthesis of *P-enol*pyruvate carboxykinase *in vivo* and of translatable mRNA for the enzyme in the livers of 21-day fetal rats. The synthesis of *P-enol*pyruvate carboxykinase and the level of mRNA for the enzyme in the livers of 21-day-old fetal rats were measured after injection *in utero* of 1 μmol of Bt_2cAMP per fetus. The animals were killed at various times after Bt_2cAMP injection and enzyme synthesis was measured as outlined by Hanson *et al.* (15). The level of translatable mRNA was determined as described. Values for *P-enol*pyruvate carboxykinase mRNA (●) are the means ± SEM for three experiments using, in each experiment, RNA extracted from livers of 15–24 animals. Enzyme synthesis values (□) are from Hanson *et al.* (15).

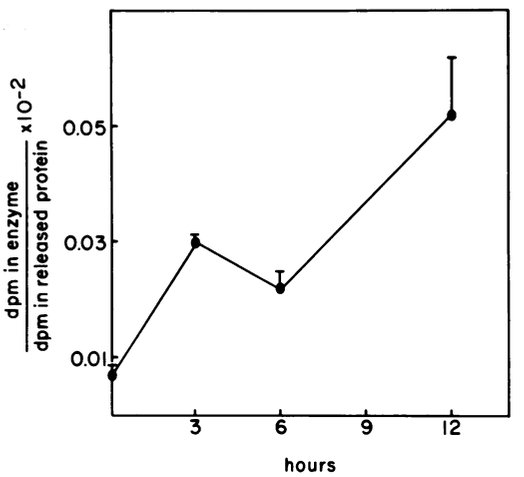

FIG. 3. Time course of development of *P-enol*pyruvate carboxykinase mRNA in rat liver after premature delivery. Eight pregnant rats at 21 days of gestation were surgically delivered and kept in a humidity incubator at 39°. The animals were killed 0, 3, 6, and 12 hr after delivery, the livers were removed, and total RNA was extracted. Each RNA extraction was from animals pooled to comprise 25% of each litter (usually three to four newborns from each litter). Poly(A)-RNA was isolated and *P-enol*pyruvate carboxykinase mRNA assayed in the wheat-germ system with 10 and 20 μg of mRNA as described in the legend of Fig. 1. The radioactivity in total and released protein was similar to that shown in Tables 1 and 2. In this figure, the data are presented as a percentage of the [^{3}H]leucine incorporated into newly synthesized *P-enol*pyruvate carboxykinase relative to that incorporated into released protein at different times after delivery. Values are the means ± SEM for two experiments.

for specific enzyme template activity in a wheat-germ, cell-free protein-synthesizing system. The data shown in Fig. 3 are expressed as a percentage of radioactivity in *P-enol*pyruvate carboxykinase as compared to that in released protein and give a relative measure of the development of the enzyme as compared to other proteins synthesized by the wheat-germ system in response to the addition of 10 μg of poly(A)-RNA. The increase in template activity for *P-enol*pyruvate carboxykinase shown in this figure is not due to a decrease in the template activity for released proteins since the latter did not vary significantly over the 12 hr after birth ($17.4 \times 10^6 \pm 3.4$ dpm at 0 time compared to $20.7 \times 10^6 \pm 0.54$ dpm at 12 hr). However, the mRNA template activity for the enzyme increased 10-fold over this same time period. The rate of accumulation *P-enol*-pyruvate carboxykinase mRNA after birth was not linear. Between 3 and 6 hr, the level of enzyme template decreased slightly before increasing during the next phase, 6–12 hr.

Inhibition by Actinomycin D of *P-Enol*pyruvate Carboxykinase mRNA Induction by Bt_2cAMP. The absence of functional *P-enol*pyruvate carboxykinase mRNA in the livers of fetal rats and its induction in the presence of cAMP suggest that RNA synthesis may be required for the initial appearance of enzyme mRNA. To test this, we injected actinomycin D (100 μg/100 g of body weight) into 21-day-old fetal rats *in utero* prior to Bt_2cAMP. In these experiments we used starved and refed animals induced by Bt_2cAMP and theophylline (8) concurrently as controls. This concentration of inhibitor was sufficient to completely block the appearance of newly synthesized poly(A)-RNA in the cytoplasm in both fetal and adult rats as measured by the incorporation of [^{3}H]orotic acid into RNA isolated by chromatography on oligo(dT)-cellulose columns (Fig. 4). The effect of actinomycin D on the induction of hepatic *P-enol*pyruvate carboxykinase mRNA by Bt_2cAMP, either alone in fetal rats or in combination with theophylline in

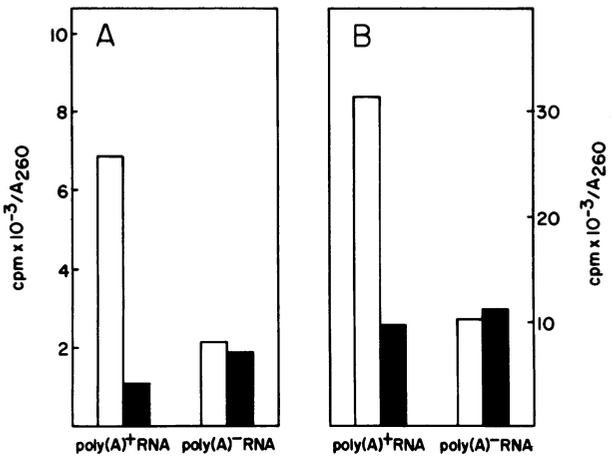

FIG. 4. Effect of actinomycin D on labeling of hepatic RNA in fetal or adult rat liver. Actinomycin D (100 μg/100 g of body weight) or 0.154 M NaCl was administered intraperitoneally 30 min prior to Bt_2cAMP in 21-day-old fetuses or Bt_2cAMP plus theophylline in 24-hr starved and 2-hr refed rats. After 30 min for starved and refed rats and 2 hr for fetuses, [^{3}H]orotic acid (1.5 mCi/kg of body weight) was injected and the animals were killed 1 hr later. The procedure for the extraction and fractionation of poly(A)-RNA and RNA without poly(A) has been reported in detail (8). Open bars, controls; closed bars, actinomycin D-treated. Data are the averages of four litters (*A*) and four starved and refed rats (*B*).

starved and refed adult animals, is shown in Table 2. There is a virtually complete block in the Bt_2cAMP induction of enzyme mRNA in livers of fetal and adult animals. In both groups, the template activity for total and released protein assayed in the wheat-germ system did not change with the actinomycin D treatment.

DISCUSSION

The results of this study extend our understanding of the regulation of hepatic *P-enol*pyruvate carboxykinase by concentrating on the period near birth when the enzyme is being initially synthesized. One major question addressed in this study is whether there is a preexisting pool of functional mRNA for *P-enol*pyruvate carboxykinase in fetal rat liver before birth. If the answer were affirmative, it would be a compelling argument in favor of a translational control of the enzyme synthesis. On the basis of the results of the present study, it is clear that there is no pool of preexisting functional messenger for the enzyme that could account for the rapid induction of the enzyme after administration of cAMP. There are only marginal levels of active mRNA for the enzyme before birth, which increased markedly after birth or after injection of glucagon or Bt_2cAMP into 21-day-old fetal rats. In this case, as was noted earlier by Iynedjian and Hanson (8, 9) with both hepatic and renal *P-enol*pyruvate carboxykinase induction, the change in the level of enzyme mRNA directly parallels the change in synthesis rate of the enzyme. Furthermore, Killewich and Feigelson (23) have also noted a direct relationship between the synthesis of hepatic tryptophan 2,3-dioxygenase and the level of the mRNA after induction of the enzyme by glucocorticoids administered to 4-day-old rats.

Besides the parallelism between the rates of enzyme synthesis and changes in the level of *P-enol*pyruvate carboxykinase mRNA, we observed that the induction of hepatic enzyme mRNA by Bt_2cAMP, either in starved and refed adult animals or in fetal animals *in utero*, can be blocked by cordycepin (8) or actinomycin D (Table 2). Kioussis *et al.* (10) have shown that the administration of cordycepin to starved rats caused an 85%

Table 2. Effect of actinomycin D on induction of *P-enol*pyruvate carboxykinase mRNA in fetal and adult rat liver by Bt_2cAMP

Treatment	Poly(A)-RNA tested, μg	[^{3}H]Leucine incorporated into Total protein*	Released protein*	Enzyme†
Adult				
Bt_2cAMP + theophylline	10	17.3 ± 0.5	4.6 ± 0.2	19.6 ± 0.2
Actinomycin D + Bt_2cAMP + theophylline	10	19.5 ± 2.6	5.2 ± 0.7	2.4 ± 0.1
Fetus				
Bt_2cAMP	10	26.1 ± 1.1	12.4 ± 1.4	7.9 ± 0.6
Actinomycin D + Bt_2cAMP	10	29.0 ± 0.2	12.6 ± 1.8	1.1
Adult				
Bt_2cAMP + theophylline	20	27.2 ± 3.7	8.0 ± 1.3	28.5 ± 4.6
Actinomycin D + Bt_2cAMP + theophylline	20	30.4 ± 4.0	10.0 ± 0.7	3.8 ± 0.6
Fetus				
Bt_2cAMP	20	39.2 ± 0.1	17.7 ± 0.1	10.0 ± 0.7
Actinomycin D + Bt_2cAMP	20	38.5 ± 0.5	18.8 ± 3.7	1.8 ± 0.5

Actinomycin D (100 μg/100 g of body weight) or 0.154 M NaCl was injected into 21-day-old fetal rats 30 min before Bt_2cAMP (1 μmol per fetus) or Bt_2cAMP plus theophylline (15 mg/kg each) in 24-hr starved and 2-hr refed adult animals. Total liver RNA was extracted and poly(A)-RNA was isolated and assayed in a wheat-germ protein-synthesizing system. Values are for total incorporation of [^{3}H]-leucine into released protein and *P-enol*pyruvate carboxykinase after correction for endogenous incorporation of radioactivity in the absence of added poly(A)-RNA. Data are the means ± SEM of two experiments. For each RNA extraction, 15–24 fetal livers or 2 adult livers were used.

* dpm × 10^{-6}.

† dpm × 10^{-3}.

decrease in *P-enol*pyruvate carboxykinase mRNA within 90 min after injection. This decrease in the level of translatable mRNA for the enzyme also closely parallels the change in enzyme synthesis found under the same condition and suggests that continued mRNA processing is required for the maintenance of induced levels of *P-enol*pyruvate carboxykinase mRNA in the liver. A similar conclusion was also drawn by Noguchi *et al.* (7) for the mechanism of Bt_2cAMP induction of rat liver cytosol tyrosine aminotransferase.

Taken together, the available evidence for the mechanism by which cAMP induces the synthesis of two hepatic enzymes, *P-enol*pyruvate carboxykinase and tyrosine aminotransferase, suggests an effect on transcription or on mRNA processing. This is at variance with earlier proposals for a direct effect of cAMP on the translation of mRNA (24). The simplest interpretation of our results would argue for a *pretranslational* effect of cAMP, although an effect on the rate of mRNA degradation cannot be excluded. Further studies using hybridization of excess RNA to *P-enol*pyruvate carboxykinase cDNA will greatly clarify the present picture. The availability of DNA complementary to *P-enol*pyruvate carboxykinase mRNA would permit us to determine whether high molecular weight precursors of enzyme mRNA exist, such as recently demonstrated for globin mRNA (25–27). It is possible that *P-enol*pyruvate carboxykinase sequences are present in pre-enzyme mRNA and that this maturation to functional template is regulated by cAMP. Recent work by Melli *et al.* (28) has shown

that the controlling step regulating the presence of histone mRNA in the cytoplasm of HeLa cells is not transcriptional and may involve the stability of the processing of histone template. Such possibilities would be directly measurable in fetal rat liver when cDNA to *P-enol*pyruvate carboxykinase mRNA is available.

The direct relationship between the intracellular concentration of cAMP and its effect on enzyme synthesis remains to be firmly established. Adenylate cyclase increases in activity in fetal rat liver 4 days before birth and reaches adult levels at birth (29). Thus, the liver is capable of responding to the increased level of glucagon that occurs immediately after birth (30). Measurements of hepatic cAMP concentration both before and after birth (31) indicate that there is a significant level of the cyclic nucleotide in the liver at birth and that within the first 12 hr after birth its concentration increases approximately 75%. Why the considerable concentration of cAMP already present in fetal rat liver does not stimulate the *de novo* synthesis of *P-enol*pyruvate carboxykinase is not clear. It is probable, however, that the high levels of insulin in fetal rats sharply restricts any initiation of enzyme synthesis by cAMP. During the first 12 hr after birth the translatable mRNA for *P-enol*pyruvate carboxykinase directly follows the concentration of glucagon in the blood. In fact, the lag in the increase in *P-enol*-pyruvate carboxykinase mRNA between 3 and 6 hr after birth is accompanied by a similar lag in the increase of blood glucagon during the first 3–6 hr after birth (30). Thus, the capability for the initial induction of *P-enol*pyruvate carboxykinase is present in the liver prior to birth and awaits only the stimulus of a rise in intracellular cAMP levels caused by the increased glucagon to insulin ratios observed in newborn rats. Whether a critical level of cAMP is required to initiate the appearance of *P-enol*pyruvate carboxykinase mRNA remains to be established.

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