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cAMP stimulates transcription of the gene for cytosolic phospho*enol*pyruvate carboxykinase in rat liver nuclei

(nuclear RNA precursor/phospho*enol*pyruvate carboxykinase mRNA turnover/blot hybridization)

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ABSTRACT **The effects of starvation, glucose refeeding, dibutyryl cAMP, and dexamethasone on expression of the gene for phospho*enol*pyruvate carboxykinase (GTP) [GTP:oxaloacetate carboxy-lyase (transphosphorylating), EC 4.1.1.32] from rat liver cytosol was studied by using a cloned cDNA probe. The rate of transcription of the gene for phospho*enol*pyruvate carboxykinase in hepatic nuclei isolated from starved rats decreased rapidly after refeeding with glucose. Administration of dibutyryl cAMP to glucose-refed animals increased the rate of phospho*enol*pyruvate carboxykinase gene transcription seven-fold within 20 min. Phospho*enol*pyruvate carboxykinase mRNA in the cytosol is 2.8 kilobases long whereas liver nuclei contain four precursor RNA species that are up to 6.5 kilobases long. Feeding glucose to starved rats rapidly decreased the sequence abundance of enzyme mRNA in both nuclei and cytosol. However, the decrease in cytosolic phospho*enol*pyruvate carboxykinase mRNA was preceded by a transient increase in enzyme mRNA over the first 20 min after glucose refeeding. Administration of dibutyryl cAMP to glucose-refed starved animals increased the concentration of the nuclear RNA precursors of phospho*enol*pyruvate carboxykinase five- to eight-fold within 30 min and induced the mRNA for the cytosolic enzyme over a period of 60 min. We conclude that cAMP induces phospho*enol*pyruvate carboxykinase mRNA by increasing the rate of gene transcription.**

The mechanism by which cAMP controls the synthesis rate of hepatic enzymes such as phospho*enol*pyruvate (*P-enol*pyruvate) carboxykinase (GTP) [GTP:oxaloacetate carboxy-lyase (transphosphorylating), EC 4.1.1.32] has not been directly determined. $N^6,O^{2'}$-Dibutyryladenosine 3′:5′-cyclic monophosphate (Bt_2cAMP) administration to a glucose-fed rat rapidly increases the levels of cytosolic *P-enol*pyruvate carboxykinase mRNA (1–3) and this effect can be blocked by simultaneous administration of cordycepin or actinomycin D (1), suggesting that cAMP acts at the level of gene transcription. However, there are also a number of reports indicating that cAMP regulates the translation of both *P-enol*pyruvate carboxykinase (4) and tyrosine aminotransferase (5, 6) mRNA. The availability of a cDNA probe to *P-enol*pyruvate carboxykinase from rat liver cytosol (2) provides a direct method to determine the mechanism of action of cAMP on expression of the gene for this enzyme. In this paper, we present evidence that administration of Bt_2cAMP induces a rapid increase in the rate of transcription of the *P-enol*pyruvate carboxykinase gene as measured in rat liver nuclei *in vitro*.

MATERIALS AND METHODS

Chemicals. The following nucleotides and reagents were purchased from New England Nuclear or Amersham Searle: [^{3}H]UTP (52 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels), [α-^{32}P]dCTP (400–600 Ci/mmol), [α-^{32}P]UTP (410 Ci/mmol), and DNA polymerase I. Nitrocellulose (BA-85) was from Schleicher & Schuell; nucleotides and dextran sulfate were from Boehringer Mannheim and Pharmacia, respectively. Guanidinium thiocyanate was purchased from Kodak and salmon testis DNA, α-amanitin, and Bt_2cAMP were from Sigma. Formamide (MCB Chemical, Norwood, OH) was deionized before use with Bio-Rad AG 501 × 8 resin. DNase I (Worthington Biochemicals) was found to be contaminated with RNase, as measured by loss of trichloroacetic acid-precipitable radioactivity from [^{3}H]RNA. RNase was removed by incubating with 0.1% diethyl pyrocarbonate overnight (7). *Escherichia coli* RNA polymerase was the gift of D. Anthony. Sucrose-containing solutions were treated with 0.5% diethyl pyrocarbonate to inactivate RNase. Other solutions were autoclaved. All other reagents were of the highest purity available.

Animals. Male Sprague–Dawley rats, either adrenalectomized (7 or 8 days after adrenalectomy) or normal, were fed Wayne Lab Blox ad lib; the adrenalectomized animals received 0.9% NaCl in their drinking water. Glucose (5 g/kg of body weight) administered by gavage and other compounds were administered as indicated in the table and figure legends.

Preparation and Isolation of cDNA Plasmid. The cloned cDNA used was a 600-base-pair insert in the *Pst* I- site of pBR322 and was prepared by treating *P-enol*pyruvate carboxykinase mRNA from rat kidney with RNA-dependent DNA nucleotidyltransferase (reverse transcriptase) as described by Yoo-Warren *et al.* (2).

Isolation of Nuclear and Cytosolic RNA. Nuclei were prepared by a modification of the citric acid procedure (8). Livers were isolated and homogenized in 10 vol of ice-cold 0.25 M sucrose/75 mM citric acid, pH 2.3, the homogenate was filtered through two layers of cheesecloth, and Triton X-100 was added to 0.5%. The mixture was layered onto a 10-ml cushion of 1.8 M sucrose/25 mM citric acid/0.1% Triton X-100 and a 5-ml cushion of 1.0 M sucrose/75 mM citric acid/0.5% Triton X-100 and centrifuged at 2,000 × *g* for 30 min at 0°C. The 0.25 M and 1.0 M sucrose layers were combined and centrifuged for 30 min at 20,000 × *g* to obtain the cytosolic RNA fractions. The nuclear pellet was suspended in 20 ml of 0.25 M sucrose/10 mM citric acid/0.1% Triton X-100, layered onto a 10-ml cushion of 1.0 M sucrose/20 mM KOAc, pH 4.5/0.1% Triton X-100, and centrifuged for 20 min at 1,000 × *g*. The pellet from this step was suspended in 0.25 M sucrose/20 mM KOAc, pH 4.5/0.1 Triton X-100, and centrifuged for 10 min at 600 × *g*. The yield from

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Abbreviations: *P-enol*pyruvate, phospho*enol*pyruvate; Bt_2cAMP, dibutyryl cAMP.

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this procedure was approximately 10^8 nuclei per g (wet weight) of liver.

The nuclear pellet was dissolved in 4 M guanidinium thiocyanate/0.5% sodium *N*-lauroylsarcosine/20 mM EDTA/0.2 M 2-mercaptoethanol/50 mM Pipes, pH 7 [approximately 5 ml/g (wet weight) of liver] (9), and CsCl was added to a final concentration of 1.4 M. The extract was layered onto a 2-ml cushion of 5.7 M CsCl/0.1 M EDTA, pH 7.0, and centrifuged at 25°C for 16–18 hr at 150,000 × *g*. The pelleted RNA was dissolved in 10 mM Hepes/1 mM EDTA/0.1% $NaDodSO_4$, pH 7.5, and the solution was extracted with chloroform/isoamyl alcohol (24:1). The yield of RNA was 50–100 μg per g (wet weight) of liver.

Cytosolic RNA was extracted from the 20,000 × *g* pellet with 5 M guanidinium thiocyanate/0.75% sodium *N*-lauroylsarcosine/30 mM EDTA/150 mM $NaHCO_3$/0.3 M 2-mercaptoethanol [approximately 2 ml/g (wet weight) of liver] (9). RNA was precipitated with EtOH at −20°C for 30 min, pelleted by centrifugation for 10 min at 3,000 × *g* (−10°C), and dissolved in 7 M guanidinium chloride/20 mM EDTA/0.2 M 2-mercaptoethanol, pH 7.0. The RNA was acidified with HOAc and precipitated with EtOH at −80°C for 10 min, and the mixture was centrifuged. The pellet was dissolved in the guanidinium chloride solution, the solution was extracted with an equal volume of chloroform/isoamyl alcohol (24:1), and the RNA was precipitated again. The RNA was washed successively with 0.2 M NaOAc, pH 5.0/75% ethanol, 3.0 M NaOAc (pH 6.0) (10), and 0.2 M NaOAc, pH 5.0/75% EtOH, and then the RNA was dissolved in 10 mM Hepes, pH 7.5/1 mM EDTA/0.1% $NaDodSO_4$. The yield of RNA from this procedure was 4 to 5 mg per g (wet weight) of liver.

Isolation of Nuclei for Determination of RNA Synthesis. Livers were homogenized at 4°C in 5–10 vol of 0.3 M sucrose/5 mM dithiothreitol/5 mM $MgCl_2$/10 mM Tris·HCl, pH 7.5/0.1% Triton X-405 (medium A). The homogenate was filtered through four layers of cheesecloth, and the crude nuclear fraction was isolated by centrifugation at 800 × *g* for 5 min. The nuclei were then homogenized in medium A lacking Triton X-405, sucrose was added to a final concentration of 1.65 M, and the mixture was centrifuged at 25,000 × *g* for 60 min at 4°C through 1 ml of 2 M sucrose/2 mM $MgCl_2$/10 mM Tris·HCl, pH 7.5 (11). The pellet was homogenized in 50% glycerol/5 mM $MgCl_2$/0.1 mM EDTA/50 mM Hepes, pH 7.5, and the nuclei were counted by using phase-contrast microscopy and frozen at −80°C at a concentration of 5–10 × 10^8/ml. Recovery of nuclei averaged 0.1–0.7 × 10^8/g (wet weight) of tissue.

Measurement of RNA Transcription. Rates of RNA transcription by isolated frozen rat liver nuclei were measured with 25 μCi of [^{32}P]UTP in 25% glycerol/2.5 mM $MgCl_2$/0.05 mM EDTA/75 mM Hepes, pH 7.5/100 mM KCl/4 mM dithiothreitol/0.5 mM CTP/0.5 mM GTP/1.0 mM ATP/creatine kinase (0.04 mg/ml)/8.8 mM creatine phosphate containing 2–4 × 10^7 nuclei in a total volume of 200 μl (12). RNA synthesis was terminated at 60 min by incubating with RNase-free DNase (15 μg/ml)/5 mM $MgCl_2$ for 10 min at 37°C. The mixture was digested with proteinase K (90 μg/ml)/0.5% $NaDodSO_4$/5 mM EDTA for 20 min at 37°C. RNA was extracted with phenol, several times with phenol/chloroform, 1:1 (vol/vol), and precipitated in 0.2 M NaOAc with 2 vol of absolute EtOH at −20°C for several hours. The mixture was centrifuged, the pellet was dried, digested with DNAse (15 μg/ml) in 0.1 M NaOAc, pH 6/5 mM $MgCl_2$/1 mM UTP for 20 min at 37°C, and the digest was extracted with phenol, washed with ether, and EtOH precipitated. The final pellet was dissolved in 100 μl of water.

Hybridization of the [^{32}P]RNA to nitrocellulose-bound cDNA was carried out as described by Harpold *et al.* (13). The cDNA was boiled 5 min in 0.1 M NaOH and cooled, and 2.0 μg in 20 μl was applied directly to 1-cm^2 nitrocellulose filters that had previously been boiled, soaked in 3.0 M NaCl/0.30 M Na citrate, and baked at 75°C. The cDNA-containing filters were dried, baked 2 hr at 75°C, and stored. Recovery of filter-bound DNA after hybridization and washing, as measured by A_{260} material released by perchloric acid, was quantitative. To estimate the hybridization efficiency, [^{3}H]cRNA (specific activity, 5 to 6 × 10^5 cpm/μg) was made according to Lis *et al.* (7) using the 600-base-pair cDNA insert isolated by *Pst* I digestion of pPCK2 as template. Filters containing either pBR322 or pPCK2 were prehybridized at 65°C for 2–4 hr in 0.5 M NaCl/10 mM Hepes, pH 7.5/10 mM EDTA/0.2% $NaDodSO_4$/0.2% ficoll/0.02% polyvinylpyrollidone (medium B). The prehybridization medium was discarded, 0.3 ml of medium B/[^{32}P]RNA/[^{3}H]cRNA was added, and the mixture was layered with silicone oil and hybridized for 36 hr at 65°C. Filters were washed five times at 45°C with 0.75 M NaCl/75 mM Na citrate/0.5% $NaDodSO_4$/0.5 mM EDTA, twice at 45°C with 0.30 M NaCl/30 mM Na citrate/5 mM EDTA, and once for 20 min at 24°C with 0.30 M NaCl/30 mM Na citrate containing RNase A at 10 μg/ml. The filters were then boiled in 0.2 ml of water twice for 2 min each, and the radioactivity in the eluted RNA was determined by liquid scintillation counting.

Blot Hybridization Analysis of RNA. Total RNA isolated as outlined above was subjected to electrophoresis on agarose gels containing 2.2 M formaldehyde. The RNA was transferred to nitrocellulose and hybridized to [^{32}P]pPCK2 (14). Hybridization was quantitated by cutting out the appropriate lanes and the radioactivity determined by liquid scintillation counting was shown to be linear over a RNA concentration range of 15–45 μg.

RESULTS

Transcription of the *P-enol*pyruvate Carboxykinase Gene by Isolated Nuclei. The transcription medium was adjusted to give a linear rate of uptake of UTP into total RNA. In preliminary experiments, it was found that 2.5 mM $MgCl_2$ gave a maximal rate of RNA synthesis and inclusion of either Ca^{2+} or Mn^{2+} had no further stimulatory effect. A relatively high glycerol concentration (25%) was also critical to achieve maximal RNA synthesis. Incorporation of [^{3}H]UTP into trichloroacetic acid-precipitable RNA is nearly linear for 60 min at 22°C and is inhibited 50% by α-amanitin at 0.5 μg/ml. When this medium is used, routine incorporation is 9–14% of [^{32}P]UTP (5–7 pmol) into total RNA per 2–4 × 10^7 nuclei per hr.

The effects of Bt_2cAMP and theophylline on transcription of *P-enol*pyruvate carboxykinase RNA in isolated nuclei from glucose-refed starved rats are shown in Table 1. The absolute rate of RNA synthesis was determined by including [^{3}H]cRNA in each sample to measure the efficiency of hybridization. The basal rate of *P-enol*pyruvate carboxykinase RNA synthesis was about 0.05%, or 500 ppm, in both adrenalectomized (group A) and control (group E) glucose-refed rats, suggesting that glucocorticoids are not necessary to maintain this basal rate of enzyme RNA synthesis. In glucose-refed adrenalectomized rats, injection of Bt_2cAMP and theophylline increased the synthesis rate of *P-enol*pyruvate carboxykinase mRNA seven-fold after 20 min, to 3,415 ppm (0.34%) but, by 60 min, the rate had decreased to 1,779 ppm. In intact rats injected with Bt_2cAMP/theophylline (group G), there was a four-fold increase in the transcription rate of the *P-enol*pyruvate carboxykinase gene after 20 min. In dexamethasone-treated rats, the increase in the rate of transcription was only 35% above the control value at 60 min (group D). Finally, α-amanitin at 0.5 μg/ml reduced basal synthesis of both total RNA and *P-enol*pyruvate carboxykinase

Table 1. Effect of Bt_2cAMP on rate of synthesis of *P-enol*pyruvate carboxykinase RNA by liver nuclei isolated from intact and adrenalectomized rats

Group	Treatment	Total RNA, cpm × 10^6	Incorporation, cpm		Synthesis rate,* ppm
			pPCK2	pBR322	
A (adexed)	Glucose only	9.35	731	123	474
B (adexed)	Glucose, Bt_2cAMP/ theophylline (20 min)	5.75	2,950	310	3,415
C (adexed)	Glucose, Bt_2cAMP/ theophylline, Bt_2cAMP (60 min)	5.50	1,298	110	1,779
D (adexed)	Glucose, dexamethasone (60 min)	9.45	932	127	621
E (control)	Glucose only	6.85	582	100	522
F (control)	Glucose, α-amanitin	3.8	174	66	260
G (control)	Glucose, Bt_2cAMP/ theophylline (20 min)	7.15	2,031	170	2,218

Adrenalectomized (adexed; groups A–D) and nonadrenalectomized (control; groups E–G) Sprague–Dawley rats weighing 250–300 g were starved for 24 hr and then fed glucose at 5 g/kg of body weight for 2 hr. Then, groups B, C, and G were given Bt_2cAMP and theophylline, each at 30 mg/kg of body weight, intraperitoneally; 20 and 40 min later, group C was given additional doses of Bt_2cAMP; group D was given dexamethasone at 5 mg/kg of body weight intraperitoneally. For group F, α-amanitin at 0.5 μg/ml was added to the isolated nuclear transcription system. RNA synthesis results are based on duplicate samples. Efficiency of hybridization was assessed by adding 910 cpm of [^{3}H]cRNA prepared from *P-enol*pyruvate carboxykinase cDNA (pPCK2) to each assay vial.

* Synthesis rate = [cpm(pPCK2 − pBR322)/cpm in total RNA] × (100/efficiency) × 2,800/600, where 2,800 is the length of mature *P-enol*pyruvate carboxykinase mRNA in nucleotides and 600 is the length of pPCK2 in base pairs.

RNA about 50% (group F) to about 260 ppm.

Reversal of induction of *P-enol*pyruvate carboxykinase mRNA synthesis by glucose refeeding of starved rats is shown in Table 2. The rate of *P-enol*pyruvate carboxykinase RNA synthesis in starved rats (group A) is 3,676 ppm, which is close to the transcription rate of nuclei from animals injected with Bt_2cAMP (group F). Addition of α-amanitin at a concentration sufficient to block RNA polymerase II activity inhibits transcription of *P-enol*pyruvate carboxykinase RNA by 92%. Refeeding with glucose (groups C, D, and E) produced a rapid decrease in enzyme RNA synthesis, reaching basal levels after 2 hr. Again, Bt_2cAMP/theophylline stimulated synthesis about six-fold within 20 min after administration (Table 1, groups E and G).

Table 2. Effect of refeeding on rate of synthesis of *P-enol*pyruvate carboxykinase RNA by isolated rat liver nuclei

Group	Treatment	Total RNA, cpm × 10^6	Incorporation, cpm		Synthesis rate, ppm
			pPCK2	pBR322	
A	Starvation	7.25	3,119	348	3,676
B	Starvation, α-aminitin	3.80	318	188	306
C	Glucose (20 min)	7.78	2,070	157	2,208
D	Glucose (60 min)	12.0	1,450	242	895
E	Glucose (120 min)	9.35	927	214	653
F	Glucose (120 min), Bt_2cAMP (20 min)	7.35	3,495	132	3,519

Sprague–Dawley rats weighing 200–250 g were starved for 48 hr and then fed glucose at 5 g/kg of body weight for the indicated times. Synthesis of *P-enol*pyruvate carboxykinase RNA was determined with duplicate samples. For group B, α-amanitin at 0.5 μg/ml was added to the isolated nuclei to inhibit RNA polymerase II-directed transcription. Efficiency of hybridization was determined for each sample and was 52–60%. Synthesis rate was calculated as in Table 1.

Regulation of Levels of *P-enol*pyruvate Carboxykinase mRNA in the Nucleus and Cytosol. Liver nuclei contain several RNA species that hybridize to our *P-enol*pyruvate carboxykinase cDNA probe (Fig. 1). The nuclear RNAs range in size from approximately 1.0 to 6.5 kb and as many as four nuclear RNA species are considerably larger than the 2.8 kb of mature cytosolic *P-enol*pyruvate carboxykinase mRNA. We routinely note three smaller nuclear RNA species, possibly breakdown products of the larger RNAs, that hybridize with our cDNA probe. Both the putative *P-enol*pyruvate carboxykinase mRNA precursors and the degradation products are polyadenylylated (3) and are not observed in cytosolic RNA isolated from rat liver (lane C). Feeding glucose to a starved rat rapidly decreased the levels of hybridizable *P-enol*pyruvate carboxykinase mRNA precursors detectable in the nucleus so that, within 2 hr, the sequence abundance of these RNAs was barely evident by blot analysis. Bt_2cAMP administration increased all of these nuclear precursors five- to eight-fold within 20 min, after which there was a marked decrease in the sequence abundance of these precursor RNA species. When adrenalectomized rats were injected with dexamethasone, there was an increase in the sequence abundance of *P-enol*pyruvate carboxykinase RNA precursors in the nucleus, but this induction was not as marked as that after administration of Bt_2cAMP.

*P-enol*pyruvate carboxykinase mRNA in the cytosol is a single species, 2.8 kb long (Fig. 2). Feeding glucose to a starved rat caused a transient increase (see Fig. 3) followed by a rapid decrease in the sequence abundance of enzyme mRNA as measured by blot hybridization ($t_{1/2}$, 20 min). After administration of Bt_2cAMP to a starved animal that had been refed glucose for 2 hr, there was a lag phase of approximately 10 min followed by a rapid induction of *P-enol*pyruvate carboxykinase mRNA in the cytosol. This increase was approximately linear for the 60-min duration of the experiment shown in Fig. 2. Dexamethasone also increased the sequence abundance of cytosolic *P-enol*pyruvate carboxykinase mRNA in the livers of carbohy-

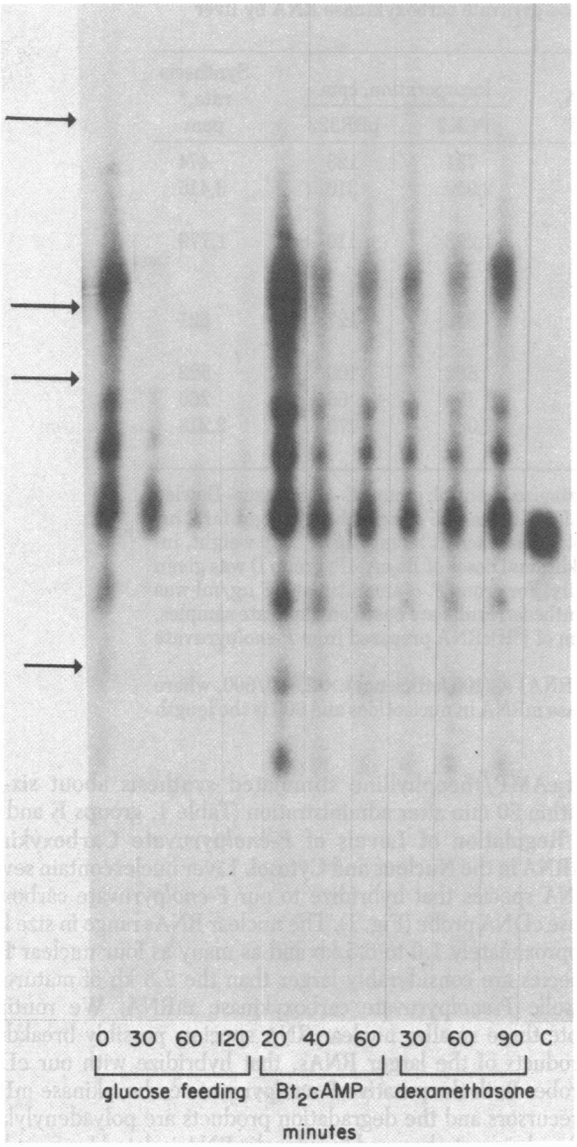

FIG. 1. Blot hybridization analysis of *P-enol*pyruvate carboxykinase mRNA in rat liver nuclei. Starved adrenalectomized rats were refed with glucose (at 0 time) and, 120, 140, and 160 min later, injected with Bt_2cAMP at 30 mg/kg of body weight, or 120 min later, injected with dexamethasone at 5 mg/kg of body weight. At the indicated times, livers were removed from the animals and homogenized, and nuclear and cytosol fractions were isolated. RNA was extracted from the nuclei and separated by agarose gel electrophoresis, transferred to nitrocellulose, and hybridized to nick-translated pPCK2. Arrows indicate positions of 18, 28, 32, and 45S rRNAs. For comparison, cytosolic *P-enol*pyruvate carboxykinase mRNA (2.8 kilobases) from a starved rat is also shown (lane C).

drate-refed starved rats but to a lesser extent.

The effects of starvation, glucose feeding, and administration of Bt_2cAMP on the rate of transcription of the *P-enol*pyruvate carboxykinase gene by isolated rat liver nuclei, as well as on the levels of enzyme mRNA in the nuclei and cytosol of these animals, are summarized in Fig. 3. After glucose refeeding, the RNA polymerase II-dependent transcription of the *P-enol*pyruvate carboxykinase gene decreases with a half-time of about 20 min, and this is accompanied by a similar reduction in enzyme RNA sequences in the nucleus. However, the levels of cytosolic *P-enol*pyruvate carboxykinase mRNAs increase over the first 20–30 min and then decreased rapidly for the next 1.5

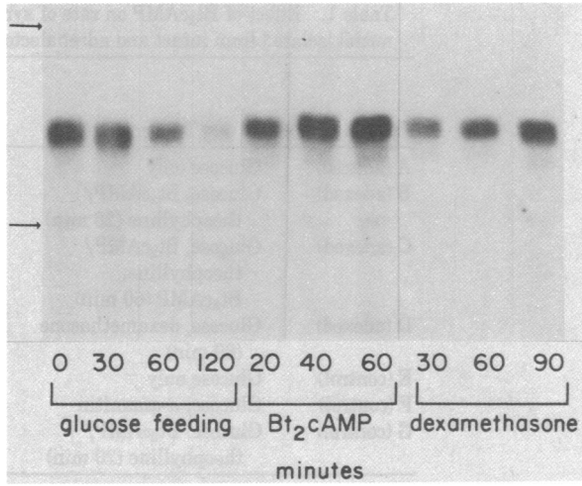

FIG. 2. Blot hybridization analysis of *P-enol*pyruvate carboxykinase mRNA levels in the cytosol of livers from starved adrenalectomized, refed starved, and Bt_2cAMP- and dexamethasone-treated starved and refed rats. Animals and experimental conditions are as in Fig. 1 except that the RNA was extracted from the cytosol. Arrows indicate positions of 18 and 28S rRNAs.

hr. Administration of Bt_2cAMP to these starved glucose-refed rats rapidly elevated both the rate of *P-enol*pyruvate carboxykinase gene transcription and the sequence abundance of enzyme mRNA in the nucleus and cytosol. Maximal increase in both the rate of gene transcription and the concentration of enzyme mRNA in the nuclei occurred about 20 min after the injection of Bt_2cAMP, but these effects markedly decreased over

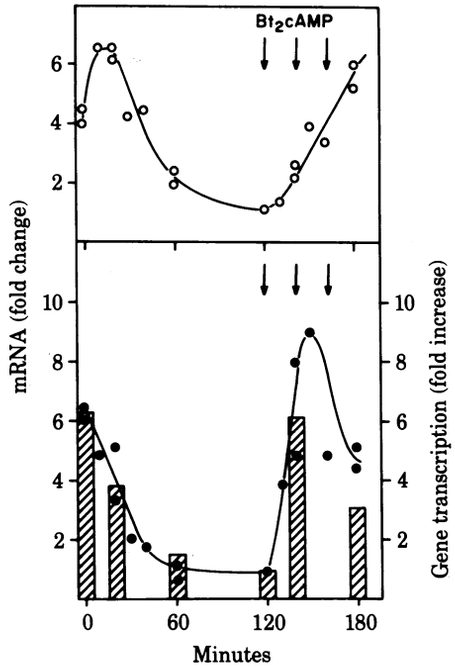

FIG. 3. Time course of changes in rates of *P-enol*pyruvate carboxykinase gene transcription (▨) and in sequence abundance of enzyme mRNA in isolated rat liver nuclei (●) and cytosol (○) after refeeding with glucose and Bt_2cAMP treatment. Experimental conditions are as in Fig. 1. Rate of transcription of the *P-enol*pyruvate carboxykinase gene and the levels of enzyme mRNA in cytosol and nuclei were determined at various times. Data were obtained from four different experiments and are normalized to the value for rats fed glucose for 2 hr.

the next 40 min despite the fact that Bt_2cAMP was subsequently injected twice at 20-min intervals. In contrast, *P-enol*pyruvate carboxykinase mRNA in the cytosol increased linearly for the 60-min duration of the experiment.

DISCUSSION

The results presented here provide direct evidence that cAMP can alter the level of *P-enol*pyruvate carboxykinase mRNA by stimulating the rate of gene transcription. This effect was suggested in earlier studies using inhibitors of transcription, such as actinomycin D (15), and is supported by more recent work in which Bt_2cAMP was shown to increase the concentration of hepatic mRNA for both *P-enol*pyruvate carboxykinase (1, 16) and tyrosine aminotransferase (6). However, there are also reports of an effect of cAMP at the level of mRNA translation (4–6, 17). For example, Roper and Wicks (5) have reported that addition of Bt_2cAMP to cultured hepatoma cells accelerates the rate of elongation of tyrosine aminotransferase nascent chains 6- to 10-fold, while Snoek *et al.* (17) noted, using the same experimental system, that Bt_2cAMP doubled the number of enzyme nascent chains. In contrast, dexamethasone did not alter the translation of enzyme mRNA, despite the fact that it elevated the activity of tyrosine aminotransferase 5- to 10-fold in hepatoma cells. Although the present study shows that Bt_2cAMP can induce transcription of the *P-enol*pyruvate carboxykinase gene, our data do not exclude the possibility that it acts at more than one site in regulating the level of the enzyme in the liver.

On feeding glucose to a starved animal, there is a rapid decrease ($t_{1/2}$, 30 min) in the synthesis rate of *P*-enolpyruvate carboxykinase (18) and an equally rapid decrease in the level of translatable mRNA for the enzyme (19). The rate of decay of translatable enzyme mRNA became exponential only after an initial lag period of 20–30 min (19, 20). One explanation for this lag period came from the experiments of Reshef *et al.* (21), who have presented evidence that this transient increase in *P-enol*pyruvate carboxykinase synthesis may be due to the shift of a substantial subpopulation of enzyme mRNA into polysomes, thereby temporarily protecting it from degradation. The concomitant decrease of transcription rate and levels of nuclear and cytosolic RNA for *P-enol*pyruvate carboxykinase suggests that the RNA that is synthesized during the first 60 min after refeeding is immediately degraded. On treatment of glucose-refed starved rats with cAMP or dexamethasone, the transcription rate and levels of nuclear RNA increase without an appreciable lag time while the cytosolic RNA level begins to increase only after 10–20 min (Fig. 3). Since relatively high levels of precursor RNA compared with mature *P-enol*pyruvate carboxykinase mRNA are detected in nuclei at this time (Fig. 1), the observed lag time for cytosolic RNA appears to be due not only to transport but also to processing of the RNA.

Both the rate of *P-enol*pyruvate carboxykinase gene transcription and the level of enzyme mRNA in the nuclei decreased sharply approximately 30 min after Bt_2cAMP administration (Fig. 3). This is not due to a decrease in the hepatic concentration of cAMP since the cyclic nucleotide was injected every 20 min and the enzyme mRNA in the cytosol continued to increase for 2 hr. Dexamethasone treatment also caused a similar transient increase in nuclear *P-enol*pyruvate carboxykinase mRNA (data not shown). The cytosolic mRNA for the enzyme remained constant between 30 and 60 min after hormone administration, after which it decreased, presumably due to the glucocorticoid-induced elevation of insulin secretion.

The stimulatory effect of cAMP on expression of the *lac* operonin *E. coli* is well known (22) but, until recently, a direct effect of cAMP on RNA transcription in eukaryotes had been observed only in the slime mold *Dictyostelium* (23). Recently, Maurer (24) has found that Bt_2cAMP added to ergocryptine-treated pituitary cells restores prolactin RNA transcription to normal levels (400–500 ppm). In this paper, we report that *P-enol*pyruvate carboxykinase gene expression is increased four- to seven-fold by Bt_2cAMP and inhibited over 80% by refeeding starved rats with glucose. The marked effect of Bt_2cAMP on the rate of transcription of the *P-enol*pyruvate carboxykinase gene indicates that this gene has a very active promoter region.

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