Increase in Level of Functional Messenger RNA Coding for Phosphoenolpyruvate Carboxykinase (GTP) during Induction by Cyclic Adenosine 3':5'-Monophosphate*

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The administration of \(N^{\beta},O^{\gamma}\)-dibutyryl cyclic AMP and theophylline to fasted-refed rats produces an 8-fold stimulation of the relative rate of hepatic phosphoenolpyruvate carboxykinase synthesis in 90 min, as measured by isotopic immunchemical techniques in vitro. The mechanism of this induction was studied first by using a homologous, noninitiating cell-free protein-synthesizing system derived from the liver of fasted-refed, cyclic AMP-treated rats. In such a system, a 5-fold increase in phosphoenolpyruvate carboxykinase synthesis is observed at 20 min post-treatment and a 9-fold stimulation at 75 min, indicating a rapid increase in the number of ribosomes engaged in the translation of the enzyme mRNA after exposure to cyclic AMP. The level of functional mRNA coding for phosphoenolpyruvate carboxykinase was then assayed in a wheat germ protein-synthesizing system capable of using rat liver mRNA as template. The template activity for phosphoenolpyruvate carboxykinase synthesis is greatly increased in the poly(A)-containing RNA isolated from cyclic AMP-induced animals. Both the increase in the capacity of the liver extract for in vitro phosphoenolpyruvate carboxykinase synthesis and the emergence of enzyme mRNA detected in the wheat germ assay are completely prevented by a pretreatment with cordycepin at doses which inhibit the appearance in the cytoplasm of newly synthesized poly(A)-containing RNA. These data demonstrate that the induction of hepatic phosphoenolpyruvate carboxykinase by cyclic AMP is characterized by the rapid build-up of newly synthesized, actively translated mRNA coding for the enzyme. The messenger accumulation could be due to an increase in the rate of its production or a decrease in the rate of its degradation.

The development in recent years of improved methods for the extraction (1-3) and isolation (4, 5) of eukaryotic mRNAs and the use of efficient cell-free protein-synthesizing systems for the translation of heterologous mRNAs (6-9) have permitted significant advances in the understanding of the hormonal control of gene expression in nucleated cells. With few exceptions (10), however, the models of specific protein induction studied at the molecular level have involved proteins which are under steroid hormone control (for reviews see Refs. 11 to 13). In the present paper, we deal with the cyclic AMP-dependent induction of P-enolpyruvate carboxykinase in the liver.

It has been known for several years that P-enolpyruvate carboxykinase activity increases in the rat liver after injection of cyclic AMP or dibutyryl cyclic AMP (14). Studies using specific immunoprecipitation of the enzyme after labeling with radioactive amino acid showed that a stimulation of enzyme synthesis accounts for the increase in enzyme level (15-17). P-enolpyruvate carboxykinase induction, defined here as a specific increase in the rate of enzyme synthesis, was also observed in vitro in Reuber H-35 hepatoma cells incubated in the presence of dibutyryl cyclic AMP (18, 19). The increase in the hepatic concentration of cyclic AMP in starvation, diabetes, or after glucagon administration is probably instrumental in the increase in P-enolpyruvate carboxykinase level originally observed in these conditions by Shrago et al. (20).

In the experiments to be described we have used two cell-free systems capable of synthesizing P-enolpyruvate carboxykinase in order to define the mechanisms by which cyclic AMP causes an increase in the rate of the enzyme synthesis. A homologous, noninitiating system derived from the liver of dibutyryl cyclic AMP-treated rats served to quantitate the ribosomes engaged in P-enolpyruvate carboxykinase synthesis at various times after treatment. Secondly, the level of functional P-enolpyruvate carboxykinase mRNA during the course of induction was determined by measuring the amount of enzyme synthesized in a wheat germ system programmed with the poly(A)'RNA fraction of rat liver RNA. Finally, using both cell-free systems, we tested the effect of cordycepin (3'-deoxyadenosine), an inhibitor of RNA synthesis, on the induction process. Some results were published previously in abstract form (21).

EXPERIMENTAL PROCEDURES

Materials-Dibutyryl cyclic AMP and theophylline were purchased from Sigma Chemical Co. Cordycepin was supplied by Sigma or kindly donated by Dr. Robert J. Suhadolnik of our department.

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1 Abbreviations used are: P-enolpyruvate carboxykinase, phosphoenolpyruvate carboxykinase (GTP) (EC 4.1.1.32); Poly(A)'RNA, RNA containing a polyadenylic acid sequence; Poly(A)+RNA, RNA without polyadenylic acid sequence; Hepes, N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid; SDS, sodium dodecyl sulfate; dibutyryl cyclic AMP, \(N^{\beta},O^{\gamma}\)-dibutyryl adenosine 3',5'-monophosphate.

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Cyclic AMP and P-enolpyruvate Carboxykinase mRNA

The absorbance of the eluate at 260 nm was 0.05 or less. Elution of the

mixture of phenol/chloroform/isoamyl alcohol (50:15:1) for 2 min in a

oligo(dT)-cellulose. After the sample was passed through at a rate of

25 ml/min, the absorbance of the eluate was measured. The fraction with an

absorbance at 260 nm of 0.5 or less was collected. The radioactivity of the

fractions containing P-enolpyruvate and pyruvate kinase were from Boehringer

and kept in liquid nitrogen until used for translation.

The precipitation of total protein from the incubation mixture was

performed at 28°C in the presence of (final concentration): 20 mM Tris-

HCl, pH 7.6, 10 mM potassium acetate, 24 mM NaCl, 2.3 mM dithiothreitol, 1.9 mM 2-mercapto-

ethanol, and 1.4 mM MgCl₂. The gradient (50-0% sucrose) was loaded with 1.36 ml of the

supernatant from cell-free protein synthesis experiments. The precipitates were washed twice with 20% (w/v) glycerol and layered on top of 20% to 50% (w/v) linear sucrose gra-

dients. After centrifugation, the gradient fractions were collected, and the radioactivity of the fractions was measured. The amount of radioactivity in the slices of the gradient was computed by adding the radioactivity of the fractions corresponding to the enzyme band (usually five 1-mm slices) and subtracting a background activity estimated from the gradient fractions corresponding to the enzyme band.

The precipitation of total protein from the incubation mixture and the estimation of their radioactivity were performed as described by Ballard et al. (22) in the presence of carrier bovine serum albumin.

RESULTS

Synthesis of P-enolpyruvate Carboxykinase in Vivo after Administration of Dibutyryl Cyclic AMP — The effect of the injection of dibutyryl cyclic AMP plus theophylline on P-
enolpyruvate carboxykinase synthesis in vivo is illustrated in Fig. 1. For reasons discussed elsewhere (25), the rate of enzyme synthesis is presented as the ratio of [3H]leucine incorporated into the enzyme to [3H]leucine incorporated into total protein. The changes shown reflect an absolute increase in radioactivity incorporated into P-enolpyruvate carboxykinase rather than depression of general protein synthesis. In agreement with previous data (15, 17), the relative rate of synthesis of P-enolpyruvate carboxykinase increases in a modest (less than 3-fold) and variable way when rats fed ad libitum are used (Fig. 1A). By contrast, the stimulation of enzyme synthesis is presented as the ratio of [3H]leucine incorporated into total protein. The changes shown reflect an absolute increase in radioactivity incorporated into P-enolpyruvate carboxykinase during incubation of liver S-17. The upper panels show the distribution of radioactivity in SDS-polyacrylamide gels of the immunoprecipitated products by antibody precipitation. In good agreement with previously published data (22), increasing amounts of enzyme are detected during 20 min of incubation (Fig. 2, lower frames). Sucrose gradient analysis of the incubated polyribosomes reveals that, concomitantly, the quantity of monosomes and ribosomal subunits increases at the expense of the larger polyribosomal aggregates (Fig. 2, upper frames). The progressive disaggregation of polyribosomes indicates that the incubated postmitochondrial fraction has a limited capacity to initiate polypeptide chain synthesis, if initiation occurs at all. Most or all of the amino acid incorporation into protein, included into P-enolpyruvate carboxykinase, must, therefore, result from the "run-off" of ribosomes from polyribosomes preformed in vivo. The maintenance of active protein synthesis for as long as 20 min, thus, probably reflects a rate of peptide elongation slower than in vivo, as has been reported for other cell-free protein-synthesizing systems (26, 27).

The capacity of the S-17 fraction of the liver to carry out in vitro P-enolpyruvate carboxykinase synthesis is markedly increased after the administration of dibutyryl cyclic AMP plus theophylline (Table I). The nearly 5-fold increase observed 20 min after injection of these compounds demonstrates the striking rapidity of the effect. At 75 min post-treatment, the stimulation of in vitro P-enolpyruvate carboxykinase synthesis is 9-fold, which is in agreement with the magnitude of the induction observed in vivo (compare Table I and Fig. 1B). Since protein synthesis by the liver extract reflects mostly the completion of nascent polypeptide chains originally attached to the ribosomes.
polyribosomes, the number of enzyme chains initiated in vivo must have rapidly increased after exposure to cyclic AMP. In contrast with the abrupt increase in P-enolpyruvate carboxykinase synthesis, note the overall depression in protein synthesis, especially at 20 min after dibutyryl cyclic AMP. This general inhibitory effect is in agreement with previous observations of other investigators (28, 29).

Effect of Cordycepin on Induction of P-enolpyruvate Carboxykinase by Dibutyryl Cyclic AMP—Although the mechanism of action of cordycepin and its triphosphate derivative at the enzymatic level is still debated (30, 31), a net result of the drug action in HeLa cells (32) or mouse sarcoma 180 cells (33) is an inhibition of mRNA synthesis. In Fig. 3, we show that cordycepin, injected into the rat 30 min prior to a pulse of [3H]orotic acid, prevents the appearance of the tracer in the cytoplasmic poly(A)+RNA of the liver. Inasmuch as poly(A)+RNA represents most of the mRNA (for a review see Ref. 34), the data indicate that cordycepin can substantially inhibit mRNA synthesis in the rat liver. The labeling of cytoplasmic poly(A)+RNA, reflecting predominantly RNA synthesis after a short term pulse (35), was little affected by cordycepin in those experiments.

The effect of cordycepin on the cyclic AMP induction of P-enolpyruvate carboxykinase is illustrated in Fig. 4. In these experiments, cordycepin was administered 30 min before the injection of dibutyryl cyclic AMP plus theophylline and the liver S-17 fraction was prepared for \textit{in vitro} protein synthesis 45 min after the latter injection. Exposure to cyclic AMP occurred, therefore, during a period in which the synthesis of poly(A)+RNA was inhibited. Clearly, pretreatment with cordycepin prevents the increment in the cell-free synthesis of P-enolpyruvate carboxykinase normally observed after dibutyryl cyclic AMP administration (Fig. 4). Three lines of evidence suggest that the suppression of P-enolpyruvate carboxykinase induction is related to the inhibition of poly(A)+RNA synthesis. First, as the comparison of the two log-dose effect plots shows (Figs. 3 and 4), both effects manifest themselves within the same dosage range. Second, the overall capacity of the liver polyribosomes to synthesize protein is not signifi-

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**Table I**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time</th>
<th>Propriotions tested</th>
<th>[3H]leucine incorporation into P-enolpyruvate carboxykinase (dpm x 10^6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>6</td>
<td>2439 ± 178</td>
<td>1197 ± 92</td>
</tr>
<tr>
<td>75</td>
<td>6</td>
<td>1915 ± 86</td>
<td>897 ± 41</td>
</tr>
<tr>
<td>Dibutyryl cyclic AMP</td>
<td>5</td>
<td>1722 ± 44</td>
<td>760 ± 35</td>
</tr>
</tbody>
</table>

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**Fig. 3 (left).** Log dose effect of cordycepin on the labeling of liver cytoplasmic RNA. Cordycepin was administered at the doses specified 30 min before the pulse injection of [3H]orotic acid. Cytoplasmic RNA was extracted and fractionated into poly(A)+RNA and poly(A)-RNA as described under "Experimental Procedures." Data are means ± S.E. for four to six animals.

**Fig. 4 (right).** Log dose effect of cordycepin on P-enolpyruvate carboxykinase induction. The rats were fasted and refeed prior to the experiment as in Table I. Cordycepin at the doses indicated or a control injection was given 30 min before dibutyryl cyclic AMP and theophylline and the liver S-17 was prepared 45 min after the latter injection for cell-free protein synthesis. Cordycepin inhibited total soluble protein; [3H]leucine incorporation into P-enolpyruvate carboxykinase. Data are means ± S.E. for five or six S-17 extracts. The inset summarizes data on the liver glycogen level in the various experimental groups. Bars A and C illustrate the effect of dibutyryl cyclic AMP plus theophylline in rats without pretreatment. Bars B and D show the effect in rats pretreated with 34 mg of cordycepin/kg of body weight.
immunoglobulin light chain. Enolpyruvate carboxykinase; H, immunoglobulin heavy chain; L, represents a duplicate gel after staining with Coomassie blue; E, P-described under “Experimental Procedures.” Counts in the ^H channel were corrected for the spill-over of ^3S radioactivity. The top panel termination of radioactivity in the gel slices were performed as precipitate, its electrophoresis on SDS-polyacrylamide gel, and the de-carboxykinase antibody. The washing of the antigen-antibody pre-were mixed and incubated in the presence of anti-P-enolpyruvate H,O to '/5 of the original volume. The ^H- and ^3S-labeled products obtained after the incubation was freeze-dried and redissolved in sis in the presence of [^3S]methionine; the high speed supernatant rat similarly treated and was incubated for cell-free protein synthesis in the presence of poly(A) RNA isolated from the liver of dibutyryl cyclic AMP-treated rats. After incubation and ultracentrifugation of the reaction mixture, the supernatant from several individual tubes was pooled. Aliquots of this pooled material were then incubated with a fixed volume of antibody solution and with various amounts of purified P-enolpyruvate carboxykinase, as indicated on the abscissa. Antibody reaction, washing and dissociation of the immunoprecipitates, and SDS-polyacrylamide gel electrophoresis were then conducted as described under “Experimental Procedures.” The radioactivity detected in the enzyme band of the polyacrylamide gels is indicated on the far left-hand ordinate (△). The supernatants of the antigen-antibody mixtures were kept for determination of P-enolpyruvate carboxykinase activity, which is given on the right-hand ordinate (□–□). The amount of protein in replicate antigen-antibody precipitates, presented on the middle left ordinate (□–□), was determined by the Lowry method after solubilization in 0.1 N NaOH. Data are shown as the average of determinations made on two samples at each antigen level, the vertical lines indicating the range between the two measurements.

as well as of the rat liver (Fig. 5, bottom panel) protein-synthesizing systems. The two proteins migrate with identical mobility, which coincides with that of P-enolpyruvate carboxykinase. Both with the wheat germ and with the rat liver extracts, some radioactivity corresponding to shorter polypeptides is consistently present in the immunoprecipitate, as may be seen in Fig. 5 and Fig. 2. Whether these minor products originate from the premature release of partially completed P-enolpyruvate carboxykinase chains by "early quitting" ribosomes or from the hydrolysis during incubation of finished enzyme molecules, or whether they betray unspecific adsorption to the precipitate of peptides unrelated to P-enolpyruvate carboxykinase is not known. In any case, the amount of contaminating radioactivity is always very small and does not interfere with the accurate quantitation of the radioactivity in the major peak.

The demonstration that the immunoprecipitable polypeptide synthesized by the wheat germ S-23 is in fact immunologically identical with P-enolpyruvate carboxykinase is presented in Fig. 6. Aliquots of the cell-free products made by the wheat germ extract in the presence of poly(A) RNA from rat liver were incubated with a constant amount of antibody against P-enolpyruvate carboxykinase and with increasing amounts of carrier antigen in the form of purified hepatic enzyme. After completion of the antigen-antibody reaction, the immunoprecipitate was analyzed by SDS-polyacrylamide gel electrophoresis and the radioactivity in the enzyme band was determined. As may be seen, the amount of radioactive enzyme-like material precipitated is constant in the zone of antibody excess. However, in the zone of antigen excess, indicated by the progressive appearance of P-enolpyruvate carboxykinase activity in the supernatant of the antigen-antibody reaction mixture, the immunoprecipitation of the labeled product becomes incomplete. Within the limits of the experimental error, the equivalence points in the precipitin reaction for purified P-enolpyruvate carboxykinase and for the wheat germ-made, antibody-reactive protein coincide. This observation indicates that the immunoprecipitable translation product of the wheat germ S-23 is identical with rat liver P-enolpyruvate carboxykinase.

To determine the level of functional mRNA for P-enolpyruvate carboxykinase during cyclic AMP induction, several liver RNA extractions were performed using tissue from rats treated for 75 min with dibutyryl cyclic AMP and theophylline and tissue from controls. The yield of total tissular RNA and poly(A) RNA was the same in the control and experimental groups, respectively, 5.33 ± 0.32 and 5.84 ± 0.28 μg of total RNA/g of liver and 134 ± 20 and 161 ± 12 μg of poly(A) RNA/
Poly(A)+RNA was isolated from total tissue RNA extracted 75 min after a control injection or after administration of dibutyryl cyclic AMP and theophylline. When used, cordycepin was injected 30 min before the cyclic nucleotide and theophylline. The rats had been fasted and refed before treatment. Conditions for the preparation and incubation of the wheat germ S-23 are described under "Experimental Procedures." The amounts of poly(A)+RNA listed were added to a total incubation volume of 0.59 ml. The incorporation values are for the total incubation volume. Incorporation into total protein and into released protein has been corrected for endogenous incorporation measured in the absence of added poly(A)+RNA. The endogenous incorporation averaged 1460 × 10^3 dpm into total protein and 449 × 10^3 dpm into released protein. Data are means ± S.E. when relevant.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Poly(A)+RNA added</th>
<th>RNA batches tested</th>
<th>Total protein</th>
<th>Released protein</th>
<th>Enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg</td>
<td></td>
<td>dpm × 10^3</td>
<td>dpm × 10^3</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>5</td>
<td>5</td>
<td>6518 ± 1,030</td>
<td>1881 ± 194</td>
<td>0.50 ± 0.12</td>
</tr>
<tr>
<td>Dibutyryl cyclic AMP</td>
<td>5</td>
<td></td>
<td>6138 ± 217</td>
<td>1706 ± 52</td>
<td>2.86 ± 0.32</td>
</tr>
<tr>
<td>Cordycepin + dibutyryl cyclic AMP</td>
<td>2</td>
<td></td>
<td>4469</td>
<td>1379</td>
<td>0.15</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>5</td>
<td>12086 ± 1,432</td>
<td>3280 ± 250</td>
<td>0.59 ± 0.10</td>
</tr>
<tr>
<td>Dibutyryl cyclic AMP</td>
<td>5</td>
<td></td>
<td>12694 ± 1,324</td>
<td>3517 ± 292</td>
<td>0.70 ± 0.52</td>
</tr>
<tr>
<td>Cordycepin + dibutyryl cyclic AMP</td>
<td>2</td>
<td></td>
<td>8116</td>
<td>2292</td>
<td>0.25</td>
</tr>
<tr>
<td>Control</td>
<td>15</td>
<td>5</td>
<td>15910 ± 1,947</td>
<td>4289 ± 554</td>
<td>0.54 ± 0.24</td>
</tr>
<tr>
<td>Dibutyryl cyclic AMP</td>
<td>5</td>
<td></td>
<td>16873 ± 1,314</td>
<td>4285 ± 273</td>
<td>12.91 ± 1.04</td>
</tr>
<tr>
<td>Cordycepin + dibutyryl cyclic AMP</td>
<td>2</td>
<td></td>
<td>11109</td>
<td>3322</td>
<td>0.46</td>
</tr>
</tbody>
</table>

Fig. 7. Translation by the wheat germ S-23 of a mixture of induced and noninduced poly(A)+RNA. The wheat germ S-23 was incubated for protein synthesis in the presence of poly(A)+RNA from control (○○○○) and from dibutyryl cyclic AMP and theophylline-treated rats (■■■■), as well as in the presence of a 1:1 mixture of the two poly(A)+RNAs (△△△△). The amounts of RNA added to the incubation reaction are indicated on the abscissa. P-enolpyruvate carboxykinase synthesis was quantitated as described under "Experimental Procedures." Overall RNA-directed protein synthesis was similar for the three poly(A)+RNA preparations translated.
duced tissues (data not shown). The latter observation excludes the preferential loss of the mRNA coding for the enzyme during the extraction of RNA from noninduced liver.

**DISCUSSION**

The induction of hepatic P-enolpyruvate carboxykinase by cyclic AMP is well recognized (for a review see Ref. 42). However, in the experimental systems studied so far, which include the whole animal (15, 17) as well as in vitro models such as liver explants from rat fetuses (43), Reuber H-35 hepatoma cells (18, 19, 44), and the isolated perfused liver (45), the magnitude of the induction attainable has been rather limited. Here we report that the effect of dibutyryl cyclic AMP on liver P-enolpyruvate carboxykinase synthesis in the rat can be markedly accentuated by submitting the animal to a preliminary cycle of fasting and glucose refeeding. After this dietary manipulation, the stimulation of the enzyme synthesis by cyclic AMP is rapid, large, and consistent and can serve as a good experimental model for the study of the mechanisms by which the nucleotide regulates the synthesis of a specific gene product in eukaryotes.

Using a cell-free protein-synthesizing system derived from the liver of fasted-refed rats injected with dibutyryl cyclic AMP and theophylline, we noted a 5-fold increase in the synthesis of P-enolpyruvate carboxykinase within 20 min after the treatment. Given the inability of the liver extract to initiate polypeptide chain synthesis in vitro, this observation indicates that the number of ribosomes engaged in the synthesis of the enzyme rapidly increases in vivo after exposure to cyclic AMP. Evidence is presented in this paper which suggests that these ribosomes are carrying out the translation of newly synthesized rather than pre-existing P-enolpyruvate carboxykinase mRNA.

Cordycepin injected before the inducer completely abolishes the increase in the capacity of the liver postmitochondrial supernatant to synthesise P-enolpyruvate carboxykinase. This finding is in agreement with the initial observation of Yeung and Oliver (14), later confirmed by Hanson et al. (16), that actinomycin D blocks the enzyme-inductive effect of cyclic AMP in fetal rats, as well as with the results of Wicks (43) showing the pronounced inhibitory effect of the antibiotic on the induction of P-enolpyruvate carboxykinase by dibutyryl cyclic AMP in organ culture. As pointed out above, our data exclude a general impairment of the protein synthesis machinery or an unspecific inhibition of all hepatic actions of cyclic AMP by cordycepin. On the other hand, they suggest a relationship between inhibition of poly(A)+RNA synthesis and suppression of the enzyme induction. We, therefore, conclude that the induction of P-enolpyruvate carboxykinase by dibutyryl cyclic AMP in the fasted-refed rat is absolutely dependent on the synthesis of new mRNA. Even though, as discussed below, our conclusion does not preclude a purely translational effect of cyclic AMP, it is in apparent conflict with previous results of other investigators. Wicks and McKibbon (18) suggested that the early rise in P-enolpyruvate carboxykinase activity in Reuber H-35 cells incubated with dibutyryl cyclic AMP was insensitive to actinomycin D. Similarly, Krone et al. (45) reported that cordycepin did not block the increase in enzyme activity taking place in the isolated rat liver perfused with dibutyryl cyclic AMP. These data, however, may not be strictly comparable to ours because the cell systems under investigation were markedly different.

The poly(A)-containing RNA isolated from the liver of induced animals exhibits increased template activity for the synthesis of P-enolpyruvate carboxykinase in a translational assay using a wheat germ extract. We have excluded artifacts which could arise from the presence in the poly(A)+RNAs of positive or negative effectors of the translation of the enzyme mRNA or from the preferential loss of the template during the extraction of uninduced tissue. The results derived from a biological assay do not, by themselves, indicate whether a net increase in the content of mRNA coding for the enzyme or an activation of preformed messenger takes place during induction. However, the first alternative is clearly supported by the demonstration that a pretreatment with cordycepin prevents the occurrence of the change. Thus, we propose that a net increase in P-enolpyruvate carboxykinase mRNA concentration occurs in the liver during the induction caused by dibutyryl cyclic AMP.

The mechanism for the accumulation of the messenger remains hypothetical. It could involve, as in the classical case of the β-galactosidase induction in *Escherichia coli* (46), a cyclic AMP-dependent stimulation of gene transcription. In eukaryotes cyclic AMP has been implicated in the regulation of the phosphorylation of some nuclear nonhistone proteins (47, 48) and the state of phosphorylation of these proteins appears to have regulatory significance in the control of genetic expression (49–52). Recently, Costa and his colleagues (53), on the basis of their work on the cyclic AMP- mediated induction of tyrosine 3-mono-oxygenase in the rat adrenal medulla, have proposed a model in which the interaction of cyclic AMP with a cytosolic protein phosphokinase results in the translocation of the catalytic subunit of the kinase into the nucleus (see also Ref. 54), where chromatin phosphorylation and stimulation of transcription ensue (55). In addition to purely transcriptional mechanisms favoring gene read-out, the post-transcriptional modification of the primary transcript and the transport of the mRNA into the cytoplasm might be controlled by cyclic AMP. Clearly, if any of these mechanisms operates in the induction of P-enolpyruvate carboxykinase, it has to do so with extreme rapidity.

Alternatively, the build-up of mRNA coding for P-enolpyruvate carboxykinase during induction might be due to the stabilization of the messenger. Palmiter and Carey (56) have shown that the half-life of ovalbumin mRNA in the chick oviduct can vary greatly according to the hormonal status of the animal, apparently in relationship with the rate of initiation of ovalbumin synthesis. Histone mRNAs are stable for hours when translated (57), but disappear within minutes when translation ceases (58). If, in a similar manner, there is a great difference in the stability of the mRNA coding for P-enolpyruvate carboxykinase according to whether the messenger is naked or covered with ribosomes, large fluctuations in the level of the messenger might result from varying the rate of initiation of enzyme chain synthesis. It is conceivable that under uninduced conditions the initiation of the enzyme synthesis is minimal and that the enzyme mRNA is degraded within minutes of its export into the cytoplasm. The mechanism of the induction by cyclic AMP could then consist of a stimulation of the formation of initiation complexes involving the enzyme template, resulting in its stabilization and accumulation. Under these circumstances the mRNA would build up without any transcriptional or post-transcriptional stimulation. A continuous flow of newly synthesized mRNA from the nucleus would nevertheless be required, in agreement with our cordycepin data, because little mRNA would exist free in the cytoplasm before induction. This model implies an action of cyclic AMP at the initiation step of P-enolpyruvate carboxy-
kinase synthesis. Therefore, the type of mechanism based on a cyclic AMP-dependent stimulation of the release of completed protein chains from the polyribosomes, which was proposed by Chauhan and Oliver (59, 60) in their studies of the postnatal induction of tyrosine aminotransferase, does not apply in the case of P-enolpyruvate carboxykinase induction. Any model postulating an action of cyclic AMP at initiation must include features ensuring the selectivity of the effect. As discussed by Gressner and Wool (61), the rapid increase in the phosphorylation of one protein in the small subunit of the liver ribosomes after administration of cyclic AMP might provide a way by which the entrance of a few selected mRNAs into initiation complexes might be favored.

There is as yet no direct evidence favoring any one of the above mechanisms at the exclusion of the others. It is indeed a case of P-enolpyruvate carboxykinase induction. Any model after administration of cyclic AMP might provide a way by which the entrance of a few selected mRNAs into initiation complexes might be favored.

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