In order to provide further information regarding one of the 
NH₂OH-sensitive bonds, the appropriate peptides were sub-
jected to Edman degradation (16) and the phenylthiohydantoin 
amino acids identified by thin layer chromatography on sheets of 
silica gel containing a fluorescent indicator (Eastman). Chroma-
tograms were developed with Solvents D and E of Edman and 
Sjöquist (17). The results of amino acid analyses of resid-
ual peptides, performed after each degradation step of T₄, 
agreed with those obtained by thin layer chromatography of 
the phenylthiohydantoin amino acids. These experiments indi-
cated the following partial sequences:

NH₂ terminus of α₁-CB₃: Gly–Phe–Hyp–Gly–Pro–
NH₂ terminus of H₄: Gly– Ala– Hyp–
T₄: Gly– Asn– Asn– Gly– Ala– Hyn– (Gly, Aex, Ala)–Lys

The identity of the amino acid sequences of Residues 4 to 6 of 
T₄ and of 1 to 3 of H₄ further strengthens the conclusion that T₄ 
and H₄ are derived from the same area of α₁-CB₃, and that T₄ 
contains a bond susceptible to cleavage by NH₂OH. Since 
the sequences at the NH₂ ends of α₁-CB₃ and of H₄ are not iden-
tical, NH₂OH cleavage produced a new NH₂-terminal sequence. 
These observations strongly suggest that the Asn–Gly (peptide) 
bond in α₁-CB₃ (formed from Residues 3 to 4 of T₄) was cleaved 
by NH₂OH to yield H₄ as one of its products. 

Recent experiments by Volpin, Hörmann, and Kühn (18) 
involving isolation and characterization of some products of 
NH₂OH cleavage of denatured calf skin collagen have provided 
evidence for a NH₂OH-sensitive bond in the central portion of 
the α₁ chain. The bond which they are studying is perhaps 
identical with the one reported here, since α₁-CB₃ is derived 
from this same area of α₁ (10).

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An Adenosine 3',5'-Monophosphate-de-
dependent Protein Kinase from 
Escherichia coli

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SUMMARY

An enzyme present in Escherichia coli which catalyzes the 
phosphorylation of histone by ATP has been found to be 
stimulated by adenosine 3',5'-monophosphate (cyclic AMP). 
The rate of the enzyme-catalyzed phosphorylation of histone 
was increased by about 300% at concentrations of cyclic 
AMP above 7 x 10⁻⁷ M. The apparent Kₘ of the E. coli en-
zyme for cyclic AMP was about 2 x 10⁻⁷ M.

A cyclic adenosine 3',5'-monophosphate-dependent protein kinase was recently found in rabbit skeletal muscle by Walsh, 
Perkins, and Krebs (1) which catalyzes the phosphorylation of 
casein, of protamine, and of phosphorylase kinase. It was pro-
posed (1) that this enzyme serves as the link between the epi-
nephrine stimulation of adeny cyclase and the activation of 
phosphorylase kinase which occurs in skeletal muscle in vivo. 
Subsequently, cyclic AMP-dependent enzymes which catalyze 
the phosphorylation of histone and protamine have been found 
in calf liver (2) and in rabbit brain (3). Therefore, it seems pos-
sible, as the mechanism by which cyclic AMP exerts its very 
different effects in various tissues, e.g. glycosogenesis, ste-
roidogenesis, lipolysis, etc., that cyclic AMP mediates its action 
by activating specific protein kinases in these tissues. Since cyclic 
AMP is distributed very widely in nature, including microor-
ganisms, one test of this protein kinase hypothesis would be to 
examine microorganisms for the presence of a cyclic AMP-
dependent protein kinase, and for that reason, the present ex-
periments were carried out. Escherichia coli, which has been 
found to contain cyclic AMP (4), was used for the present inves-
tigation.

Preparation of Protein Kinase—Lyophilized cells (2.5 g) of 
E. coli K-12 (lot 117B-0020, Sigma) were mixed thoroughly with 
40 g of glass beads (Type 100, 5005, 3M Company) and 20 ml 
of 4 mM EDTA, pH 7.5. The cells were ruptured by homoge-

# This investigation was supported by Grant GB 8391 from the Na-
# tional Science Foundation.

* The abbreviation used is: cyclic AMP, cyclic adenosine 3',5'-monophosphate.
trifugation, dissolved in 15 ml of 5 mM Tris-Cl, pH 7.5, containing 2 mM EDTA, and followed by extensive dialysis against the same buffer. The pH of the enzyme solution was then adjusted to 5.9 with ice-cold 1 N acetic acid, frozen at -20° overnight, thawed, and centrifuged to remove precipitate formed. The supernatant, after being extensively dialyzed against 5 mM Tris-Cl, pH 7.5, containing 2 mM EDTA, was used as the source of the cyclic AMP-dependent protein kinase in the experiments reported in this communication.

The activity of the cyclic AMP-dependent protein kinase from E. coli was assayed in an incubation volume of 0.2 ml containing: Tris-Cl, pH 7.5, 5.0 μmoles; EDTA, 0.02 μmole; γ-32P-ATP, 1.1 × 10⁶ μmole (containing about 3.5 × 10⁶ cpm); MnCl₂, 0.2 μmole; histone (from calf thymus, Mann), 400 μg; protein kinase preparation, 56 μg of protein; with or without cyclic AMP (Schwarz BioResearch), 2 × 10⁻⁴ μmole. Incubation was carried out for 50 sec to 2 min at 30° in a shaking water bath. The reaction was initiated by the addition of radioactive ATP. The reaction was terminated and the protein-bound 32P was determined as described for the enzyme from skeletal muscle (1, 5).

Using the purification procedure and the assay conditions described above, the specific activity of the partially purified enzyme was increased about 78-fold over that of the crude extract. For the purpose of calculating the activity and extent of purification of the cyclic AMP-dependent protein kinase, the difference between the rate of phosphorylation in the presence and absence of cyclic AMP was used. It was not possible to determine total recovery of the enzyme with accuracy, since highly inhibitory material was removed during the course of purification; the final preparation contained 47% of the starting units of enzyme activity, accompanied by a protein recovery of about 6%. Inhibitory material was also found during the course of purification of the cyclic AMP-dependent protein kinase from brain (3).

Properties of Protein Kinase Obtained from E. coli—The activity of the protein kinase was proportional to the amount of enzyme preparation added (up to 112 μg, the highest amount tested). The time course of histone phosphorylation catalyzed by the protein kinase from E. coli is shown in Fig. 1. The cyclic AMP-stimulated reaction was linear for about 1 min. When the reaction was allowed to proceed for longer than 5 min, the cyclic AMP stimulation of phosphorylation was greatly reduced or abolished. The absolute requirement of cyclic AMP-dependent protein kinase for MnCl₂ is shown in Fig. 2. The dependence of the cyclic AMP stimulation on MnCl₂ concentration showed a rather sharp optimum at about 0.9 to 1.0 mM, with activity falling off very rapidly at higher levels of MnCl₂. When MgCl₂ replaced MnCl₂ in the incubation mixture, no effect of cyclic AMP on protein kinase activity was observed; this was true over a range of concentrations of MgCl₂ up to 10 mM. In the absence of added cyclic AMP, MgCl₂ was slightly stimulatory at low concentrations (32% stimulation at 0.25 mM) and inhibitory at somewhat higher concentrations (80% inhibition at 1.25 mM). In contrast to the situation with the enzyme from E. coli, the cyclic AMP-dependent protein kinases from skeletal muscle, liver, and brain are active in the presence of MgCl₂.

A variety of proteins were tested for their ability to act as substrates for the cyclic AMP-dependent protein kinase from E. coli and, as shown in Table I, histone was found to be the most effective phosphate acceptor of the substances tested. Histones were also found to be more effective than protamine as substrates for the cyclic AMP-dependent protein kinases from liver (2), brain (3), and skeletal muscle.

The effect of cyclic AMP concentration on the rate of histone phosphorylation catalyzed by the E. coli enzyme is shown in Fig. 3. The apparent $K_m$ for cyclic AMP was calculated to be about $2 \times 10^{-7}$ M. In comparison, the apparent $K_m$ for cyclic AMP of the muscle kinase was $6 \times 10^{-5}$ M using protamine as substrate.

\[ + \text{CYCLIC AMP} \]

\[ \text{MnCl}_2 (\text{mM}) \]

\[ \text{proteins P INCORPORATED} \]

\[ 2.4 \]

\[ 2.0 \]

\[ 1.6 \]

\[ 1.2 \]

\[ 0.8 \]

\[ 0.4 \]

\[ 0.0 \]

\[ \text{CONTROL} \]

\[ 0 \]

\[ 0.5 \]

\[ 1.0 \]

\[ 1.5 \]

\[ 2.0 \]

\[ \text{INCUBATION (MIN.)} \]

\[ \text{FIG. 1. Time course of histone phosphorylation catalyzed by E. coli protein kinase in the presence or absence of cyclic AMP. The incubation conditions were as described in the text except for varying the incubation time.} \]

\[ \text{FIG. 2. Effect of MnCl}_2 \text{ on histone phosphorylation catalyzed by E. coli protein kinase in the presence or absence of cyclic AMP. The incubation conditions were as described in the text except for varying the MnCl}_2 \text{ concentration. The incubation time was 45 sec.} \]

TABLE I
Substrate specificity of cyclic AMP-dependent protein kinase from E. coli

<table>
<thead>
<tr>
<th>Substrate</th>
<th>a3P incorporated</th>
<th>Stimulation by cyclic AMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+Cyclic AMP</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>0.375</td>
<td>0.398</td>
</tr>
<tr>
<td>Histone</td>
<td>0.532</td>
<td>1.996</td>
</tr>
<tr>
<td>Protamine</td>
<td>0.837</td>
<td>0.840</td>
</tr>
<tr>
<td>Bovine serum albumin</td>
<td>0.637</td>
<td>0.780</td>
</tr>
<tr>
<td>Phosvitin</td>
<td>0.556</td>
<td>0.649</td>
</tr>
<tr>
<td>Phosphocreatine</td>
<td>0.532</td>
<td>1.996</td>
</tr>
</tbody>
</table>

Fig. 3. Effect of cyclic AMP concentration on histone phosphorylation catalyzed by E. coli protein kinase. The incubation conditions were as described in the text except for varying the cyclic AMP concentration. The incubation time was 30 sec. The amount of a3P incorporated was corrected for that in the absence of cyclic AMP, which amounted to 0.2 pmole.

DISCUSSION

The discovery of a cyclic AMP-dependent protein kinase in E. coli lends support to the hypothesis, discussed above, that all of the wide variety of effects elicited by cyclic AMP may be mediated through stimulation of protein kinases. Such a mechanism provides a unifying theory for the action of cyclic AMP. The tissue-specific effects of cyclic AMP on a given tissue would be accounted for in terms of the specific protein kinase and its substrate in that tissue. From our work thus far, it seems clear that cyclic AMP-dependent protein kinases from muscle, brain, and E. coli differ from one another with regard to their behavior on ion exchange resins, during isoelectric precipitation, in their metal ion requirements, etc. The tissue-specific properties of the different protein kinases, indicated by these results, together with the nature of the substrate of the protein kinase present in any particular tissue, would provide the basis for the tissue-specific actions of cyclic AMP.

The mechanism by which cyclic AMP stimulates the various protein kinases remains totally unknown. The recent demonstration (6) that the standard free energy of hydrolysis of cyclic AMP is extremely high (about 12,000 calories) and that, in the presence of adenyl cyclase, cyclic AMP can adenylate inorganic pyrophosphate to form ATP, has led to the suggestion that cyclic AMP may function biologically by acting as a protein adenylylating agent (6). It seems possible that cyclic AMP functions by adenylylating tissue-specific protein kinases, and thereby activating these kinases.

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Regulation of Phosphatidylcholine Biosynthesis by the Methylation Pathway in Saccharomyces cerevisiae*

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SUMMARY

The labeling of the methylated phospholipids of Saccharomyces cerevisiae has been studied with methionine-methyl-14C, serine-3-14C, and choline-1,2-14C as precursors. It was found that inclusion of 1 mM choline in the growth media greatly lowered the rate of labeling of the methylated phospholipids by methionine and serine; sterol labeling was unaffected. It is suggested that this is a result of repression of synthesis of the specific N-methyl transferases involved in the biosynthesis of phosphatidylcholine. A study in vitro with methyl-14C-S-adenosylmethionine as methyl donor showed that particles prepared from cells grown in the ab-

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