Evidence Against the Presence of 3',5'-Cyclic Adenosine Monophosphate and Relevant Enzymes in Lactobacillus plantarum

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Analysis of cells of Lactobacillus plantarum, starved or undergoing induction, showed no 3',5'-cyclic adenosine monophosphate (cAMP). Neither adenylyl cyclase nor 3',5'-cAMP phosphodiesterase was detected in extracts. Extracts of L. plantarum did not inhibit these two enzymes of Escherichia coli K-12, strain W1435. Incubation of adenosine triphosphate (ATP)-U-14C with cells or various cell-free fractions of L. plantarum did not produce labeled 3',5'-cAMP. Of various 3',5'-cyclic and acyclic nucleotides tested, only 3',5'-cAMP, ATP, and yeast adenylic acid stimulated L-arabinose isomerase. Yeast adenylic acid was two to four times as effective as 3',5'-cAMP or ATP. 2',3'-cAMP was not effective.

Since the discovery of 3',5'-cyclic adenosine monophosphate (cAMP) in 1957, its wide distribution together with enzymes related to its synthesis, degradation, and function has been demonstrated in various tissues and organisms from mammals to bacteria.

In view of the implication of 3',5'-cAMP in the regulation of several cellular activities, we attempted to detect this compound and its enzymatic systems in the gram-positive Lactobacillus plantarum. We have also tested the effect of 3',5'-cAMP and other compounds on the activity of L-arabinose isomerase. Evidence is presented against the presence of endogenous 3',5'-cAMP, adenylyl cyclase, and 3',5'-cAMP phosphodiesterase—at least in concentrations or activities detectable by the methods used. Added 3',5'-cAMP stimulated L-arabinose isomerase, and adenosine triphosphate (ATP) was as effective as 3',5'-cAMP, whereas yeast adenylic acid was two to four times as effective as 3',5'-cAMP or ATP.

MATERIALS AND METHODS

Reagents. The sources of chemicals are: 3',5'-cAMP and N4-O-dibutyryl cAMP, Calbiochem, Los Angeles, Calif; yeast adenylic acid, a mixture of 2' and 3'-AMP·H2O, BDH Chemicals Ltd., Poole, England; 3',5'-cyclic cytidine, guanosine, inosine, deoxythymidine, and uridine monophosphates (CMP, GMP, IMP, dTMP, UMP), Boehringer, Mannheim GmbH, Germany; 2',3'-cAMP, kindly provided by Boehringer. All of these nucleotides were available as the monosodium salts except yeast adenylic acid, 3',5'-cyclic IMP, and 2',3'-cAMP, which were in the acid form. ATP·4H2O, disodium salt, was from Mann Research Laboratories, New York; L-arabinose, Nutritional Biochemical Corp., Cleveland, Ohio; phosphorylase b, Boehringer; Crotalus atrox snake venom, Sigma Chemical Co., St. Louis, Mo. All radiochemicals were obtained from the Radiochemical Centre, Amersham, England.

Growth of cells. L. plantarum (ATCC 8014) was grown for 12 hr at 30°C in two different media: medium P (20) containing 1% (w/v) glucose and with casein hydrolysate replaced by peptie peptone; or the enriched medium containing 0.5% glucose as described (2). All of the methods indicated below were repeated several times on the cells grown in each medium and on cell-free preparations thereof.

Enzyme preparations. Cells were lysed as described (8). The lysate was centrifuged at 3,000 × g for 30 min, and the pellet was discarded. The supernatant fraction was further centrifuged at 35,000 × g for 15 min. Our studies were performed on the 3,000 × g supernatant fraction or the 35,000 × g supernatant fraction and the 35,000 × g pellet. Protein was determined as described (12). Unpublished work showed that membranes were mainly recovered from the 3,000 × g supernatant fraction.

Assay of endogenous 3',5'-cAMP. Cells grown in either medium were harvested in the cold, and occasionally washed with 0.03 M tris(hydroxymethyl) aminomethane (Tris)-hydrochloride, pH 8.0. Washed or unwashed cells were then processed by the following two different methods.

An acid extract was prepared as described by...
Makman and Sutherland (13) by fixing the cells in cold 0.05 n HCl (the ratio of wet weight of cells to acid was 1:10). This was followed by placing the cell suspension in boiling water for 10 min. The acid extract was chilled and centrifuged at 3,000 × g for 15 min. The supernatant solution was neutralized before assay of its 3',5'-cAMP content.

The second method involved preparing a cell-free extract of lysozyme- and thiol-treated cells as described (7). The 3,000 × g supernatant fraction of lysed cells was placed in a boiling water bath for 5 min, and the resulting precipitate was removed by centrifugation at 3,000 × g for 15 min. Every 3 g of wet cells gave 5 ml of extract.

The assay of 3',5'-cAMP was performed by the modified (13) colorimetric method of Rall and Sutherland (18). Samples were always assayed in duplicate. The variation in absorbancy did not exceed ±5% of the average value. 3',5'-cAMP was measured in samples of the extracts representing 5 mg of wet cells. Alternatively, extracts were lyophilized so that a single determination utilized the equivalent of 250 mg of wet cells.

Chromatography and measurement of radioactivity. Radioactive products formed from ATP-U-14C were analyzed by ascending chromatography on Whatman no. 3 paper. The chromatogram was developed with ethanol–1 M ammonium acetate, pH 7.5 (75:30, v/v) for 20 hr at room temperature (10). The resulting spots were made visible under ultraviolet light, cut out, suspended in a scintillation medium, and counted by using a Packard Tri-Carb scintillation spectrometer model 3003 (80% efficiency).

Assay of adenyl cyclase. Cells of L. plantarum were lysed as described (8). Adenyl cyclase was assayed in the following preparations: (i) The 3,000 × g supernatant fraction; (ii) the 35,000 × g supernatant fraction; (iii) a suspension of the pellet in a volume of buffer equal to that of the 35,000 × g supernatant fraction. The amounts of protein used in the assay were respectively 30, 17, and 13 mg per reaction mixture. The enzyme was assayed (21) as follows: 1.0 ml of the desired fraction of the enzyme preparation was incubated with 1.4 ml of a reaction mixture containing 0.04 mg Tris-hydrochloride (pH 7.5), 3.5 × 10⁻⁸ M MgSO₄, 7 × 10⁻⁸ M caffeine (an inhibitor of some phosphodiesterases), and 2.5 × 10⁻⁸ M ATP at 30 C for 30 min. The incubation was terminated by placing the reaction tubes in a boiling water bath for 5 min. After chilling and centrifugation, 0.05-ml samples of the supernatant fluid were tested for 3',5'-cAMP production by the phosphorolysis β kinase activation method (19).

Adenyl cyclase also was assayed (10) using ATP-U-14C. The 0.5-ml incubation mixture containing 8 × 10⁻⁸ M MgSO₄, 0.05 M Tris-hydrochloride (pH 7.2 or 7.8), 3 × 10⁻⁴ M ATP-U-14C (0.65 μCi/μmole), and 8 mg of protein. The reaction was terminated after 30 min at 30 C by placing the tubes in a boiling water bath for 5 min. The suspension was centrifuged, and a sample of the supernatant fluid (25 μl) was applied to Whatman no. 3 paper for chromatography and determination of radioactive products.

Adenyl cyclase was also assayed (9) at a higher pH. The 0.15-ml reaction mixture contained 0.10 mM Tris-hydrochloride (pH 9.0), 0.10 M MgSO₄, 2 × 10⁻⁴ M ATP-U-14C (0.13 μCi/μmole), and 2 mg protein of the 3,000 × g supernatant fraction were run for 30 min at 33 C and arrested by placing the tubes in a boiling water bath for 5 min. Twenty-four such incubation mixtures were run simultaneously, pooled, and centrifuged. The supernatant fraction was lyophilized to 0.2 ml. Portions (5 μl) were analyzed by paper chromatography and scintillation counting to determine the possible formation of radioactive 3',5'-cAMP.

Assay of phosphodiesterase. The enzyme was assayed essentially as described (4). The reaction mixture contained 1.5 mg of protein (35,000 × g or 3,000 × g supernatant fraction, 1 μmole of 3',5'-cAMP, 0.2 μmole of MgSO₄, 20 μmoles of Tris-hydrochloride buffer (pH 8.5), in a final volume of 0.5 ml. In several experiments various concentrations of the ingredients were used. The extracts were also tested before or after passing them through Sephadex G-25. The extracts were also assayed within a range of pH 7.3 to 8.4. The incubation was for 20 min at 30 C. The reaction was terminated by immersing the tube in boiling water for 5 min. The cooled mixture was centrifuged and the supernatant fluid was adjusted to pH 7.3 with 1 N HCl. Crotalus atrox snake venom (0.1 mg in 0.10 ml of 0.05 M Tris-hydrochloride, pH 7.3) was immediately added. After 10 min, the reaction was arrested by placing the tube in a boiling water bath for 5 min. The clear supernatant fluid recovered following centrifugation was assayed for its content of inorganic phosphate as described (7). Three controls containing boiled extract, boiled venom, or no substrate were used. The enzyme was also assayed (15) at pH 8 using 5 mM 3',5'-cAMP.

L-Arabinose isomerase induction. The inducibility of L-arabinose isomerase has been demonstrated in L. plantarum (2) and in other bacteria as well (3, 6, 19). Methods used previously (2) were adopted. Cells grown for 10 hr on the enriched medium (2) with 0.5% glucose were harvested in the cold, and washed with a volume of enriched medium (not containing glucose) equal to the volume of the growth culture. The cells were then resuspended in the same medium not containing glucose to an optical density of 0.11 to 0.12 at 660 nm. The suspension was then supplemented with 0.1% L-arabinose and any one of the nucleotides to be tested. The control was similarly treated, but no nucleotide was added. Incubations (in 100-ml Erlenmeyer flasks) were at 35 C with shaking. The pH of all incubations was 6.2 to 6.6. Growth and enzyme levels were checked at various time intervals (see Table 4). The treatment of cells for enzyme assay involved (i) removing 1-ml samples of the culture, (ii) adding 0.2 ml of toluene, mixing continuously for 20 min on a rotator, (iii) centrifuging for 15 min at 3,000 × g, (iv) resuspending the pellet in a volume of 0.05 M Tris-hydrochloride (pH 7.5), such that 0.10 ml of the suspension corresponds to 0.125 mg of cells. The assay mixture consisted of 0.10 ml of the toluenized cell
suspension, 0.10 ml of a solution of L-arabose (10 mg/ml), and 0.80 ml of 0.08 M Tris-hydrochloride, pH 7.5. Incubation was for 15 min at 35 C. The production of L-ribulose was determined by the carboxy-vol color test (5). The control for color determination contained L-arabinose and buffer, the tolenuenized cells had no significant effect on the development of the color.

Lack of ATP conversion into 3',5'-cAMP in cells undergoing induction of L-arabinose isomerase. Since investigators reported high levels of 3',5'-cAMP in bacterial cells undergoing induction, it became of interest to determine whether 3',5'-cAMP could be detected in cells of L. plantarum undergoing induction of L-arabinose isomerase. Consequently, cells were grown on the enriched medium, harvested, washed, suspended, and supplemented with arabinose as described above. ATP-U-14C (100 amoles, 0.3 μCi) was added to each 50 ml of such suspensions. After 1, 2.5, and 3.5 hr of incubation, cells were collected and acid-extracted. The cells were not washed before extraction to avoid any possible leakage of radioactive compounds into the washing buffer. A 25-μliter amount of each acid extract (i.e., about one-tenth of the total extract volume) was chromatographed and analyzed for radioactive 3',5'-cAMP as described earlier.

RESULTS

Assay of endogenous 3',5'-cAMP. The assay method relying on phosphorylase b kinase activation was tested on two other biological systems before it was applied to L. plantarum. The results obtained with glucose-fed or glucose-starved E. coli K-12, W1435, agreed with those reported earlier (18). The 3',5'-cAMP content of the superior cervical ganglia of cats with or without electrical stimulation (30 sec-1) was also measured by this method, yielding a significant and consistent basal content of the cyclic nucleotide with at least a twofold increase after electrical stimulation. Similarly, significant activities of adeny1 cyclase and phosphodiesterase in the ganglia were detected by the corresponding methods of assay (4, 21; and Atweh and Sahyoun, unpublished experiments). Every experiment was accompanied by a standard curve as described (18). Furthermore, no inhibitor of the phosphorylase system in our bacterial extracts was detected. This was determined by adding concentrates of L. plantarum extracts to controls containing 3',5'-cAMP and the assay mixture. 3',5'-cAMP could not be detected in acid extracts of the cells grown in either medium or in 3,000 x g supernatant or pellet fractions of lysed cells. Typical results are shown in Table 1. The possibility that such extracts were too dilute to allow detection was eliminated by concentrating the extracts such that each determination was done on the equivalent of 250 mg of wet cells, which also gave negative results.

It has been reported that glucose starvation increases 3',5'-cAMP concentrations in E. coli (13). Hence L. plantarum was grown on Skegg’s medium P (20) containing various concentrations of glucose (0.1 to 1%, w/v) for 12 hr. The harvested cells were washed and resuspended in a volume of 0.03 M Tris-hydrochloride, pH 7.5. (Optical density of the suspension at 660 nm was 40.) The cells were starved at 30 C for 3.5 and 7 hr. Subsequently an acid extract was prepared. Neither the extract nor the buffer in which the cells were suspended had any detectable 3',5'-cAMP.

Some bacteria release relatively large quantities of 3',5'-cAMP when grown in the presence of adenine or adenosine (T. Ishiyama, T. Yokatsuka, and N. Saito, West German patent no. 1,926,072; see also Chem. Abstr. 73;2676f). Consequently, L. plantarum cells were grown in the enriched medium with either 0.1% glucose, 0.1% glucose and 0.2% adenine, or 0.1% glucose and 0.2% adenosine for 14 hr. Neither the acid extract nor the growth medium had any detectable 3',5'-cAMP.

Similarly, a high level of 3',5'-cAMP was expected during periods of enzyme induction. Hence, acid extracts of cells were prepared at 1, 2.5, and 3.5 hr during an L-arabinose isomerase induction experiment (the inducer being arabinose). These periods correspond to zero induction, and a progressively increasing level of enzyme induction. None of the samples had any demonstrable 3',5'-cAMP.

Phosphodiesterase. The possibility that 3',5'-cAMP was not detected because of a relatively high 3',5'-cAMP phosphodiesterase activity was investigated by adding 3',5'-cAMP to extracts of L. plantarum. A 3',5'-cAMP-dependent release of inorganic phosphate would then indicate the presence of the

<table>
<thead>
<tr>
<th>Material assayed</th>
<th>Absorbancy at 660 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank (water)</td>
<td>0.225</td>
</tr>
<tr>
<td>Standard (7 x 10^-1 M 3',5'-cAMP solution)</td>
<td>0.325</td>
</tr>
<tr>
<td>Bacterial deproteinized 3,000 x g supernatant fraction</td>
<td>0.215</td>
</tr>
</tbody>
</table>

* See reference 13 for assay.
phosphodiesterase in *L. plantarum*. Applications of methods for the detection of the bacterial enzyme or the rat brain enzyme to 3,000 × g supernatant fluid or 35,000 × g supernatant fluid and the pellet thereof failed to detect a 3',5'-cAMP-dependent activity.*<sup>1</sup>

Although extracts of *L. plantarum* were passed through Sephadex, nevertheless the possibility that an inhibitor in extracts of *L. plantarum* could have interfered with either enzyme activity or analytical methods was investigated. Cells of *E. coli* K-12, W1435, were grown and an extract was prepared as described (10). Phosphodiesterase of *E. coli* was then assayed (4) in the extract of *E. coli* and simultaneously in a mixture of extracts prepared from *E. coli* and *L. plantarum*. Results (Table 2) indicate that no such inhibitor existed.

**Adenyl cyclase.** Incubation of ATP or ATP-U-<sup>14</sup>C with various fractions of the cell-free extract of *L. plantarum* also failed to demonstrate the formation of 3',5'-cAMP, thereby providing evidence against the presence of adenyl cyclase in *L. plantarum*.

A possible inhibitor for adenyl cyclase was also investigated as described for the phosphodiesterase. Results (Table 3) indicate that the enzyme of *E. coli* was not inhibited.

**Other studies with ATP-U-<sup>14</sup>C.** Since 3',5'-cAMP and enzymes of cAMP metabolism from cells of *L. plantarum* and extracts thereof were not detected, the possible formation of other nucleotides or related compounds were investigated. ATP-U-<sup>14</sup>C (0.65 μCi/μmole) was incubated with the described cell-free preparations of *L. plantarum*. The conditions and ingredients of the incubation (10) were described in Materials and Methods. After the incubation, samples of the deproteinized supernatant fluid were chromatographed, and the paper was scanned for radioactive spots. Only one distinct radioactive spot (*R<sub>e</sub> 0.14*) which co-chromatographed separately and in a mixture with 5'-cAMP was detected. Only the 35,000 × g pellet catalyzed this conversion. The remaining radioactivity was mainly (over 70%) recovered in ATP which remained at the starting line. None of the cyclic nucleotides or yeast adenyl acid had any radioactivity.

**1-Arabinose isomerase induction.** In view of the inducibility of L-arabinose isomerase, the effect of various nucleotides was determined. Table 4 shows data on the effect of 3',5'-cAMP, yeast adenyl acid, and ATP, at a concentration of 3.0 mM, on the enzyme levels. The compound to be tested was added to a 10-ml growth culture of enriched medium

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**Table 2. Lack of an inhibitory effect of lysates of *L. plantarum* on phosphodiesterase of *E. coli*<sup>2</sup>**

<table>
<thead>
<tr>
<th>Source of extract</th>
<th>Inorganic P released (μmoles)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. plantarum</em>, 35,000 × g</td>
<td>0</td>
</tr>
<tr>
<td><em>L. plantarum</em>, 3,000 × g</td>
<td>0</td>
</tr>
<tr>
<td><em>E. coli</em>, 35,000 × g</td>
<td>1.0</td>
</tr>
<tr>
<td><em>E. coli</em>, 3,000 × g</td>
<td>0.95</td>
</tr>
<tr>
<td><em>E. coli</em> + <em>L. plantarum</em>, 35,000 × g</td>
<td>0.90</td>
</tr>
<tr>
<td><em>E. coli</em> + <em>L. plantarum</em>, 3,000 × g</td>
<td>1.0</td>
</tr>
</tbody>
</table>

* For experimental details see text (and reference 4); 1.5 mg of protein was used.

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**Table 3. Lack of an inhibitory effect of lysates of *L. plantarum* on adenyl cyclase of *E. coli*<sup>2</sup>**

<table>
<thead>
<tr>
<th>Source of extract</th>
<th>Counts per min per mg of protein</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. plantarum</em>, 35,000 × g</td>
<td>0</td>
</tr>
<tr>
<td><em>L. plantarum</em>, 3,000 × g</td>
<td>0</td>
</tr>
<tr>
<td><em>E. coli</em>, 35,000 × g pellet</td>
<td>1,500</td>
</tr>
<tr>
<td><em>E. coli</em>, 3,000 × g supernatant</td>
<td>1,700</td>
</tr>
<tr>
<td><em>E. coli</em> + <em>L. plantarum</em>, 35,000 × g</td>
<td>1,800</td>
</tr>
<tr>
<td><em>E. coli</em> + <em>L. plantarum</em>, 3,000 × g</td>
<td>1,550</td>
</tr>
</tbody>
</table>

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**Table 4. Effect of yeast adenyl acid, 3',5'-cAMP, and ATP on L-arabinose isomerase induction**

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>None</th>
<th>Yeast adenyl acid</th>
<th>3',5'-cAMP</th>
<th>ATP</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.6</td>
<td>2.0</td>
<td>11.2</td>
<td>0.14</td>
</tr>
<tr>
<td>2.5</td>
<td>0.4</td>
<td>6.4</td>
<td>11.2</td>
<td>0.14</td>
</tr>
<tr>
<td>3.75</td>
<td>4.8</td>
<td>12.0</td>
<td>8.8</td>
<td>11.2</td>
</tr>
<tr>
<td>5.0</td>
<td>6.8</td>
<td>12.0</td>
<td>8.8</td>
<td>10.8</td>
</tr>
<tr>
<td>6.25</td>
<td>10.0</td>
<td>24.0</td>
<td>14.8</td>
<td>16.4</td>
</tr>
<tr>
<td>7.25</td>
<td>6.0</td>
<td>14.8</td>
<td>7.8</td>
<td>12.4</td>
</tr>
</tbody>
</table>

* One unit of specific activity corresponds to a net change of 0.1 unit of absorbancy per 1 mg of cell under the experimental conditions of induction. Yeast adenyl acid is an equal mixture of 2'-AMP and 3'-AMP.
in a 100-ml Erlenmeyer flask with 0.10% L-arabinose. In a series of six experiments yeast adenylic acid was two to four times as effective as ATP or 3',5'-cAMP. At a concentration of 5 x 10^{-8} \text{ M}, the indicated nucleotides had no effect on the induction of the enzyme.

Other compounds have been tested for their effect on the induction of L-arabinose isomerase. At concentrations of 3 \text{ mm}, 5'-AMP, adenosine, adenine, and phosphate, 3',5'-cyclic GMP, IMP, CMP, and dTMP, N^6-O^2-dibutyryl 3',5'-cAMP, and 2',3'-cAMP did not have any effect. Adenosine diphasphate (ADP) was slightly stimulatory.

Catabolite repression was consistently effected by using growth medium containing 0.1% glucose and 0.1% L-arabinose. 3',5'-cAMP, yeast adenylic acid, and ATP at 3.0 \text{ mm} were unable to reverse the catabolite repression partially or completely and did not affect the enzyme levels after the period of catabolite repression ended at about the fourth hour.

Furthermore, none of the compounds tested, regardless of their effect on enzyme level, had any consistent effect on growth rate.

**DISCUSSION**

Established methods of analysis applied to bacteria and mammalian tissues were used to assay for endogenous 3',5'-cAMP, adenyl cyclase, and 3',5'-cAMP phosphodiesterase in *L. plantarum*. The validity of such methods was confirmed in our laboratory by applying them to two biological systems, *E. coli* and the superior cervical ganglia of the cat. Neither 3',5'-cAMP nor the enzymes could be detected in *L. plantarum*. The possibility that extracts of *L. plantarum* contained an inhibitor which interfered with the analytical methods or activities of enzymes was ruled out since the addition of such extracts to standards or to an extract of *E. coli* neither affected the determinations nor inhibited the activities of adenyl cyclase or phosphodiesterase of *E. coli*. Further, cells of *L. plantarum* were grown in various media and for various lengths of time. Analysis for 3',5'-cAMP was made under various conditions (starvation, addition of adenine or adenosine, and during periods of maximal enzyme induction), all known to stimulate 3',5'-cAMP formation. But no 3',5'-cAMP could be detected.

Concentrations of 3',5'-cAMP in other bacterial species were found to vary from 5 x 10^{-8} \text{ m} to 5 x 10^{-1} \text{ m} depending on growth conditions (16). Methods of assay applied here were sensitive enough to have detected such concentrations in *L. plantarum*, particularly when such methods were applied to extracts equivalent to 5 and up to 250 mg (wet weight) of cells. Methods for the assay of adenyl cyclase (9, 10) using ATP- U-^{14}C could have detected a 0.1% conversion of ATP to 3',5'-cAMP, whereas the method (13, 18) using phosphorylase b kinase activation could have detected a 0.02% conversion. In other bacterial systems 0.6 to 20% conversion of ATP to 3',5'-cAMP has been detected (9, 10). Our system lacked even one-one hundredth of the reported activities although several conditions and methods of assays were used.

The fact that no conversion of ATP to 3',5'-cAMP could be detected, within the limits of the established methods, suggested that either 3',5'-cAMP was formed but was quickly degraded or it was not formed at all. Again, repeated analysis failed to show that extracts of *L. plantarum* catalyzed a 3',5'-cAMP-dependent release of inorganic phosphate. In other bacterial extracts, the release of inorganic phosphate accounted for the hydrolysis of 20% or more of the added 3',5'-cAMP (14, 15).

In search for possible regulators of metabolism, in the absence of 3',5'-cAMP, the effect of various compounds on the induction of L-arabinose isomerase was investigated, including: 2',3'-cAMP, 3',5'-cAMP, 3',5'-cyclic GMP, IMP, CMP, and dTMP, N^6-O^2-dibutyryl 3',5'-cAMP, ATP, ADP, 5'-AMP, yeast adenyl acid, and adenosine, adenine, and inorganic phosphate. Of these compounds, 3',5'-cAMP, ATP, and yeast adenyl acid stimulated the induction of L-arabinose isomerase. The magnitude of this stimulation by 3',5'-cAMP was comparable to that reported for the same enzyme in *Salmonella typhimurium* (1) and for \(\beta\)-galactosidase in *E. coli* (17). However, ATP had quantitatively the same effect, and yeast adenyl acid surprisingly was two to four times as effective. That ATP exerted any effect on whole cells is surprising in view of possible permeability barriers to the triphosphate nucleotides. The stimulatory effect of yeast adenyl acid is hard to explain. Chromatography of the acid did not show more than the expected equal mixture of 2'-AMP and 3'-AMP. The specificity of the isomer as well as its possible formation in the cell is being studied. Neither of these isomers was formed from exogenously added radioactive ATP. Nevertheless, our results show that the role of compounds other than 3',5'-cAMP in regulating cellular function has to await diversification of studies on systems other than *E. coli* and a few other gram-negative bacteria.
and one gram-positive organism, *Streptococcus salivarius* (11).

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**LITERATURE CITED**


