THE EFFECTS OF 6-MERCAPTOPURINE ON BIOSYNTHESIS IN ESCHERICHIA COLI

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The purine analogue, 6-mercaptopurine (6-MP) (1), has been the subject of considerable study, but the mechanisms of its actions are still not clear. This drug inhibits the proliferation of acute and chronic myelocytic leucemias (2) and the growth of bacteria (3). It interferes with the utilization of formate for nucleic acid synthesis (4) and suppresses the formation of adaptive enzymes (5). However, it has not been shown whether the various biochemical effects of the drug could be observed in a single species, or whether the biochemical effects reflected one or many sites for the drug’s action. Therefore, a systematic study of the effects of 6-MP on the biosynthesis in Escherichia coli B was undertaken. This strain of bacteria has been the subject of extensive biochemical investigations (6).

A preliminary report on the results of these experiments has been presented (7).

Materials

6-MP was obtained from Burroughs Wellcome and Company, Tuckahoe, New York, courtesy of Dr. G. H. Hitchings. Aliquots of solutions of 6-MP (1 mg. per ml.) in 0.1 per cent Na₂CO₃ were added to bacterial cultures as required. Unless otherwise specified, the concentration of the drug in the medium was 10 γ per ml. Radioactive sulfate and phosphate were obtained from the Atomic Energy Commission at Oak Ridge, Tennessee. C¹⁴-formate and C¹⁴-formaldehyde were purchased from the Isotopes Specialties Company, Inc., Glendale, California, and 1-C¹⁴-acetate from Tracerlab, Inc., Boston, Massachusetts. 8-C¹⁴-Adenine and 2-C¹⁴-uracil were products synthesized as described previously (8, 9). Glucose-salts media as described by Roberts et al. (6) were used for culturing the bacteria. E. coli B (ATCC 11303) was obtained from the American Type Culture Collection, Washington, D. C.

Methods

Growth of Bacteria—E. coli cells were grown in 500 ml. polyethylene bottles and aerated by mechanical shaking at 37°. Exponentially growing
cultures were sampled through a spout into cuvettes of a Beckman model
DU spectrophotometer merely by squeezing the flexible bottles. Bacterial
growth was represented by the increase in optical density at 650 m\(\mu\) \((\Delta \rho)\),
\textit{i.e.} the difference between the measured optical density \((\rho)\) and that at
the beginning of the experiment \((\rho_0)\). Viable bacterial counts of normal
and 6-mercaptopurine-inhibited cultures compared at selected optical
densities showed no differences between the two cultures, justifying the
use of optical density measurements as criteria of growth (10). When
required, aliquots were withdrawn from the Beckman cells and further
analyzed.

**Chemical Fractionation**—Bacteria were harvested and washed in the
centrifuge and fractionated by a modification (6) of the Schneider (11)
procedure.

When chemical estimations of the bacterial nucleic acid content were
required, the cells were extracted successively with cold 5 per cent tri-
chloroacetic acid (TCA), 80 per cent ethanol, and boiling 10 per cent NaCl
(12) for 30 minutes to release nucleic acids. The absorption of light at
260 m\(\mu\) by the 10 per cent NaCl extract was taken as a measure of the
nucleic acid content.

Kinetic studies of the utilization of labeled compounds were made by
using the technique developed by Britten, Roberts, and French (13).
Bacterial samples were withdrawn at intervals, and the optical density
was measured. 2 ml. of each sample were added to an equal volume of
TCA at room temperature, and the resulting suspension was filtered through
Schleicher and Schuell membrane filters and washed with 5 ml. of 5 per
cent TCA. After drying, the filters were assayed for radioactivity.

**Radioactivity Measurements**—All radioactivity determinations were made
with a thin mica, end window counter, by using the procedures previously
described (6).

**Results**

**Effect of 6-MP on Bacterial Growth**—Fig. 1 shows the growth curves of a
control culture of \textit{E. coli} (Curve A) and a parallel culture treated with
10 \(\gamma\) of 6-MP per ml. (Curve E). 6-MP decreased the growth rate of the
bacteria within a few minutes after its addition to the cultures.

Since vitamins and coenzymes are known to promote the growth of
bacteria or to interfere competitively with growth inhibition brought on
by drug treatment (14), a number of substances and mixtures were tested
for their ability to reverse the inhibitory effect of 6-MP. Coenzyme A,
folic acid, biotin, niacin, riboflavin, a mixture of the B vitamins, adenosine
triphosphate, thiamine, pyridoxine, vitamin B\(_12\), glutathione, casein hydrol-
ysat, a mixture of diphosphopyridine nucleotide, asparagine, and gluta-
mine; carbamyl phosphate, and uracil were without effect upon the growth curves of 6-MP-inhibited bacteria.

Hypoxanthine, adenine, or guanine added to cultures of 6-MP-inhibited bacteria restored the growth rate to that of the control cultures. The effect of adenine on the growth rates of 6-MP-inhibited cultures is also shown in Fig. 1. The simultaneous addition of adenine and 6-MP to a bacterial culture prevented the inhibitory effect of the latter compound. If the cells were allowed to grow for 10, 30, or 60 minutes in the presence of the drug and were then treated with adenine, reversal of inhibition took place only after a considerable delay (Fig. 1, Curves B, C, and D), which was directly related to the period of exposure of the cells to 6-MP. 6-MP, therefore, specifically affected the biosynthesis of cell components from purines.

Reversal could be achieved also by subculturing 6-MP-treated cells in 6-MP-free culture media. Furthermore, when the medium concentration of 6-MP was 1.5 γ per ml. or less, the growth rate returned to that of the control after 2.5 hours of inhibition. It is evident, therefore, that the cell damage caused by 6-MP was not permanent.

Effect of 6-MP on β-Galactosidase Synthesis—The production of the inducible enzyme, β-galactosidase, was examined to determine whether 6-MP influenced the extent of biosynthesis of a specific protein. E. coli
cells growing exponentially with glucose as the energy source were harvested, washed, and resuspended in a culture medium lacking an energy source. 50 ml. aliquots of the suspension were placed in each of five culture bottles. A mixture of 10 mg. of glucose and 20 mg. of lactose was added to each of three of the suspensions (Fig. 2, Curves A, B, and C). 20 mg. of lactose were added to the remaining two cell suspensions (Fig. 2, Curves D and E). No 6-MP was added to the control cultures (Curves A and D). 0.5 mg. of 6-MP was added to the cultures represented by Curves C and E at the same time that the energy sources were added and to the culture of Curve B when the growth curve indicated that the glucose of the culture was exhausted. Optical densities of each culture were measured periodically, and aliquots, after injection into a solution of chloramphenicol (50 γ per ml.) to prevent further enzyme synthesis, were analyzed for β-galactosidase essentially by the method of Koppel et al. (15).

Curve A of Fig. 2 followed the typical diauxic growth pattern expected for cultures growing on the glucose-lactose combination (16). Curve B followed Curve A (the control), until the addition of 6-MP. Thereupon the rate of growth decreased, reaching finally the typical 6-MP-inhibited rate. The cells of the culture of Curve C grew at the 6-MP-inhibited rate during the metabolism of glucose and, after adaptation, lactose. Curves D and E show that 6-MP inhibited the growth of bacteria when lactose alone provided the energy source. The β-galactosidase determinations showed that the enzyme was formed after the bacteria had exhausted the glucose and were growing on lactose whether or not the cultures contained 6-MP. The enzyme content was found to be proportional to the amount of growth on lactose irrespective of the presence of 6-MP. It is concluded that 6-MP inhibited the synthesis of the adaptive enzyme β-galactosidase only in accord with its inhibitory effect upon cell growth.

Utilization of Radioactive Sulfate—The uptake of radioactive sulfate was used as a measure of total protein synthesis. A control culture of bacteria and a culture inhibited with 6-MP were grown in the glucose-salts media to which had also been added $^{35}\text{S}{\text{O}}_4^{2-}$. Samples were withdrawn at intervals from each culture, and the TCA-extracted cell residues containing the protein sulfur of the bacteria (17) were analyzed for radioactivity. The data are presented in Fig. 3, where the uptake of $^{35}\text{S}$ by a control and a treated culture are compared for similar extents of growth. By this means of presentation the general decrease in tracer uptake expected as a result of the slower growth of inhibited cells is compensated, and specific effects become apparent. The results for the control and the 6-MP-inhibited culture were clearly the same. Since the optical density of the culture is proportional to the bacterial mass (6) and since the incorporation of sulfate sulfur is directly proportional to the amount of new protein
synthesized (17), it is concluded that 6-MP influenced the utilization of sulfate sulfur for protein synthesis only in accord with its effect upon the rate of bacterial growth. These results are in agreement with those for β-galactosidase.

Utilization of 8-C\(^{14}\)-Adenine, 2-C\(^{14}\)-Uracil, and C\(^{14}\)-Formate—These compounds contribute carbon chiefly to the nucleic acids of growing *E. coli* (6). Since 6-MP was suspected to interfere with nucleic acid synthesis (3, 18), it was of interest to examine the incorporation of these nucleic acid precursors in the presence of the drug by using the membrane filter technique. Since adenine reversed the effects of 6-MP, no effect upon the uptake of C\(^{14}\)-adenine was expected, and none was observed. Fig. 4 shows the inhibitory effect of 6-MP on the utilization of C\(^{14}\)-formate by *E. coli*. The uptake of formate carbon is directly proportional to the amount of new growth (Δρ) whether or not 6-MP is present. In the presence of the drug, however, the uptake of C\(^{14}\) is less per unit growth than in the control culture. These results agree with those of Skipper (4) regarding the inhibition by 6-MP of the *de novo* synthesis of purines from labeled formate in the mouse. 6-MP also suppressed the utilization of exogenous 2-C\(^{14}\)-uracil to a greater extent than expected as a result of mere growth inhibition.

To determine the effect of non-radioactive adenine supplementation on 2-C\(^{14}\)-uracil utilization by bacteria treated with 6-MP, two bacterial cultures were prepared as above with 2-C\(^{14}\)-uracil. 6-MP was added to one of the cultures, and the membrane filter analysis was carried out. Non-radioactive adenine (10 γ per ml.) was then added to both cultures after 70 minutes, and the analysis was continued. The results (Fig. 5) show
initially the characteristic depression in the uptake of uracil in the case of the 6-MP-treated bacteria. Following the addition of adenine, however, there was an increase in the amount of C\textsuperscript{14}-uracil utilized per unit of new growth until the slopes of the uptake curves became the same for both cultures. It is apparent that adenine reversed the 6-MP-induced inhibition of uracil uptake as well as of growth.

The results with the labeled nucleic acid precursors suggest that one of the effects of 6-MP upon bacterial metabolism is the suppression of nucleic acid synthesis. This hypothesis was directly tested by measuring the nucleic acid content of control and 6-MP-inhibited bacteria.

**Nucleic Acid Content of 6-MP-Inhibited E. coli—**

$E$. coli cells were grown in glucose-salts media in the presence and absence of 6-MP. After 1.5 hours of growth the cells from each culture were harvested, washed, and extracted with TCA, ethanol, and 10 per cent NaCl as described. The optical density readings of the salt extracts at 260 m\(\mu\) were determined on bacterial samples before and after growth in the presence of 6-MP. Similar determinations were carried out for the control culture. The nucleic acid contents were then computed for the same extent of growth by dividing the increase in absorption at 260 m\(\mu\) by the increase in growth measured at 650 m\(\mu\). It was found that for eight separate experiments the nucleic acid content of cells grown in the presence of 6-MP ranged from 58 to 76 per cent (mean = 65 per cent) of that determined for untreated bacteria. It was concluded that 6-MP at 10 \(\gamma\) per ml. decreased the nucleic acid content of $E$. coli.

As a test of this conclusion, identical inocula of bacteria were grown to the same extent in the presence and absence of 6-MP in cultures which contained a mixture of randomly labeled C\textsuperscript{14}-fructose and P\textsuperscript{32}O\textsubscript{4}. The nucleic acids were extracted with 10 per cent NaCl as described above, and the ultraviolet absorption spectra of the extracts were measured. Aliquots were taken for radioactivity determinations with and without an aluminum absorber in order to measure both the total radioactivity and that from the

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![Fig. 5. Adenine reversal of 6-MP-induced inhibition of uracil-C\textsuperscript{14} uptake in $E$. coli. \(\bigcirc\), control; \(\times\), 6-MP-inhibited. Adenine added at arrow.](http://www.jbc.org/)

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P^{32} alone. It is evident from the results in Table I that the bacteria grown in the presence of 10 y per ml. of 6-MP contained approximately one-half as much nucleic acid, phosphorus, and carbon plus phosphorus as did cells grown in the absence of 6-MP. These results confirm the conclusion that 6-MP treatment decreased the nucleic acid content of E. coli, but the composition of the polynucleotides seemed unchanged. A similar effect of 6-MP on both bacterial ribonucleic acid and deoxyribonucleic acid has been observed in other investigations (10).

The absorption spectra of NaCl extracts containing approximately 0.25 mg. of nucleic acid per ml. were also examined in the region of 300 to 340 μm. Since 6-MP exhibits a characteristic absorption peak in this region (1), any increase in absorption at this region might be evidence for incorporation of the 6-MP into nucleic acids. No such indication of absorption due to the drug could be found. The possibility of more than a small amount of the purine analogue being incorporated is thus precluded. The incorporation of the drug into nucleic acids has been proposed as a possible explanation for its mechanism of action (10).

<table>
<thead>
<tr>
<th>Table I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleic Acid Content and Specific Radioactivity of 6-MP-Inhibited E. coli</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Newly formed nucleic acid (arbitrary units)</th>
<th>C^{4} + P</th>
<th>Specific radioactivity (2) ÷ (1)</th>
<th>P^{32}</th>
<th>Specific radioactivity (4) ÷ (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
<td>457</td>
<td>4.57</td>
<td>60.6</td>
<td>0.606</td>
</tr>
<tr>
<td>6-MP</td>
<td>51</td>
<td>243</td>
<td>4.75</td>
<td>29.4</td>
<td>0.575</td>
</tr>
</tbody>
</table>

Effect of 6-MP on Utilization of 1-C^{14}-Acetate—McQuillen and Roberts have shown (20) that 1-C^{14}-acetate contributes radioactive carbon principally to the proteins and lipides of E. coli. Since no effect of 6-MP upon protein synthesis had been observed in the present studies (Figs. 2 and 3) and since 1-C^{14}-acetate does not efficiently label the nucleic acids, it was thought that the total uptake of 1-C^{14}-acetate would provide a convenient indicator of the effect of the drug upon lipid synthesis. Accordingly, the uptake of 1-C^{14}-acetate by control and 6-MP-treated (10 y per ml.) bacterial cultures was determined by the membrane filter technique. Sufficient carrier acetate was added to minimize isotopic dilution by C^{12}-acetate produced from glucose (6). For the same extent of growth 6-MP-inhibited E. coli incorporated only about one-third as much C^{14} as did control cells. Since the radiocarbon from 1-C^{14}-acetate is approximately equally distributed between lipide and protein in control cells (20), the suppression
observed was greater than could be accounted for as a result even of complete lack of lipide synthesis. The results suggest that, in spite of the absence of effect of 6-MP on protein synthesis as measured by sulfur uptake (Fig. 3), acetate utilization for protein synthesis was curtailed by 6-MP. In order to test this suggestion and to determine the distribution of carbon among the different chemical fractions in the cell a series of analyses with various C¹⁴-labeled compounds was carried out.

**Effect of 6-MP on Distribution of Carbon in E. coli**—6-MP-treated and untreated cultures of *E. coli* were grown to an equal extent in the presence of 1-C¹⁴-acetate, randomly C¹⁴-labeled glucose, C¹⁴-formate, or 2-C¹⁴-uracil.

### Table II

**Effect of 6-Mercaptopurine on Utilization of C¹⁴-Labeled Compounds**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>C¹⁴-Acetate</th>
<th>C¹⁴-Glucose</th>
<th>C¹⁴-Formate</th>
<th>C¹⁴-Uracil</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>10</td>
<td>30</td>
<td>80</td>
<td>65</td>
<td>45</td>
</tr>
<tr>
<td>Cold TCA-soluble</td>
<td>5</td>
<td>18</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>15</td>
<td>2</td>
<td>1.5</td>
</tr>
<tr>
<td>Alcohol-soluble</td>
<td>53</td>
<td>35</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>30</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Hot TCA-soluble</td>
<td>3</td>
<td>2</td>
<td>58</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>34</td>
<td>28</td>
<td>40</td>
</tr>
<tr>
<td>Residue</td>
<td>37</td>
<td>52</td>
<td>27</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>34</td>
<td>28</td>
<td>1.5</td>
</tr>
</tbody>
</table>

* *Micrograms of 6-MP per ml. added.*

† The values are given in per cent of the radioactivity incorporated by the cells in the control culture. Bacteria in the inhibited cultures were grown for a longer time than those in the control cultures in order to provide the same amounts of cells in each case.

At the conclusion of the growth period the cells were harvested, washed, and fractionated by the chemical fractionation procedure described above. Radioactivity of each of the fractions was determined and calculated as per cent of the radioactivity incorporated by the bacteria in the control cultures.

It is evident from the results (Table II) that 6-MP markedly suppressed the incorporation of C¹⁴ from 1-C¹⁴-acetate into both lipides (alcohol-soluble) and proteins (residue).

In the case of C¹⁴-glucose, which randomly labels all of the carbon of the cell (6), the major effect of 6-MP was found in the combined hot TCA and residue fractions. This is probably due to the low nucleic acid content of the 6-MP-treated cells since the sulfur incorporation studies (Fig. 3) indicated a normal protein content in bacterial cells exposed to 6-MP. The small effect on the lipide fraction shows that the lipide content is
only slightly decreased, in spite of the suppression of 1-C\textsuperscript{14}-acetate utilization.

The results for C\textsuperscript{14}-formate labeling show that the nucleic acid fraction contained most of the C\textsuperscript{14} and that this fraction was the one most affected by 6-MP treatment. Incorporation of formate carbon into the cold TCA fraction, which contains small amounts of purine compounds, was apparently also suppressed. Considerable C\textsuperscript{14} was found in the protein fraction in this experiment, arising from the incorporation of C\textsuperscript{14}O\textsubscript{2} produced from the labeled formate (6). The proteins of a similar preparation were hydrolyzed to amino acids, and the hydrolysates were chromatographed on paper. The distribution of C\textsuperscript{14} among the amino acids was typical of that for cells grown in the presence of C\textsuperscript{14}O\textsubscript{2} (21). In addition, non-radioactive Na\textsubscript{2}CO\textsubscript{3} added to cultures containing labeled formate suppressed the incorporation of C\textsuperscript{14} into protein. These results indicate that amino acid syntheses involving CO\textsubscript{2} utilization were not much affected by 6-MP.

Paper chromatographic analysis of the hydrolyzed nucleic acid in the 2-C\textsuperscript{14}-uracil experiments showed that the radioactivity was contained entirely in the pyrimidine nucleotide residues. The principal effect of 6-MP was to suppress the incorporation of 2-C\textsuperscript{14}-uracil into the nucleic acid fraction. Since the cold TCA fraction contains pyrimidine compounds, it was also affected by 6-MP treatment although the amounts of radioactivity present were too low to assure accuracy.

**Effect of 6-MP Concentration on Growth, Nucleic Acid Content, and 1-C\textsuperscript{14}-Acetate Utilization**—Three series of cultures were grown in the presence of 0, 0.5, 1, 1.5, 2, and 5 \( \gamma \) per ml. of 6-MP. The nucleic acid content of one series was estimated by 10 per cent NaCl extraction and spectrophotometry. The distribution of C\textsuperscript{14} between the lipides (alcohol-soluble fraction) and proteins (residue) was determined on the second series in which 1-C\textsuperscript{14}-acetate was used as the labeled compound. The third series was analyzed by means of the membrane filter technique to detect the uptake of 1 C\textsuperscript{14} acetate. The results of these analyses are shown in Table III and Fig. 6.

0.5 \( \gamma \) per ml. of 6-MP inhibited the growth of *E. coli* but had no dis-

![Fig. 6. The effect of 6-MP on 1-C\textsuperscript{14}-acetate utilization in *E. coli*.](http://www.jbc.org/)
cernible effect upon the nucleic acid content (Table III). In addition, other experiments showed that this low dosage of drug suppressed the utilization of C$^{14}$-formate or C$^{14}$-formaldehyde only in accord with the effect upon growth. It would seem therefore that a decreased nucleic acid content is not the cause of growth inhibition.

The suppression of acetate utilization for both lipide and protein synthesis became progressively greater as the 6-MP concentration was increased. On the other hand, the lipide content as measured by the incorporation of C$^{14}$-glucose (Table II) or the protein content as measured by the incorporation of S$^{35}$O$_4^-$ (Fig. 3) was not appreciably decreased by 6-MP treatment even at 10 $\gamma$ per ml. Thus, 6-MP strongly suppressed acetate utilization without much altering the content of lipide or protein.

### Table III

<table>
<thead>
<tr>
<th>6-MP, $\gamma$ per ml.</th>
<th>0</th>
<th>0.5</th>
<th>1</th>
<th>1.5</th>
<th>2</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>( p:p_0 ) (2 hrs.)</td>
<td></td>
<td>3.9</td>
<td>3.0</td>
<td>2.5</td>
<td>2.5</td>
<td>2.1</td>
</tr>
<tr>
<td>Nucleic acid content*</td>
<td></td>
<td>100</td>
<td>105†</td>
<td>75†</td>
<td>57</td>
<td>50</td>
</tr>
<tr>
<td>C$^{14}$ into lipides*</td>
<td></td>
<td>100</td>
<td>94</td>
<td>54</td>
<td>41</td>
<td>26</td>
</tr>
<tr>
<td>&quot; &quot; proteins*</td>
<td></td>
<td>100</td>
<td>88</td>
<td>48</td>
<td>47</td>
<td>47</td>
</tr>
</tbody>
</table>

* Expressed as per cent of that in the untreated cells for the same extent of growth.
† In other experiments the nucleic acid content at these levels of 6-MP did not differ from that of the control, nor was any specific effect on the uptake of C$^{14}$-formate observed.

**DISCUSSION**

The experimental results reported in the present paper show that growth of *E. coli* was markedly inhibited by 6-MP at the lowest level (0.5 $\gamma$ per ml.) tested. However, no specific effect of this level of the drug on the utilization of various labeled compounds or on protein, lipide, or nucleic acid content was observed. Acetate utilization for protein and lipide synthesis was specifically suppressed, nucleic acid content was decreased, and the utilization of formate was specifically suppressed only at 6-MP levels of 1 $\gamma$ per ml. or more. No decrease in protein or lipide content was observed even at the highest level tested (10 $\gamma$ per ml.). It is clear, therefore, that the several metabolic properties examined differ in sensitivity to the drug. In order of decreasing sensitivity they fall in the following sequence: growth, acetate utilization, formate utilization and nucleic acid content, protein and lipide content.
6-MP treatment of *E. coli* specifically suppressed the utilization of exogenous acetate carbon for the synthesis of protein and lipid without also decreasing the protein or lipid content. It is evident, therefore, that one site of 6-MP action involves the mechanisms which control the flow of acetate carbon for synthesis. McQuillen and Roberts (20) have shown, however, that the pathways for the flow of acetate carbon into lipidic and protein are largely independent. Since 6-MP affects both pathways, it may be inferred that its influence is exerted on the early stages of acetate carbon utilization. The ready reversibility of the inhibitory effects of 6-MP by purines suggests that the analogue antagonizes the synthesis or utilization of a purine-containing compound. Coenzyme A, adenosine triphosphate, and diphosphopyridine nucleotide might be involved specifically, since they take part in the early stages of acetate utilization and also contain purine residues in their structures. Coenzyme A in addition contains a sulfhydryl group which could conceivably be inactivated by coupling with 6-MP. It is suggested, therefore, that 6-MP exerts its effects on *E. coli* by interfering with the function and synthesis of purine-containing coenzymes. The reversal of 6-MP-induced inhibition of mitosis in sarcoma 180 (22), as well as lipogenesis in embryo skin fibroblasts (23) by coenzyme A, and the inhibition of CoA-mediated acetylation by the drug in normal and tumor tissue (24) lend support to this hypothesis. The demonstration of 6-MP inhibition of diphosphopyridine nucleotide synthesis and breakdown is further corroboration (25).

Since the effect of 6-MP on growth inhibition occurred at the lowest concentration of the drug, it is likely that the effects on nucleic acid synthesis and acetate utilization are secondary. It is very probable, however, that a comparable effect of the drug on one or more specific cofactors may influence the growth rate, even though such responses may be of a magnitude outside the range of the present techniques.

**SUMMARY**

6-Mercaptopurine (6-MP) inhibits the growth of *Escherichia coli* B. Growth inhibition is reversed by adding purines to cultures of the bacteria. It may also be reversed by subculture of the cells in 6 mercaptopurine-free media or by prolonged incubation of the bacteria in low concentrations of the drug. The capacity of the microorganisms to form the inducible enzyme, β-galactosidase, is unimpaired. Protein synthesis as measured by the incorporation of 35S from labeled sulfate is identical in inhibited and control cultures for the same extent of growth. The utilization of acetate for protein and lipid syntheses is strongly suppressed. Nucleic acid synthesis, as measured spectrophotometrically or by the incorporation of radioactivity from labeled glucose, formate, uracil, or phos-
phate in cultures treated with 10 γ per ml. of 6-MP, is almost half that found in control cultures for the same amount of growth. At low levels of 6-MP a decrease in growth rate is produced, although no effect on acetate utilization, formate uptake, or nucleic acid content is observed. It is suggested that 6-MP exerts its effects by interfering with the function and synthesis of purine-containing cofactors.

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