STUDIES ON THE RELATIONSHIP OF POTASSIUM TO METABOLISM AND PURINE BIOSYNTHESIS IN ESCHERICHIA COLI

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During inhibition of the growth of Escherichia coli by sulfonamides, a diazotizable amine is formed (Fox, 1942). Following its isolation (Stetten and Fox, 1945) and identification as 4-aminooimidazolecarboxamide (Shive et al., 1947), its important roles in purine (Buchanan, 1952) and nucleic acid metabolism (Brown et al., 1952) were disclosed. Accordingly the relationships of the chief intracellular cation, potassium to growth, metabolism and the bacterial synthesis of this metabolite were investigated.

The importance of potassium in cellular physiology has received an increasing appreciation (McQuarrie, 1953; Sheppard, 1951). In contrast to sodium, potassium functions in numerous metabolic reactions. Its role in phosphorylations, i.e., in fructokinase, phosphofructokinase, and pyruvate phosphopherase activity, as well as stimulation of oxidative phosphorylation, carbohydrate metabolism, respiration, fatty acid oxidation, and decarboxylation of malate has been reviewed (Lardy, 1951; Lehninger, 1950). Potassium also stimulates metaphosphate (Schmidt et al., 1949), acetoctin (Noossal, 1952), and pantetheate formation (Maass, 1952); aldehyde dehydrogenase (Black, 1951) and lactase activity (Cohen and Monod, 1951); acetate activation by coenzyme A (Stadtmann, 1952); various oxidations (Quastel and Webley, 1942); and this action is required for the growth of several microorganisms (MacLeod and Snell, 1947; Rahn, 1936; Falk, 1923) and other forms of life.

The present study illustrates some aspects of the role that potassium exerts on the anabolic metabolism of Escherichia coli. Data are presented indicating that within defined limits the extent of uptake of assimilable carbon, phosphorus, and nitrogen substrates and the resultant synthesis of cell mass, as well as purine synthesis by one pathway, are dependent quantitatively on potassium concentration.

MATERIALS AND METHODS

Chemicals. The inorganic salts used were either CP or reagent grade. Other substances were obtained commercially and where necessary were converted to the neutral sodium salts. Several were found to be contaminated with significant amounts of K⁺ and were treated with the cation-exchange resin IR-120(Na⁺).

Media and organisms. The growth medium designated SGH contained per liter Na₂HPO₄, 6 g; NaH₂PO₄, 3 g; NH₄Cl, 1 g; NaCl, 1 g; MgSO₄·7H₂O, 0.1 g; glucose, 4 g and histidine, 80 mg; the latter two being autoclaved and added separately. Such media on analysis contained 125 mEq Na⁺, 18.7 mEq NH₄⁺, 0.08 mEq Mg²⁺, and less than 0.03 mEq K⁺. All additions were prepared in medium SGH. In this medium histidine enhances the growth response of E. coli, strain B, and the K⁺ effects reported are independent of its presence.

E. coli, strain B, and the auxotrophs, B96 and M48A-33, were used. B96 is a purine auxotroph from E. coli, strain B, M48A-33 is a p-aminobenzoic acid (PABA) auxotroph from E. coli, strain W; both accumulate 4-aminooimidazolecarboxamide (AIC) (Gots, 1950; Gots and Chu, 1952). The organisms were maintained on agar slants of similar media containing 5.1 mEq K⁺ per L as KCl, supplemented with 5 μg per ml of xanthine for growth of B96 and 5 μg per ml of p-aminobenzoic acid for M48A-33, and stored at 5 C. To prepare inocula for experiments, about 10⁸ organisms per ml taken from week old slants were grown 17 to 19 hours in media containing 0.13 μEq K⁺ per ml. These were harvested and washed twice by centrifugation with either 0.05 M tris-(hydroxy-methyl) aminomethane buffer, pH 7.4, or distilled water and adjusted to suitable concentration. Unless

1 This investigation was supported by a grant E-352 from the National Microbiological Institute, National Institutes of Health, U. S. Public Health Service. A preliminary report of portions of this data has appeared (Friedman and Fox, 1953).
otherwise indicated, an inoculum of about $4 \times 10^4$ organisms per 5 ml was used in the actual experiments.

**Methods.** Studies were done in acid cleaned test tubes, suitably rinsed and covered with aluminum caps. Turbidity was read on a Klett-Summerson photoelectric colorimeter with a 54 filter. Reducing sugar was determined by the method of Somogyi (1952) and Nelson (1944), 4-amino-5-imidazolecarboxamide of Somogyi (1952) and Nelson (1944), method of Drury (1948) and Stumpf (1947) following acetylation with acetic anhydride at pH 7 for 30 minutes (Stetten and Fox, 1945) using a synthetic hydrochloride salt as standard, phosphorus by the methods of Fiske-SubbaRow (1925) or of Lowry and Lopez (1946), adenosine triphosphate pyrophosphate as 7 minute hydrolyzable phosphate (LePage and Umbreit, 1945), and ammonia by a micro-nesslerization technique (Johnson, 1941). Pentose and deoxypentose nucleic acids were determined by the methods of Drury (1948) and Stumpf (1947) following hydrolysis at 100 C for 30 minutes in 5 per cent trichloracetic acid, using commercially obtained nucleic acids with known phosphorus contents as standards.

For analysis of intracellular potassium, 19 to 22 hour old cells were harvested by centrifugation and washed twice with distilled water which was retained for analysis of the K+ released during this manipulation. The washed cells were dried to constant weight in vacuo over P2O5 and defatted according to Lowry and Hastings (1942). The defatted cells were extracted with 0.75 N nitric acid (Lowry and Hastings, 1942) using approximately 1 ml of acid per 10 mg defatted cells. The wash water and ether extract used in defatting were evaporated, and each residue extracted with acid. The K+ concentrations of all the extracts were determined by an internal standard flame photometer (Fox, 1951).

**RESULTS**

(1) **Potassium requirement in medium SGH.** In view of the metabolic importance of K+, the optimal concentrations required for cellular synthesis, expressed as growth in medium SGH, were determined and found to range from 2.6 to 40 μEq per ml. Half maximal growth occurred with less than 0.13 μEq per ml (figure 1a). This stimulation was exerted early in the growth cycle as evidenced by the increased number of cells in exponential growth at 6 hours; neither anaerobiosis nor vigorous aeration altered this effect. Similar type curves were obtained with sulfate or acetate as the anion except that there was less growth at 24 hours, and the inhibitory effects with higher concentrations were more marked.

At 10.2 μEq K+ per ml which is optimal, the equivalent ratios of cations to K+ in medium SGH are Na+ 12.2, NH4+ 1.8, and Mg++ 0.08. Apparently K+ deficient cells are not inhibited strongly by Na+ since the Na+:K+ ratio can be increased to 16.9 (ionic strength 0.23) with no inhibition of growth (figure 1b). In contrast increases in NH4+ to 5.6 (ionic strength 0.235) or Mg++ to 8.2 (ionic strength 0.23) caused marked inhibition of growth. Increasing concentrations of K+ up to 250 μEq per ml did not reverse
growth inhibitions of 15 or 40 per cent caused by the addition to medium SGH of up to 300 μEq per ml of Na⁺, NH₄⁺, or Mg²⁺ ions. This inability of increased K⁺ to reverse inhibitions by these cations at optimal ratios makes unlikely the possibility that the K⁺ effect is primarily due to a competitive ion antagonism.

(2) Potassium partition in E. coli. Intracellular K⁺ in E. coli is partitioned between a freely diffusible fraction and a retained or "bound" fraction (Cowie et al., 1949) so that accurate measurement of the internal concentration of this cation is difficult. For example, 19 hour cells grown on an optimal concentration of 10.2 μEq K⁺ per ml contained an estimated total of 0.378 μEq K⁺ per mg fat-free dry weight (1.47 per cent) when cells equivalent to 61.2 mg were analyzed. Under the conditions of these experiments, 35 per cent (0.135 μEq) was retained in the bacterial cells and 65 per cent (0.242 μEq) was diffusible and recovered in the wash water (0.234 μEq) and the ether extract (0.008 μEq). The diffusible fraction was extractable also at 5°C. No difference in K⁺ concentration could be found in the retained fraction of 19 to 22 hour cells grown on median and optimal concentrations of cation (table 1, column a).

(3) Potassium as a metabolic regulator. The available supply of K⁺ regulates the uptakes of glucose, ammonia, and inorganic phosphate and the resultant synthesis of cell mass in medium SGH. This effect with 19 hour dense cell suspensions is shown in figure 2. Total uptake was greatest with 10.2 μEq K⁺ per ml, but maximum rate of activity occurred below 3 μEq. In contrast to the uptakes of glucose and phosphate, less ammonium ion disappeared at each concentration of K⁺. This relationship was similar when cell suspensions three times as dense (21.2 mg dry weight) were tested between 0 and 10 μEq K⁺. Total uptake was maximal at 40 μEq K⁺, although at least 25 per cent of the initial concentrations of glucose, ammonia, and inorganic phosphate remained.

In an attempt to replace the K⁺ requirement for growth, various metabolites or cofactors were tested in SGH media to which were added 0.002 μEq K⁺ per ml, an amount insufficient for growth. The following were unable to replace the need for this cation when added singly, or in a wide variety of combinations, and through a range of concentrations of which the highest tried per 5 ml medium SGH is indicated: 2 μg adenine, 2.2 μg adenosine, 6 μg adenosine triphosphate (ATP), 3 μg diphosphopyridine nucleotide (DPN), 400 μg desoxypentose nucleic acid hydrolyzate, 200 μg pentose nucleic acid hydrolyzate, 5 mg of casein hydrolyzate, 2.1 μg 1-glutamic acid, 3.9 μg glutamine, a multivitamin preparation, 10 μg p-aminobenzoic acid, 30 μg p-hydroxybenzoic acid, 30 μg biotin, 30 μg folic acid, 1.3 μg vitamin B₁₂, 200 μg citrate, 10 μg isocitrate, 200 μg pyruvate, 200

A 1:50 dilution of "pacebrin" (Lilly) injectable containing 40 μg thiamin, 88 μg riboflavin, 12 μg pyridoxine, 12 μg pantothenate, 80 μg nicotinamide, 240 μg ascorbic acid, 8 μg α-tocopherol, 4 units of vitamin A, and 4 units of vitamin D was used.

Figure 1b. Antagonism by other cations in medium SGH of the growth stimulation by K⁺. Inhibition is plotted against the ratio of equivalents of other cations to optimal K⁺ concentration (10.2 μEq per ml).

Note: A primary grown dried beer yeast powder intended for pharmaceutical purposes, Fleischmann Laboratories, Type 200B, Saccharomyces, was also analyzed. The value found for several portions was 0.582 μEq potassium per mg (2.3 per cent).
TABLE 1

Intracellular contents of cells grown in median and optimal concentrations of K+

<table>
<thead>
<tr>
<th>µEq/ml</th>
<th>µEq retained K⁺</th>
<th>µg 7 MIN HYDROLYZABLE P</th>
<th>µg PENTOSE NUCLEIC ACID</th>
<th>µg DESOXYDIPENTOSE NUCLEIC ACID</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experiment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.113</td>
<td>0.112</td>
<td>4.5</td>
<td>69.7</td>
</tr>
<tr>
<td>2</td>
<td>0.112</td>
<td>0.108</td>
<td>4.0</td>
<td>69.3</td>
</tr>
<tr>
<td>3</td>
<td>0.164</td>
<td>0.176</td>
<td>4.7</td>
<td>86.8</td>
</tr>
<tr>
<td>Mean</td>
<td>0.130</td>
<td>0.132</td>
<td>4.4</td>
<td>75.3</td>
</tr>
<tr>
<td>SD</td>
<td>±0.030</td>
<td>±0.043</td>
<td>±0.36</td>
<td>±9.9</td>
</tr>
</tbody>
</table>

All analytical values are expressed as per mg dry weight and retained K⁺ as fat-free dry weight. Turbidity of cells at optimal K⁺ was 1.6 times greater than median cells, i.e., at 19 hours turbidities were 98 and 62.

µM α-ketoglutarate, 200 µM fumarate, 200 µM succinate, and 200 µM aspartate. All combinations were tested in the presence of 3 µM adenine triphosphate and 1 µM diphosphopyridine.

In view of K⁺ involvement in synthesis and transfer of high energy phosphate (Lardy, 1951) and the alleged impermeability of cells to extracellular adenine triphosphate, the adenine nucleotide pyrophosphate contents of dried cells grown on median and optimal concentrations of K⁺ were determined. Small but comparatively greater amounts of nucleotide pyrophosphate were found in cells grown on optimal K⁺ concentrations (table 1, column b).

(4) Potassium and purine biosynthesis. The relationship of K⁺ to the synthesis of intracellular components was studied in a representative process: accumulation of bacterial derived 4-amino-5-imidazolecarboxamide (Fox 1942; Stetten and Fox, 1945; Shive et al., 1947) as an index of the purine synthesizing processes by two auxotrophs. The K⁺ requirement for their growth was similar to that for the wild type when tested at concentrations of auxotrophic substance giving maximum accumulation (table 2).

Stimulation by K⁺ of 4-amino-5-imidazolecarboxamide accumulation, however, and of growth is different. With 0.03 µEq per ml, 50 per cent of total growth but only 3 per cent of total 4-amino-5-imidazolecarboxamide are obtained. With 10× as much K⁺ (0.3), growth is only 1.6× greater, but 4-amino-5-imidazolecarboxamide accumulation increased about 20×. Maximal accumulation of 4-amino-5-imidazolecarboxamide by the p-aminobenzoic acid auxotroph was but ½ that of the purine mutant and only ½ as much K⁺ was required (0.3 to 1.3 µEq K⁺ per ml versus more than 10.2 for B96). A similar dependence on K⁺ for 4-amino-5-imidazolecarboxamide formation also was observed in sulfadiazine inhibited cultures.

Evidence that K⁺ participates in cellular

Figure 2. K⁺ as a metabolic regulator. Washed cells (0.5 ml equivalent to 0.86 mg dry wt) were added to 4.5 ml of medium SGH containing increasing K⁺ concentrations. At zero time, the turbidity was 106 and 22.4 µM of glucose, 18.6 µM of NH₄⁺ and 63 µM of inorganic phosphate (Pi) were present. The amount of substrate that disappeared and the turbidity increment, at 3 hours, are plotted against the K⁺ concentration of the medium.

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TABLE 2
Potassium stimulation of growth and 4-amino-5-imidazolecarboxamide (AIC) accumulation by auxotrophs

<table>
<thead>
<tr>
<th>µEq K⁺</th>
<th>P-AMINO BENZOIC ACID AUXOTROPH M48A-33</th>
<th>PURINE AUXOTROPH B96</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Turbidity</td>
<td>AIC</td>
</tr>
<tr>
<td>0</td>
<td>18</td>
<td>0.04</td>
</tr>
<tr>
<td>0.03</td>
<td>74</td>
<td>0.06</td>
</tr>
<tr>
<td>0.3</td>
<td>103</td>
<td>1.29</td>
</tr>
<tr>
<td>1.3</td>
<td>118</td>
<td>1.30</td>
</tr>
<tr>
<td>2.6</td>
<td>125</td>
<td>1.04</td>
</tr>
<tr>
<td>10.2</td>
<td>140</td>
<td>0.84</td>
</tr>
<tr>
<td>10.2-p-aminobenzoic acid or xanthine</td>
<td>0 trace</td>
<td>0 trace</td>
</tr>
</tbody>
</table>

System: 19 hour culture in 5.0 ml of medium SGH containing 20 µg glutamic acid and glycine per ml (Ravel et al., 1948). M48A-33 supplemented with 5 mg p-aminobenzoic acid per ml, B96 with 10 µg xanthine per ml. Data µg per ml except turbidity.

...syntheses independent of growth may be obtained by utilizing auxotrophs which accumulate precursor but do not grow in the absence of auxotrophic substance. In this way the K⁺ dependence of 4-amino-5-imidazolecarboxamide accumulation in the absence of growth was demonstrated with dense cell suspensions of the purine and p-aminobenzoic acid auxotroph (figure 3). Furthermore, in experiments with metabolic inhibitors which block 4-amino-5-imidazolecarboxamide accumulation by B96 in such a system, K⁺ in high concentrations was able to lessen the extent of inhibition of 4-amino-5-imidazolecarboxamide accumulation by azide and fluoride (table 3). Inhibition by 1 × 10⁻⁴ M iodoacetic acid, 1 × 10⁻³ M sodium cyanide, and 4 × 10⁻⁴ M dinitrophenol created blocks in metabolism not reversible by similar concentrations of K⁺.

In keeping with this data, wild type E. coli, strain B, grown in optimal K⁺ concentrations had relatively higher pentose nucleic acid contents than cells grown in median K⁺ concentrations; desoxypentose nucleic acid contents were almost equal (table 1, columns c and d).

* Dr. J. S. Gots kindly made available his data with the auxotroph B96 (Gots and Love, 1954). The p-amino-benzoic acid auxotroph was similarly found to accumulate 4-amino-5-imidazolecarboxamide in the absence of growth.

Figure 3. The effect of K⁺ on 4-amino-5-imidazolecarboxamide accumulation by purine (B96) and p-aminobenzoic acid (M48A-33) mutants in the absence of auxotrophic substance. Washed cells (0.5 ml equivalent to 0.28 mg dry weight B96 and 0.78 mg M48A-33) were added to 4.5 ml medium SGH supplemented with 20 µg per ml of glutamic acid and glycine (Ravel et al., 1948) containing increasing concentrations of K⁺. In absence of auxotrophic substance turbidity remained constant and the histidine in medium SGH did not relieve the purine requirement of B96. Nonacetylatable diazotizable amine per ml determined at 3 hours is plotted against K⁺ concentration.

TABLE 3
The effect of K⁺ on inhibitors of 4-amino-5-imidazolecarboxamide (AIC) accumulation by purine auxotroph B96

<table>
<thead>
<tr>
<th>INHIBITOR ADDED</th>
<th>MOLES/L. X 10⁻⁴</th>
<th>AIC ACCUMULATED IN PRESENCE OF K⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0.0001 mEq K⁺</td>
</tr>
<tr>
<td>None</td>
<td>-</td>
<td>2.9</td>
</tr>
<tr>
<td>Na fluoride</td>
<td>100</td>
<td>2.1</td>
</tr>
<tr>
<td>Na azide</td>
<td>10</td>
<td>1.0</td>
</tr>
<tr>
<td>2,4-Dinitrophenol</td>
<td>4</td>
<td>0.1</td>
</tr>
</tbody>
</table>

System: 0.5 ml cells (0.8 mg dry wt), 0.1 ml inhibitor, K⁺ as KCl, 20 µg/ml glutamic acid and glycine (Ravel et al., 1948), plus SGH media containing no auxotrophic substance to 5 ml volume. At 3.5 hours samples centrifuged 6,500 rpm for 15 minutes, and nonacetylatable diazotizable amine determined. Data µg per ml.
DISCUSSION

The data presented illustrate the importance of $K^+$ in the synthetic reactions of *E. coli*. This is expressed as a $K^+$ requirement for optimal exponential growth and attainment of maximal cell yield during the growth cycle and for the proper uptake and assimilation of carbon, phosphorus, and nitrogen substrates by the cell. The relationship found in dense suspensions of 19 hour cells between $K^+$ concentrations and the rates of assimilation of the substrates tested may provide a biochemical explanation for the well known dependence of normal growth of *E. coli* upon the $K^+$ concentration in the medium (Hotchkiss, 1923; Winslow and Dolloff, 1928; Roberts et al., 1949).

The free outward diffusion of $K^+$ which occurs during harvesting and washing of the cells in preparation for analysis may account for the wide range of values of $K^+$ reported in microorganisms. The higher total value of $K^+$ in *E. coli*, strain B, reported here is based on a more critical accounting for the freely diffusible $K^+$ fraction and supersedes values previously reported by this laboratory (Friedman and Fox, 1953). The retained $K^+$ fraction would appear essential for metabolic competence but insufficient for optimal functioning. As evidence for this, no difference was found in the retained fraction of median and optimal grown cells, indicating that quantitative intracellular incorporation of $K^+$ is not the sole basis for its stimulating effect. Washed dense cell suspensions containing only such a store of $K^+$ function at suboptimal levels; furthermore, the addition of various substrates including $K^+$ mediated reaction products does not relieve this requirement. The addition of small quantities of $K^+$ to such cells, however, causes a marked rise in activity, suggesting a catalytic function and attesting to the importance of the freely diffusible fraction for optimal metabolism.

The comparatively increased adenine nucleotide pyrophosphate content of cells grown with optimal $K^+$ and the failure of $K^+$ mediated reaction products to relieve this $K^+$ requirement suggest that this cation may function at several levels: in energy synthesis or its transport, or in coupled reactions between energy and substrate levels leading to assimilation. Involvement of $K^+$ in such functioning would explain adequately the metabolic role of this cation in the processes illustrated in this paper: in assimilation of substrates during and in the absence of growth. Some extension is required also of the common concept that $K^+$ transport is dependent on metabolism since optimal metabolism itself is dependent on $K^+$.

A requirement for $K^+$ in purine synthesis as measured by bacterial 4-amino-5-imidazolecarboxamide accumulation which is independent of growth and occurs prior to closure of the purine ring also has been demonstrated. This is reflected in the comparatively increased syntheses of more actively metabolized pentose nucleic acid in cells grown in optimal $K^+$ concentrations and in the ability of $K^+$ to relieve partially the inhibition of 4-amino-5-imidazolecarboxamide accumulation by azide or fluoride. Ability of high concentrations of $K^+$ to influence these two inhibitors may further imply a role for $K^+$ in phosphorylation reactions involved in purine synthesis at sites not influenced by dinitrophenol.

ACKNOWLEDGMENT

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SUMMARY

Some aspects of the importance of potassium to the anabolic metabolism of *Escherichia coli* have been examined. Narrow concentration ranges of potassium were required for optimal exponential growth, attainment of maximum cell yield and efficient uptakes of ammonia, glucose and inorganic phosphate from salts-glucose media by suspensions of wild type *E. coli*, strain B, as well as for 4-amino-5-imidazolecarboxamide accumulation by a purine and *p*-aminobenzoic acid auxotroph. Analysis of 19 hour cells showed that a greater portion of
intracellular potassium is freely diffusible and a lesser fraction retained. No quantitative difference could be found in the retained fraction of potassium in median and optimal grown cells. Optimal grown cells, however, had comparatively increased pentose nucleic acid and adenine nucleotide pyrophosphate contents. A variety of metabolites or cofactors including potassium mediated reaction products was unable to replace the potassium requirement of low potassium cells. The general metabolic significance of these findings is discussed.

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