SUMMARY

1. A study has been made of the effect on the respiration and adenosine-triphosphatase activity of rabbit-kidney-cortex homogenates and mitochondria of some factors (Na^+ ions, K^+ ions, Ca^{2+} ions and ouabain) that influence respiration and ion movements in tissue slices.

2. The Q_{O_2} of the homogenate showed a dual response to variations in K^+ ion and Na^+ ion concentrations. First, when the K^+ ion concentration was increased from 2.5 to 32.5 mM there was a marked rise in Q_{O_2} , the magnitude of which was dependent on the relative concentrations of Na^+ and K^+ ions; it was not prevented by ouabain or the absence of Na^+ ions, and was found with different respiratory substrates. Secondly, a further increase in K^+ ion concentration caused an additional rise in Q_{O_2} which was abolished by ouabain or the absence of Na^+ ions. Ca^{2+} ions prevented both responses to increased K^+ ion concentrations.

3. The respiration of mitochondria was stimulated by K^+ ion concentrations up to 30 mM; ouabain or the lack of Na^+ ions was without effect. A further increase in K^+ ion concentration did not alter the respiration.

4. Adenosine-triphosphatase activity in homogenates was stimulated by Na^+ and K^+ ions together, and was inhibited by ouabain or Ca^{2+} ions and by the absence of either Na^+ or K^+ ions; that of the mitochondrial fraction was unaffected by Na^+ ions, K^+ ions or ouabain.

5. Concentrations of ouabain giving submaximal inhibition caused parallel decreases in the oxygen consumption and adenosine-triphosphatase activity of homogenates.

6. It is concluded that an extramitochondrial adenosine triphosphatase, sensitive to Na^+ ions, K^+

ions, Ca^{2+} ions and ouabain, is one of the pace-makers of the respiration of kidney cortex.

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Repressors of Sulphate Activation in *Escherichia coli*

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The formation of the enzyme system (ATP-sulphate adenyllyltransferase, EC 2.7.7.4, and ATP-adenyllylsulphate 3'-phosphotransferase, EC 2.7.1.25) that catalyses the synthesis of PAPS* from inorganic sulphate and ATP (Lipmann, 1958) is repressed in *Escherichia coli* by the addition of L-cystine to the growth medium (Pasternak, 1961,

* Abbreviation: PAPS, adenosine 3'-phosphate 5'-sulphatophosphate.

1962). The suggestion (Peck, 1962) that these enzymes are not repressed by cystine needs revision. The addition of probable intermediates in the reduction of sulphate to cysteine and of other sulphur-containing compounds also represses the sulphate-activating system (Ellis & Pasternak, 1962). The experiments reported below make it likely that a common metabolite, namely cysteine, is the actual repressing agent in every case (Ellis & Pasternak 1962).

MATERIALS AND METHODS

Organisms. The wild-type organisms used were *Escherichia coli* ATCC 9723, *E. coli* K₁₂ 701 and *Salmonella typhimurium* LT-2. *E. coli* mutants 8/6G and 9/20, obtained from the Unit for Chemical Microbiology, Oxford University, were cysteine-less mutants obtained from *E. coli* 9723 by ultraviolet irradiation. They were classified as sulphide-less by their failure to grow (in liquid or on solid medium) on sodium sulphate or sodium sulphite. Growth on sodium thiosulphate, sodium sulphide, glutathione or L-cystine was normal. Another sulphide-less mutant of *E. coli* 9723 with similar growth characteristics was *E. coli* 1590-230 (Lampen, Roepke & Jones, 1947), given by Dr J. O. Lampen. *E. coli* mutant K₁₂ 415, given by Dr R. C. Clowes, was a cysteine-less mutant derived from *E. coli* K₁₂ 701 by ultraviolet irradiation and was classified as sulphide-less by its failure to grow on sodium sulphate or sodium sulphite. Growth on sodium thiosulphate, sodium sulphide, glutathione or L-cystine was normal. All cultures were maintained on slopes of Oxoid nutrient agar CM4 at 4°. The sulphur-free minimal medium described by Pasternak (1962) was used throughout.

Liquid media. Sodium sulphate, potassium sulphate, L-cystine (British Drug Houses Ltd. or Roche Products Ltd.) and reduced glutathione (Sigma Chemical Co., St Louis 18, Mo., U.S.A.) were added as described by Pasternak (1962). Aqueous solutions of Na₂SO₃ (anhydrous or AnalaR Na₂SO₃·7H₂O, British Drug Houses Ltd.), Na₂S₂O₃·5H₂O (AnalaR, British Drug Houses Ltd.), Na₂S₉H₂O (crystals, of AnalaR grade, British Drug Houses Ltd., were blotted to remove moisture before weighing), L-cysteine hydrochloride (British Drug Houses Ltd.) and D-cysteine hydrochloride (California Corp. for Biochemical Research, Los Angeles, Calif., U.S.A.) were prepared fresh just before use, sterilized by filtration through Oxoid membrane filters and added to cooled autoclaved medium. D-Cystine (Mann Research Laboratories Inc., New York 6, N.Y., U.S.A.), mesocystine (L. Light and Co. Ltd.), selenocystine and selenocystamine dihydrochloride, prepared (Günther & Mautner, 1960) and given by Dr H. G. Mautner, were added to the medium before autoclaving. L-Methionine, DL-homocysteine, L-serine, α-aminobutyric acid, taurine (all British Drug Houses Ltd.), L-cysteic acid (L. Light and Co. Ltd.), L-cysteinesulphinic acid (California Corp. for Biochemical Research; purified by passage through Dowex 50 resin to remove traces of cystine as described by Singer & Kearney, 1956) and cystamine dihydrochloride, prepared and given by Dr H. G. Mautner, were sterilized by filtration through Oxoid membrane filters. LL-Djenkolic acid (L. Light and Co. Ltd.) was dissolved (0.02M) by heating, rapidly filtered to sterilize and the resulting suspension used. Glucose, sterilized by autoclaving a 10% (w/v) solution, was added to autoclaved medium. The final concentration was varied so as to limit growth and decrease the removal of added sulphur compounds by metabolism. Media were inoculated, incubated and bacterial growth was measured as described by Pasternak (1962).

Solid media. The sulphur-free minimal medium containing added sodium sulphate (0.85 mM) or L-cystine (0.42 mM) and Oxoid agar no. 3 (1.8%) was sterilized by autoclaving. Sterile glucose (0.2%) was added to the hot medium which was poured on to Oxoid sterile plastic Petri dishes DP2.

Solutions of sodium sulphite (final concn. 0.85 mM) and sodium sulphide (final concn. 0.21 mM) were freshly prepared as described above and added to Petri dishes, followed by agar (1.8%) in sterile sulphur-free minimal medium to which glucose (0.2%) had been added. Plates were stored at 4°. Cultures were streaked on to plates and incubated at 37° overnight. In all experiments with mutants samples were removed from flasks before harvesting and their growth characteristics checked. Reversion to prototrophy was rare and when this occurred the experiment was discarded.

Measurement of evolution of hydrogen sulphide. Cultures (300 ml. in 500 ml. flasks) were grown with aeration overnight. The effluent stream of air was passed through Wolf bottles containing 200 ml. of 5% (w/v) (CdSO₄)₂(H₂O)₈ and the precipitated CdS estimated by titration with iodine and Na₂S₂O₃ (Saz & Brownell, 1954).

Preparation and assay of enzyme extracts. The methods described by Pasternak (1962) were used. Where indicated, cell suspensions were disrupted by passage through a French pressure cell (American Instrument Co. Inc., Silver Spring, Md., U.S.A.) instead of by ultrasonic oscillation. For experiments on end-product inhibition extracts were prepared in 10 mM-EDTA, pH 7.5, instead of 10 mM-cysteine-10 mM EDTA (Pasternak, 1962); solutions of L-cysteine hydrochloride, D-cysteine hydrochloride, glutathione and β-mercaptoethanol (L. Light and Co. Ltd.) in 0.1 M-tris were freshly prepared and the pH was adjusted to 8.8 before use. L-Cystine was dissolved by autoclaving a 0.1% suspension in water. Radioactivity on paper strips after electrophoresis was measured with an automatic scanning and print-out device (Isotope Developments Ltd., Beenham, Berks.) operated on stepwise drive.

RESULTS

Repression by various sulphur compounds. Table 1 shows the effect on the synthesis of the sulphate-activating system of a variety of sulphur-containing compounds added to the growth medium. Although the more unstable compounds (see Postgate, 1963) were prepared immediately before use, some loss during incubation was inevitable. The degree of repression indicated is therefore a minimal value. Mixing experiments (Pasternak, 1962) were carried out (Table 1) to eliminate the possibility that the lowered activity was due to the presence of inhibitory substances or to the absence of thermostable cofactors. Those compounds that repress are also capable of supporting growth as sole sulphur source, and it is possible that the actual repressor may be a metabolite such as cysteine (see below). Repression by inorganic sulphate was greater (Table 1) than previously (Table 3 of Pasternak, 1962) noted, but the reason for this is not clear. Inorganic sulphite and cysteinesulphinic acid showed similar degrees of repression and it is likely that the latter substance acts by way of metabolic conversion through sulphite (Leinweber & Monty, 1962) (see also Table 2). Inorganic thiosulphate is a weaker repressor than sulphite,

sulphide or L-cystine, and is therefore unlikely, especially in view of recent enzymic evidence (Schlossmann & Lynen, 1957; Brüggemann, Schlossmann, Merckenschlager & Waldschmidt, 1962), to be an intermediate of this pathway in *E. coli*, though it is converted into sulphide and sulphite (Kaji & McElroy, 1959; Dreyfuss & Monty, 1963) by reduction (Artman, 1956). Repression by low concentrations of sulphide is particularly dependent on the extent to which growth is allowed to occur, since it is readily removed from the growth medium. Cystamine, L-methionine and LL-djenkolic

acid, like glutathione (Pasternak, 1962), do not repress the sulphate-activating enzymes, presumably being converted into cysteine at a slow rate. However, methionine does repress in the presence of sulphate. LL-Djenkolic acid was used in place of reduced glutathione for experiments in which repression by low concentrations of L- and D-cystine was studied to prevent possible thiol exchange reactions. The repression by autoclaved, as opposed to filtered, glutathione is probably due to the formation of cysteine during autoclaving, in view of the known instability of the compound

Table 1. *Repression of adenosine 3'-phosphate 5'-sulphatophosphate formation by sulphur compounds in Escherichia coli 9723*

Cultures were grown overnight in the presence of the compounds indicated and harvested, and extracts were assayed for ability to synthesize PAPS.

Compounds added	Concn. (mM)	Concn. of glucose (%)	Extent of growth (mg. dry wt./ml.)	Enzymic activity (μ m-moles of PAPS synthesized/mg. of protein/15 min.)
Glutathione	0.1	0.05	0.13	17.8
Glutathione	0.85	0.05	0.15	18.0
Glutathione	0.85	0.25	0.52	20.1
Glutathione (autoclaved)	0.82	0.25	0.36	< 0.01
Glutathione + L-serine	0.1 + 0.8	0.05	0.12	22.2
Glutathione + L-serine	0.1 + 8.0	0.25	0.28	25.2
Glutathione + α -aminobutyric acid	0.1 + 2.0	0.05	0.10	17.9
Glutathione + selenocystamine	0.42 + 0.21	0.03	0.17	17.4
Glutathione + L-methionine	0.42 + 2.0	0.075	0.14	16.5
Glutathione + L-cysteic acid	0.1 + 1.0	0.05	0.21	12.0
Glutathione + taurine	0.1 + 1.0	0.05	0.22	10.9
Glutathione + DL-homocysteine	0.1 + 0.45	0.05	0.15	6.9
L-Methionine	0.85	0.25	0.16	17.9
LL-Djenkolic acid	0.22	0.05	0.13	18.4
Cystamine	0.42	0.05	0.03	18.2
Sodium sulphate	0.85	0.05	0.21	2.3
Sodium sulphate + glutathione	0.85 + 0.85	0.05	0.20	3.0
Sodium sulphate + L-methionine	0.85 + 2.0	0.075	0.39	0.11
Sodium sulphite	4.0	0.05	0.20	0.33
Sodium sulphite + glutathione	0.81 + 0.075	0.1	0.23	0.03
Sodium sulphite + glutathione	4.0 + 0.067	0.1	0.22	< 0.01
Sodium sulphite + glutathione	4.0 + 0.067	0.1	0.22	18.0*
L-Cysteinesulphinic acid + glutathione	0.85 + 0.1	0.05	0.18	0.53
Sodium thiosulphate	0.42	0.25	0.16	3.0
Sodium sulphide	0.85	0.25	0.42	< 0.03
Sodium sulphide	0.85	0.25	0.42	20.4*
Sodium sulphide	0.85	0.25	0.42	< 0.03†
Sodium sulphide + glutathione	0.021 + 0.2	0.025	0.11	5.8
Sodium sulphide + glutathione	0.052 + 0.2	0.025	0.07	< 0.03
Sodium sulphide + glutathione	0.105 + 0.2	0.025	0.08	< 0.02
Sodium sulphide + glutathione	0.105 + 0.2	0.25	0.23	19.1
L-Cystine + glutathione	0.005 + 0.42	0.02	0.09	1.1
L-Cystine + glutathione	0.01 + 0.42	0.02	0.07	< 0.05
L-Cystine + LL-djenkolic acid	0.05 + 0.14	0.025	0.08	< 0.04
D-Cystine + glutathione	0.01 + 0.42	0.02	0.07	16.0
D-Cystine + LL-djenkolic acid	0.05 + 0.14	0.025	0.05	1.4
D-Cystine + glutathione	0.105 + 0.42	0.015	0.03	< 0.04
L-Cysteine + LL-djenkolic acid	0.02 + 0.14	0.025	0.09	5.3
L-Cysteine + LL-djenkolic acid	0.1 + 0.14	0.025	0.09	< 0.04
L-Cysteine + glutathione	0.21 + 0.42	0.05	0.05	< 0.05
D-Cysteine + LL-djenkolic acid	0.02 + 0.14	0.025	0.07	20.0
D-Cysteine + LL-djenkolic acid	0.1 + 0.14	0.025	0.07	4.8
D-Cysteine + glutathione	0.21 + 0.42	0.05	0.06	< 0.08

* Repressed extract (0.1 ml.) mixed with 0.1 ml. of non-repressed extract.

† Repressed extract (0.1 ml.) mixed with 0.1 ml. of boiled non-repressed extract.

(Hopkins, 1929). Dreyfuss & Monty (1963) have used djenkolic acid to study the activation and subsequent reduction of sulphate by *S. typhimurium*, in which cysteine is a repressor of the enzyme sequence from sulphate to sulphide. The work of Mager (1960) and our own preliminary experiments on sulphite reductase show this to be true of *E. coli* also. Selenocystamine, which unlike selenocystine is not toxic to *E. coli* growing on glutathione, does not repress sulphate activation.

Repression in sulphide-less mutants. To test whether some of the inorganic sulphur compounds are themselves repressing agents or whether they are converted into a common metabolite such as cyst(e)ine, use was made of several mutants blocked between sulphite and sulphide. Such mutants, grown on glutathione, possess the sulphate-activating enzymes (Table 2). The addition of sodium sulphite is without effect, suggesting that its repressing action on wild-type *E. coli* 9723 or K_{12} is indeed due to a metabolic product. All the mutants show higher enzymic activity when grown on glutathione than do the parent strains. This may be due to traces in the growth medium of inorganic sulphate, which itself represses PAPS synthesis in wild-type organisms only (Table 2). The low synthesis of PAPS by *E. coli* K_{12} compared with *E. coli* 9723 is interesting in view of the fact that *S. typhimurium* LT-2, which resembles *E. coli* K_{12}

genetically (Zinder, 1960), was found to have a similarly low level of PAPS-synthesizing ability ($6 \mu\text{m-moles/mg. of protein/15 min. on glutathione}$, $0.8 \mu\text{m-mole/mg. of protein/15 min. on sulphate}$, less than $0.02 \mu\text{m-mole/mg. of protein/15 min. on L-cystine}$).

Formation of hydrogen sulphide from cyst(e)ine. During this work it was noted that wild-type *E. coli* cultures grown on cystine smelt strongly of hydrogen sulphide, confirming the presence of cysteine desulphhydrase [L-cysteine hydrogen sulphide-lyase (deaminating), EC 4.4.1.1] in *E. coli* (Desnuelle & Fromageot, 1939). It was therefore considered possible that sulphide, which was almost as effective a repressor as cystine (Table 1) despite its rapid loss from cultures during growth at 37° , was the actual repressor of the sulphate-activating system. Experiments were carried out in which hydrogen sulphide production and sulphate activation were measured concomitantly (Table 3). The removal of hydrogen sulphide by bubbling is so efficient that sodium sulphide does not repress at all. On the other hand, D-cyst(e)ine, and L-methionine plus sulphate, which are almost as effective as L-cystine in repressing the sulphate-activating enzymes, give rise to only very low amounts of hydrogen sulphide, which suggests that sulphide is not the repressor in this case. It is possible that a prior racemization of D- to L-cystine occurs, though growth on D-cystine

Table 2. *Repression of adenosine 3'-phosphate 5'-sulphatophosphate formation in sulphide-less mutants of Escherichia coli*

Cultures were grown overnight in the presence of glutathione (0.85 mM), glucose (0.05%) and the compounds indicated, the cells were harvested and extracts were assayed for ability to synthesize PAPS.

Organism	Compound added	Concn. (mM)	Extent of growth (mg. dry wt./ml.)	Enzymic activity ($\mu\text{m-moles of PAPS synthesized/mg. of protein/15 min.}$)
ATCC 9723	—	—	0.14	32.5
ATCC 9723	Sodium sulphate	0.85	0.19	3.3
ATCC 9723	Sodium sulphite	4.2	0.14	< 0.03
ATCC 9723	L-Cysteinesulphinic acid	0.85	0.18	< 0.03
ATCC 9723	L-Cystine	0.42	0.18	< 0.03
9/20	—	—	0.15	42.7
9/20	Sodium sulphate	0.85	0.17	46.6
9/20	Sodium sulphite	4.2	0.13	48.0
9/20	L-Cysteinesulphinic acid	0.85	0.09	44.7
9/20	L-Cystine	0.42	0.13	< 0.03
8/6G	—	—	0.10	54.0
8/6G	Sodium sulphite	4.2	0.09	45.0
8/6G	L-Cystine	0.42	0.13	< 0.02
1590-230	—	—	0.08	53.0
1590-230	Sodium sulphate	0.85	0.05	78.0
1590-230	Sodium sulphite	4.2	0.05	75.0
1590-230	L-Cystine	0.42	0.17	< 0.08
K_{12} 701	—	—	0.11	2.3
K_{12} 701	Sodium sulphate	0.85	0.16	< 0.14
K_{12} 701	Sodium sulphite	4.2	0.12	< 0.07
K_{12} 701	L-Cystine	0.42	0.09	< 0.02
K_{12} 415	—	—	0.11	2.6
K_{12} 415	Sodium sulphite	4.2	0.09	1.6
K_{12} 415	L-Cystine	0.42	0.11	< 0.02

Table 3. *Evolution of hydrogen sulphide during growth of Escherichia coli 9723 on repressors of sulphate activation*

Cultures were grown in the presence of 0.03% or 0.05% glucose and the compounds indicated with aeration overnight and harvested, and extracts were assayed for ability to synthesize PAPS. Evolved H₂S was trapped as CdS and measured as described in the text.

Compound added	Concn. (mM)	Extent of growth (mg. dry wt./ml.)	H ₂ S formed (μmoles/mg. dry wt. of cells)	Enzymic activity (μm-moles of PAPS synthesized/mg. of protein/15 min.)
Glutathione	0.21	0.12	< 0.03	28.0
L-Methionine	0.85	0.12	< 0.03	14.0
Sodium sulphate	0.85	0.12	< 0.03	7.0
Sodium sulphate + L-methionine	0.85 + 0.85	0.13	< 0.03	0.55
L-Cystine + glutathione	0.21 + 0.42	0.10	1.53	< 0.03
D-Cystine + glutathione	0.21 + 0.21	0.09	0.26	< 0.03
mesoCystine + glutathione	0.21 + 0.42	0.05	0.93	< 0.1
L-Cysteine + glutathione	0.21 + 0.21	0.19	0.91	< 0.02
D-Cysteine + glutathione	0.42 + 0.21	0.06	0.06	< 0.1

Table 4. *Repression and inhibition of sulphate incorporation by L-cystine in Escherichia coli 9723*

A culture (1200 ml.) was grown on glutathione (0.21 mM) and glucose (0.25%) for 3 hr. One-third (A) was harvested and the extract, prepared by pressing, was assayed for ability to synthesize PAPS. The remainder was divided into two portions (B and C), to one of which (C) was added L-cystine (final concn. 0.1 mM). To duplicate 5 ml. samples of cultures B and C was added Na₂³⁵SO₄ (final concn. 0.91 mM, 76 μCi/ml.). Cultures B and C and all the tubes were reincubated for 60–90 min. until the cell mass had doubled. Cultures B and C were then harvested, and extracts, prepared by pressing, were assayed for PAPS synthesis. The tubes were treated with 1 ml. of ice-cold 50% (w/v) trichloroacetic acid and the radioactivity in the protein fraction was measured as described by Pasternak (1962).

Culture	Cell density (mg. dry wt./ml.)	Enzymic activity (μm-moles of PAPS synthesized/mg. of protein/15 min.)	10 ⁻⁴ × ³⁵ S incorporation (counts/min.)
A	0.08	33	—
B	0.16	55	5.2
C	0.17	13	0.12

as sole sulphur source is poor. Reduction of D-cystine to D-cysteine, on the other hand, may well take place since mesocystine (the use of which was suggested by Dr A. Meister) gives rise to large amounts of hydrogen sulphide (Table 3).

Attempts to demonstrate end-product inhibition. Repression of the sulphate-activating system can account for the complete abolition of [³⁵S]sulphate incorporation by cells pregrown on cystine (Table 1 of Pasternak, 1962). In the pioneer experiments of Roberts, Abelson, Cowie, Bolton & Britten (1955) in which the simultaneous addition of [³⁵S]sulphate and cystine to cells pregrown on sulphate likewise resulted in almost complete inhibition, a mechanism

in addition to repression, such as end-product inhibition (Umbarger, 1961), must have been operative (see, e.g., Davis, 1961). The experiment of Table 4 confirms this result. The 2.6-fold decrease in enzymic specific activity during growth in the presence of 0.1 mM-L-cystine was that expected from dilution of existing enzyme, whereas the incorporation of [³⁵]sulphate was decreased 50-fold compared with the control growing on glutathione. An attempt was therefore made to demonstrate end-product inhibition *in vitro* by some of the compounds known to be active as repressors. No effective inhibitor of sulphate activation was found (Table 5). On the contrary, the stimulation of enzymic activity by L-cysteine, normally added to the extraction medium (Pasternak, 1962), and glutathione was confirmed.

DISCUSSION

It has generally been assumed (e.g. Davis, 1961; Rowbury & Woods, 1961) that, when an intermediate of a biosynthetic sequence represses the formation of an early enzyme, this is due to conversion of the intermediate into a specific metabolite. The present experiments (Tables 1, 2 and 3) are in clear support of this hypothesis and point to L-cysteine as the actual repressing agent. Hence the first stage in the synthesis of cysteine, namely the reduction of sulphate to sulphite, as well as the formation of cysteine from glutathione, L-methionine, djenkolic acid or cystamine, appear to be slow rate-limiting reactions, whereas the reduction of sulphite to sulphide and the conversion of sulphide into cysteine are processes rapid enough to give an intracellular concentration of cysteine sufficiently high to cause complete repression. If sulphate itself were able to repress completely, growth on it as sole sulphur source would be impossible. Repression by sulphide itself was not completely ruled out by the

Table 5. *Effect of sulphur compounds on sulphate activation by extracts of Escherichia coli 9723*

A culture was grown overnight on glutathione (0.16 mM) and glucose (0.1%) and harvested at a cell density of 0.14 mg. dry wt./ml. The cells were washed and suspended in cysteine-free medium and extracts, prepared by pressing, were assayed for their ability to synthesize PAPS in the presence of the compounds listed. The sulphate concentration was 0.75 mM throughout.

Compound added	Concn. (mM)	Enzymic activity (μ m-moles of PAPS synthesized/mg. of protein/15 min.)
—	—	36.6
L-Cysteine	60	83.2
L-Cysteine	120	48.5
D-Cysteine	60	50.5
D-Cysteine	120	45.4
Glutathione	60	116.5
Glutathione	120	78.8
β -Mercaptoethanol	60	53.9
β -Mercaptoethanol	120	34.4
L-Methionine	7.5	72.2
L-Cystine	0.26	44.3
L-Cysteic acid	3.3	57.2
Sodium sulphite	7.5	38.1
Sodium thiosulphate	7.5	38.2
Sodium sulphide	7.5	34.8

present experiments. All *E. coli* mutants blocked between sulphide and cysteine so far examined lack the ability to synthesize PAPS when grown on glutathione or djenkolic acid and could not therefore be used to resolve this point. It is not inconceivable that repression by hydrogen sulphide may be of physiological importance to an organism such as *E. coli* whose natural habitat is the mammalian colon.

End-product inhibition of sulphate activation, which appears to operate *in vivo* (Table 4), could not be demonstrated *in vitro* (Table 5). It is possible that the inhibitor is not one of those tested, or that if inhibitor and substrate occupy different binding sites on the enzyme, that for the former is destroyed during disruption of the cells (Gerhart & Pardee, 1962). Alternatively, the site of end-product inhibition may not be the synthesis of PAPS, but a subsequent reaction (Peck, 1962) in the biosynthesis of cysteine. This is currently under investigation.

SUMMARY

1. Several sulphur-containing compounds repress the formation of the enzymes (ATP-sulphate adenylyltransferase, EC 2.7.7.4, and ATP-adenylylsulphate 3'-phosphotransferase, EC 2.7.1.25) catalysing the synthesis of adenosine 3'-phosphate 5'-sulphatophosphate in *Escherichia coli* ATCC 9723 and K₁₂ 701.

2. Sodium sulphate and sodium sulphite, which repress in wild-type organisms, do not do so in

sulphide-less mutants, suggesting that neither compound is itself a repressor.

3. Less hydrogen sulphide is formed by *E. coli* from D-cyst(e)ine than from L-cyst(e)ine or meso-cystine, though all five compounds repress the activating enzymes.

4. It is likely that L-cysteine is the actual repressor of the sulphate-activating system.

5. End-product inhibition of sulphate activation could not be demonstrated.

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