The Natural Occurrence of Ethionine in Bacteria

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ABSTRACT Two unknown radioactive areas appeared after radioautography and two dimensional paper chromatography of culture medium in which *Escherichia coli* was grown. These materials were studied by paper chromatography and paper electrophoresis of several derivatives and identified as ethionine and ethionine sulfone, the latter an artifact. Chromatographic coincidence of the unknowns and their derivatives with authentic materials establishes the identification.

Ethionine was found in cellular extracts and in the growth media of *Escherichia coli*, *Bacillus megaterium*, *Pseudomonas aeruginosa*, and *Aerobacter aerogenes* but not in *Scenedesmus*, *Saccharomyces cerevisiae*, or bovine lymphosarcoma cells.

Ethionine was synthesized by resting *E. coli* cultures from radioactive sulfate and from radioactive methionine. Growing cells labeled ethionine within 1 minute after addition of radioactive sulfate to cultures. Levels of radioactivity in ethionine increased with time. No incorporation of this amino acid could be detected in the cellular proteins formed under the conditions of this study.

In an attempt to elucidate further the metabolic pathway of sulfur in biological systems, an investigation using radioactive sulfate was undertaken. Radioautograms showed two major radioactive areas in extracts of both the cells and culture medium of *Escherichia coli* B. The two compounds responsible for these areas were identified as ethionine and its oxidation product ethionine sulfone. This appears to be the first report of the natural occurrence of ethionine.

Ethionine, first synthesized in 1938 by Dyer (1), has been well established as an analog of methionine. Ethionine has been reported to inhibit the growth of such organisms as *Escherichia coli*, *Staphylococcus aureus*, lactic acid bacteria (2–5), and the Lansing type poliomyelitis virus (6, 7). The growth of young chicks (8) and rats (9) is depressed when they are fed ethionine. This inhibitory behavior is attributed to competition with methionine (9–12).

The ethyl group of ethionine appears to be available for the synthesis of

ethyl analogs of normally methylated metabolites (13–15). Pancreatic, liver, renal, and adrenal damage was observed with ethionine diets (16–19).

MATERIALS AND METHODS

Unlabeled ethionine, ethionine sulfone, and ethionine sulfoxide were purchased from the California Corporation for Biochemical Research and from Nutritional Biochemicals Corporation. The samples were checked against each other by paper chromatography. The carrier-free radioactive sulfate in 0.19 N HCl was obtained from the Atomic Energy Commission laboratories at Oak Ridge.

Escherichia coli strain B, obtained from the Bacteriology Department of the Pennsylvania State University, was carried on nutrient agar slants and grown for use in a glucose-salts medium.

For experimental purposes a synthetic medium of minimum sulfur content was prepared by diluting a mixture of 2.0 gm, of NH_4Cl, 6.0 gm of Na_2HPO_4, 3.0 gm of KH_2PO_4, 1.0 gm. of NaCl, 0.05 gm of $MgCl_2 \cdot 6H_2O$, 4.0 gm of glucose, and 0.005 gm of Na_2SO_4 to 1000 ml with distilled water. The resulting stock solution was passed through a sterile bacterial filter and refrigerated.

A similar medium but containing $S^{35}O_4^{=}$ in addition was prepared by evaporating to dryness a volume of radioactive sulfate in hydrochloric acid solution providing the desired level of radioactivity. The residue of $H_2S^{35}O_4$ was redissolved in a portion of the above stock medium and sterilized by filtration.

The cells were grown aerobically at 37°C in 10 ml of experimental medium in sterile 2 × 17.5 cm culture tubes with metal caps or in 50 or 250 ml of medium in 250 ml or 1 l flasks respectively. Cultures were shaken at 200 cycles per minute at an amplitude of 2 cm. Growth was followed turbidimetrically with a Coleman model 14 universal spectrophotometer at 650 mμ. Optical density (turbidity) was calibrated against plate and microscopic counts for *E. coli* (20) so that turbidity gave cell population directly. Cell growth was terminated at populations of 5.5 to 6.0 × 10^8 cells per ml by cooling the cultures rapidly to 0°C in an ice bath.

The cells were collected from the medium by centrifugation at 860 × *g* and 2°C for 15 minutes. The cell pellet was suspended in 5 ml. of 0.154 M saline and recentrifuged. This resuspension in saline was repeated twice and the washed cells suspended in the experimental medium.

For studies of the sulfur compounds in the medium, the cell suspension was centrifuged as above, and the supernatant medium was filtered through a bacterial filter. This cell-free filtrate was lyophilized, and the solid residue obtained was extracted by washing three times with 2 to 3 ml of absolute ethanol. These washings were combined and evaporated to dryness over $CaCl_2$ in a vacuum desiccator. The resulting material was reextracted with about 1 ml of absolute ethanol, and a small volume was chromatographed. Radioautographs of the chromatograms were made. The radioactive areas thus located were cut from the chromatograms and eluted for further use.

For studies of internal pools of sulfur compounds, *E. coli* B cells were grown, har-

vested, washed, suspended in 0.154 M sodium chloride, and lysed from without using the bacteriophage[1] method (21) to disrupt the cells and yield a solution of the soluble cell contents.

A Reco electrophoresis migration chamber, model E-800-2, was used in the electrophoretic studies. The eluted compounds were examined at pH 1, pH 10, and pH 11 using potassium chloride-hydrochloric acid, sodium carbonate–sodium borate, and disodium hydrogen phosphate–sodium hydroxide buffers respectively. A potential of 200 volts was applied at pH 1 and 400 volts at pH 10 and 11.

All chromatograms were run two dimensionally on Whatman No. 1 filter paper (46.4 × 57.1 cm). Unless otherwise specified, the developing solvent system for the first direction was phenol–water (100:39 *w/w*) and that for the second direction was *n*-butanol–propionic acid–water (151:75:100 *v/v*). Other developing solvents used were *t*-butanol–acetic acid–water (5:2:3 *v/v*) and *n*-butanol–pyridine–water (5:3:1 *v/v*).

Kodak single coated medical x-ray film was used in photoradioautography. Radioautograms were prepared by placing films on chromatograms and storing the pairs in Kodak lead-backed x-ray exposure holders for a period ranging from 1 to 7 days depending upon the intensity of radioactivity on the individual chromatograms. At the end of the exposure period the films were developed in a dark room with General Electric supermix x-ray developer and fixed with General Electric supermix x-ray fixer.

The radioactivities, in terms of counts per minute, were determined with an Anton electronics mica end-window Geiger-Müller tube and a model 123 Baird-Atomic scaler. The radioactivities were measured directly from the paper chromatograms. Large areas were divided by masking into smaller sections, which were then counted individually and totaled.

Radioactive methionine was prepared from yeast in accordance with the method of Wood and Perkinson (22) and isolated by streaking the hydrolysate across the top of a sheet of Whatman No. 1 filter paper. Approximately 10 μg of unlabeled authentic methionine was applied as a reference standard at a spot near the edge of the paper and the chromatogram was developed with *n*-butanol–propionic acid–water. The edge of the dried chromatogram was sprayed with ninhydrin to locate the methionine reference spot. The radioactive band corresponding in position to the methionine-ninhydrin spot had an R_f value of 0.60. This band was cut from the paper and eluted with water. A small aliquot of the eluate was cochromatographed with unlabeled methionine in two dimensions, first in phenol-water and second in *n*-butanol–propionic acid–water. The chromatogram was sprayed with ninhydrin showing two chromatographic spots, one corresponding to methionine, the other to its oxidation product. An autoradiogram showed two radioactive areas which coincided with the ninhydrin-positive areas.

All experiments were repeated at least once.

[1] The T_2r^+ bacteriophage was kindly supplied in concentrated suspension by Dr. James L. Barlow of the New York State Department of Health, Albany, New York.

EXPERIMENTAL RESULTS

E. coli B grown in 250 ml. of medium containing 3 millicuries of $S^{35}O_4^=$ was harvested by centrifugation and the supernatant medium extracted. A volume of this extract representing approximately 11,000 CPM (on the paper) of sulfur activity was chromatographed and a radioautogram made. Two prominent

TABLE I

COMPARATIVE CHROMATOGRAPHIC BEHAVIOR OF RADIOACTIVE MATERIAL FROM *ESCHERICHIA COLI* B, ETHIONINE, AND DERIVATIVES

	R_f values							
Material chromatographed	Direction 1 Phenol: water	Direction 2 *n*-Butanol: propionic acid: water	Direction 1 *t*-Butanol: acetic acid: water	Direction 2 *n*-Butanol: pyridine: water	Direction 1 *n*-Butanol: propionic acid: water	Direction 2 Phenol: water	Direction 1 *n*-Butanol: pyridine: water	Direction 2 *n*-Butanol: propionic acid: water
Compound I	0.72	0.60	0.70*	0.29*	0.71*	0.87*		
Ethionine (authentic)	0.72	0.60	0.70*	0.29*	0.71*	0.87*		
Acetylated I	0.94	0.89						
Compound I‡ methylsulfonium iodide + ethionine methyl sulfonium iodide (authentic)							0.029	0.34
Compound II*	0.72	0.33						
Ethionine sulfone (authentic)	0.70	0.33						
Compound II§ + ethionine sulfone (authentic)			0.59	0.22	0.37	0.74		
Acetylated II	0.91	0.82						
Acetylated II + *N*-C^{14}-acetyl‖ ethionine sulfone (authentic)			0.93	0.72				

* Material did not partially oxidize to give two spots under these conditions.
‡ Artifact (ethionine sulfone) formed by oxidation of ethionine during drying following phenol run.
§ After cochromatography the one radioactive spot coincided with the ninhydrin-positive area.
‖ After cochromatography a single radioactive spot was obtained.

spots having the R_f values shown in Table I appeared on the film (Fig. 1). The total radioactivity in spots I and II represented 70 per cent of that counted at the origin. Areas containing compounds I and II were cut from the chromatograms, and the material eluted with methanol. These eluates were used as stock solutions for further experimentation.

Since methionine is partially oxidized while drying following the phenol-water excursion (23) and since the chromatographic pattern (Fig. 1) somewhat resembled that of authentic methionine, an oxidation of I to II was suspected. To test this hypothesis I and II were rechromatographed separately. A radioautogram showed that I had been completely converted by the experimental

manipulation to II and that II was unchanged by repeating the chromatography.

An aliquot containing about 10 μg of added methionine and 11,000 CPM of ethanolic extract was chromatographed. The dried chromatogram was sprayed

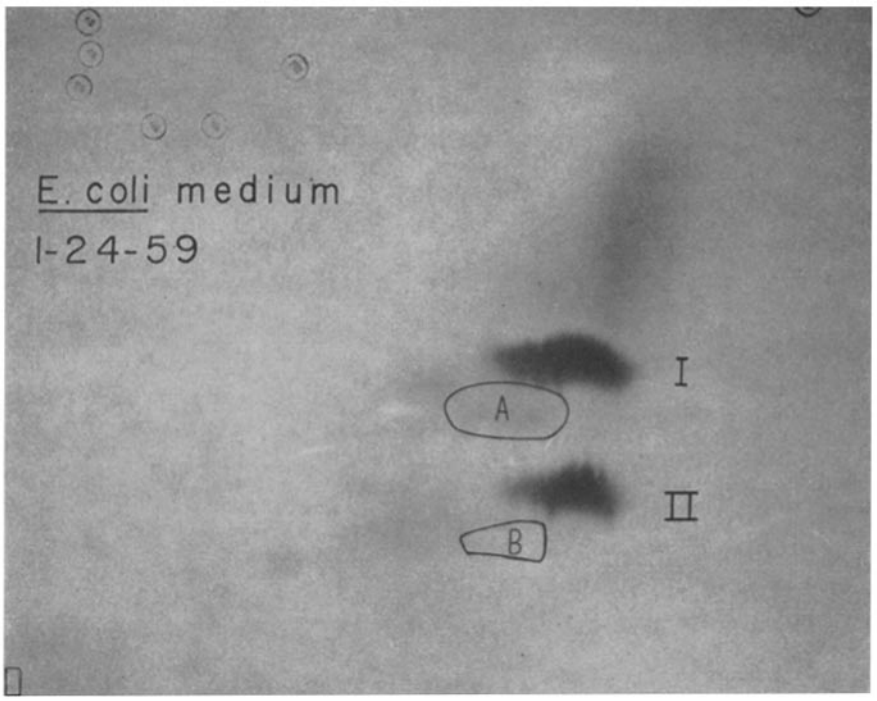

FIGURE 1. Radioautogram of medium from a culture of *Escherichia coli* B. Exposed areas I and II resulted from components labeled with S^{35} by the cells. Areas marked A and B represent the ninhydrin-positive areas on the chromatogram itself and correspond respectively to methionine and methionine sulfone. The rectangle represents the origin before chromatography first in phenol-water (left to right), then in *n*-butanol–propionic acid–water (bottom to top). The small encircled numbers are position markers applied to chromatograms with radioactive ink and they allow exact comparisons of positions on chromatograms with spots on radioautograms.

with a 0.15 per cent ninhydrin-ethanol solution containing a small amount of pyridine and radioautographed (Fig. 1). Area A represents the position of methionine and area B that of the oxidation product of methionine. Compound I appeared less polar than methionine, but nevertheless generally resembled methionine in its chromatographic behavior.

Compounds I and II were found to be insoluble in chloroform and benzene suggesting that I and II were not sulfolipids. I and II were fairly soluble in ethanol and methanol and readily soluble in water indicating a polar character.

After evaporation to dryness, compounds I and II were separately dissolved in 0.5 ml of pyridine and 0.1 ml of acetic anhydride. The resulting solutions were allowed to stand at 37°C for 10 hours and evaporated to dryness. These residues were extracted with 0.1 ml of methanol and chromatographed. The derivatives of both I and II possessed increased R_f values in both directions (Table I). The formation of these new compounds with increased R_f

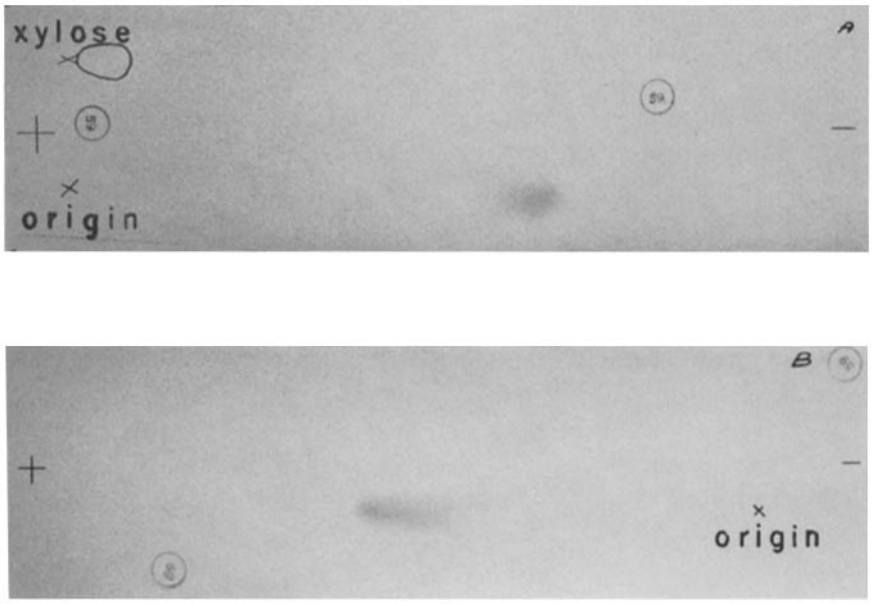

FIGURE 2. Radioautograms after paper electrophoresis of compound I. Strip A resulted from ionophoresis for 4¾ hours at 200 volts and 0.035 ampere at pH 1.0 (KCl + HCl) on Whatman No. 1 paper. The encircled area at the upper left indicates the final position of the xylose included to reveal electroosmosis. Strip B resulted after 5 hours at 400 volts and 0.015 ampere at pH 10.0 (carbonate + borax) on Whatman No. 1 paper. Plus and minus signs indicate polarity and x's mark the origins.

values suggested the presence, in both I and II, of at least one easily acetylated group.

By means of paper electrophoresis, both I (Fig. 2) and II were shown to move as anions at pH 10 and 11 and as cations at pH 1.

Approximately 10,000 CPM of I was dissolved in 0.5 ml of phosphate buffer of pH 7. One-half ml of 0.01 M sodium *p*-(hydroxymercuri)benzoate solution was added, allowed to stand at room temperature for 1 hour, and evaporated to dryness. This residue was extracted with methanol and chromatographed. A radioautogram showed only compound II. The result of this experiment indicates that the sulfur is not in the form of a sulfhydryl group or that the sulfhydryl group is not readily attacked by sodium *p*-(hydroxymercuri)benzoate.

Approximately 6,000 CPM of the extract dissolved in 0.1 ml of methanol containing about 20 μg of added authentic ethionine was chromatographed, and the chromatogram was sprayed with ninhydrin. A radioautogram (Fig. 1) showed that the radioactive areas I and II and the ninhydrin-positive areas, ethionine and its oxidation product, were coincident in both shape and position.

This cochromatography was repeated with *t*-butanol–acetic acid–water in the first direction and *n*-butanol–pyridine–water in the second direction. Both the ninhydrin-sprayed chromatogram and a radioautogram showed only single, completely coincident spots.

The above procedure was altered so that both authentic ethionine and ethionine sulfone were cochromatographed with the extract of the medium first in *n*-butanol–propionic acid–water and second in phenol-water. The only radioactive area found was coincident with the ethionine-ninhydrin area. The ninhydrin area due to added ethionine sulfone was devoid of radioactivity. The failure to find II under these conditions lends credence to the supposition that I is partially oxidized to II while drying after the phenol-water development.

Approximately 1,000 CPM of I and 10 μg of ethionine were dissolved in distilled water, and the solution studied by electrophoresis at pH 1 and at pH 10. In both the anionic and cationic migrations the ninhydrin-positive and radioactive areas were coincident (Fig. 2). Compound II and ethionine sulfone also coincided after electrophoretic migration.

Radioactive compound II (ethionine sulfone) was prepared by rechromatography of I in phenol-water. Ethionine methylsulfonium iodide was prepared by dissolving 5,000 CPM of I in 0.5 ml of methanol containing 20 μg of added authentic ethionine, and 1 ml of methyl iodide was added. The resulting solution was shaken in the dark at room temperature for 10 hours and evaporated to dryness.

N-C^{14}–acetyl ethionine sulfone was prepared by placing 0.025 ml of acetic anhydride in a 1.0 ml centrifuge tube previously dried at 170°C for 1 hour and adding 2 mg of sodium acetate-1-C^{14}. The mixture was shaken at 37°C for 2 hours. The C^{14}-labeled acetic anhydride was removed with a micropipet and added to a solution of approximately 20 μg of authentic ethionine sulfone in 2 drops of pyridine. The resulting solution was shaken at 37°C for 10 hours and evaporated to dryness.

Cochromatography of the above derivatives with the corresponding derivatives of compound I gave single radioactive areas in each case coincident with the zones due to the authentic derivatives. R_f values of the several derivatives are listed in Table I. Single values for the mixtures are the R_f values of the single areas resulting from these systems.

Production of ethionine from methionine was demonstrated by culturing *Escherichia coli* B in synthetic medium to which approximately 400,000 CPM

of methionine-S^{35} had been added. At a population of 6×10^8 cells/ml, growth was stopped and the medium freed of cells. This solution was lyophilized, the residue extracted with 1.0 ml of absolute ethanol, and an aliquot cochromatographed in two dimensions with 20 μg of authentic ethionine. The chromatogram was sprayed with ninhydrin and autoradiographed. Two of the radioactive areas were coincident with the ninhydrin-positive ethionine and

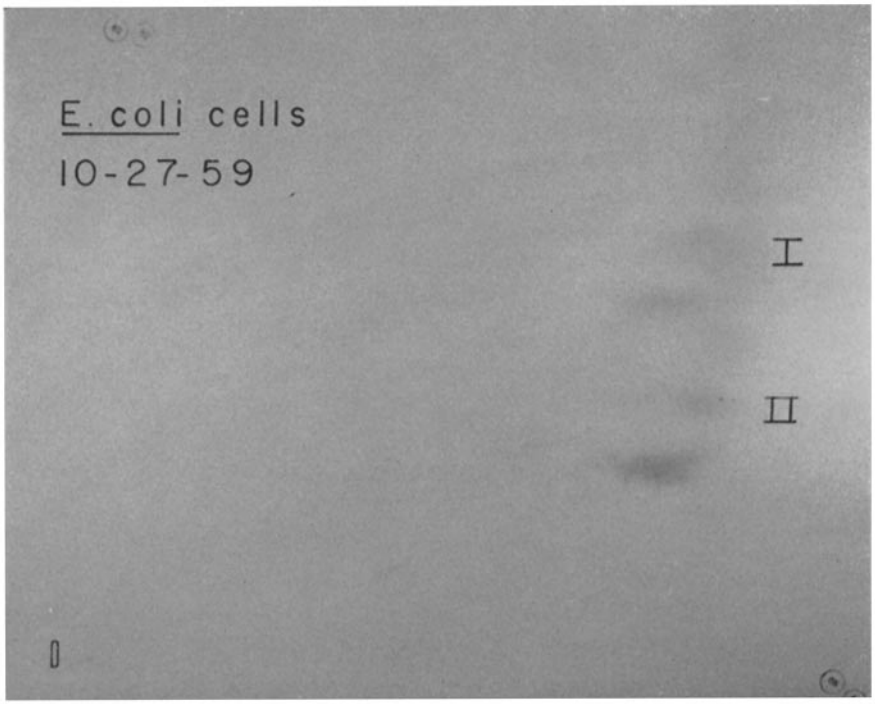

FIGURE 3. Radioautogram showing the presence of ethionine in an extract of lysed cells of *E. coli*. Exposed areas I and II resulted respectively from ethionine-S^{35} and its sulfone and exactly matched ninhydrin spots due to authentic unlabeled ethionine added to the extract just prior to chromatography. The major components slightly below and to the left of ethionine and ethionine sulfone are methionine and methionine sulfone. Chromatographic treatment was the same as that for Fig. 1. In this experiment the cells were incubated with the $S^{35}O_4^=$ for 30 minutes.

ethionine sulfone and they totalled 8920 CPM. The biosynthesis of ethionine from methionine, as well as from sulfate, suggests the presence of a system transferring ethyl groups. Search for such a process may open up a new area of bacterial metabolism.

Occurrence of ethionine intracellularly was demonstrated by culturing *E. coli* in 10 ml of synthetic medium containing 1 millicurie of $S^{35}O_4^=$ to 6×10^8 cells/ml. The cells were harvested, washed, lysed with bacteriophage,

and the insoluble debris removed by centrifuging 15 minutes at 850 × *g* and 2°C. The supernatant solution was lyophilized, and the residue extracted once with 2.0 ml of absolute ethanol. After evaporation to 0.1 ml, this extract was cochromatographed with 10 μg of unlabeled ethionine.

TABLE II

RATE OF APPEARANCE OF ETHIONINE IN THE MEDIUM OF *E. COLI**

Incubation time	Milliliters at origin	Counts per minute at origin	$S^{35}O_4^=$	Ethionine	Ratio of ethionine to CPM at origin	Counts per minute of ethionine per μl at origin
min.			*cpm*‡	*cpm*		
1	250	8400	4400	3300	0.39	13
5	150	8700	3700	4800	0.55	32
30	130	10,000	1600	6800	0.68	52
60	90	10,900	330	7800	0.71	87
90	80	11,200	300	8500	0.76	105
120	70	11,700	205	9500	0.82	136

* Based on an ethanol extract of 1.0 ml. Ethionine as reported here is the sum of the radioactive areas produced by both ethionine and ethionine sulfone. Growth had ceased by 135 minutes. All radioactive counting data corrected for decay occurring during chromatography and autoradiography.

‡ Not all the sulfate from the two earliest samples dissolved in the ethanol used in the extraction. Hence the corresponding values for the ratio of ethionine to CPM at the origin (total S^{35} in the extract) are probably higher than for the medium itself.

TABLE III

RATE OF APPEARANCE OF ETHIONINE IN *E. COLI* CELLS*

Incubation time	Microliters at origin	Counts per minute at origin	Ethionine	Ratio of ethionine to CPM at origin	Counts per minute of ethionine per μl at origin	Counts per minute at origin (total S^{35} per μl)
min.			*cpm*			
1	500	3600	700	0.20	1.4	7.2
5	500	6100	1020	0.17	2.0	12.2
30	400	12,400	2000	0.16	5.0	31
60	200	12,100	1800	0.15	9.0	61
90	150	12,300	1700	0.14	11	82
120	125	12,200	1500	0.13	12	97

* See first footnote of Table I.

Of the several radioactive areas appearing in Fig. 3, two coincided with added authentic ethionine and ethionine sulfone. Two other radioactive areas corresponded to methionine and methionine sulfone. Counts of radioactive areas and totals, using the assumption that the sulfones originated from the

methionine and ethionine, indicated approximately four times as much ethanol-soluble intracellular methionine as ethionine.

In an attempt to detect incorporation of ethionine into *E. coli* proteins, the insoluble debris from the lysis of 3.7 mg of cells in the preceding experiment was washed three times with 70 per cent ethanol. 0.1 mg of unlabeled ethionine was added to the residue and the mixture hydrolyzed by refluxing 6 hours in 6 M HCl under nitrogen. After evaporation to dryness *in vacuo*, the residue was extracted with 5.0 ml of absolute ethanol. This extract was concentrated

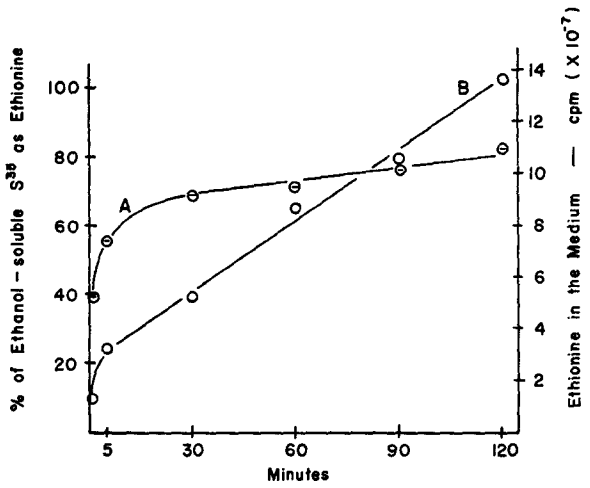

FIGURE 4. Appearance of ethionine in the culture medium of *Escherichia coli* B. Curve A reveals the increase in ethionine relative to the total S^{35} in the medium. (See second footnote of Table II.) Curve B shows the increase in the total ethanol-extractable ethionine of the medium.

to 1.0 ml, and 0.1 ml containing approximately 10,000 CPM was chromatographed as usual. No ethionine-S^{35} could be detected on the autoradiogram. However, a trace of radioactivity (17 CPM above a background of 43 CPM) was recorded by the counter. In view of experimental uncertainties and the possibility of adsorption on the cellular debris, the incorporation of ethionine into *E. coli* protein is unlikely although relatively large amounts of methionine appeared on the radiograms. Certainly the level, if any, of ethionine must be low even though appreciable quantities of free ethionine do exist inside the cell.

Synthesis of ethionine by resting cells was demonstrated as follows. A culture was grown to 4×10^8 cells/ml, harvested, and the cells washed three times with 0.154 M NaCl. These cells were suspended in synthetic medium devoid of glucose but containing 1 millicurie of $S^{35}O_4^=$ and incubated with shaking for 3 hours at 37°C. The turbidity of the suspension remained constant

throughout the experiment. Chromatographic examination of the medium with unlabeled ethionine added for reference revealed 8000 CPM of ethionine-S^{35} formed by the resting cells. Formation of ethionine under these conditions argues either an important metabolic role for the compound or its use as an excretory end product.

FIGURE 5. Radioactive ethionine in the culture medium from *Bacillus megaterium*. This experiment was conducted in the same way as were those using *E. coli*. Ninhydrin-positive spots, due to ethionine added prior to chromatography, coincided with the radioactive zones labeled I and II for ethionine and ethionine sulfone.

The rate of formation of ethionine was studied by inoculating 9.0 ml of synthetic medium containing 5.0 μg of $S^{32}O_4^=$ in each of six tubes with 0.5 ml of log phase *E. coli* in 0.154 M NaCl. This system was incubated with shaking at 37°C through one generation to a population of 1.2×10^8 cells/ml. One millicurie of $S^{35}O_4^=$ was then added to each tube and the incubation continued. Growth was stopped at timed intervals by shaking in an ice and water bath. The medium from each culture was collected in the cold, lyophilized, and extracted with 1.0 ml of absolute ethanol. Aliquots chosen to contain similar levels of radioactivity were spotted on papers and chromatographed. Autoradiograms revealed ethionine by 1 minute after addition of the radioactive

sulfate. Radioactivity was determined by counting the zones for ethionine and ethionine sulfone and adding. These results are summarized in Table II.

Cells harvested from the above experiment were washed and lysed, the soluble portion lyophilized, and the residue extracted and chromatographed. Radioactivities due to intracellular ethionine are presented in Table III.

Fig. 4 is based on data of Table II and reveals a rapid incorporation of $S^{35}O_4^=$ into the ethionine of the medium. Similarly, labeled ethionine appeared quickly inside the cells (Table III). Ethionine accumulated with time but at a decreasing rate because the medium was limiting in sulfur content.

Biosynthesis of ethionine is not peculiar to *E. coli* since this amino acid was also identified in the culture media and inside the cells of *Bacillus megaterium*, *Pseudomonas aeruginosa*, and *Aerobacter aerogenes*. For example see Fig. 5. Using the same techniques, biosynthesis of ethionine could not be detected in an alga of the genus *Scenedesmus*, the yeast *Saccharomyces cerevisiae*, or bovine lymphosarcoma cells.

DISCUSSION

The foregoing chromatographic and ionophoretic comparisons establish the presence of ethionine in the culture medium *Escherichia coli*. The ethionine sulfone, observed after certain manipulations, probably is not formed directly by this organism since it appeared only after oxidation of ethionine during the drying of phenol-water chromatograms. Ethionine sulfoxide did not correspond in chromatographic behavior to any of the radioactive materials encountered in this investigation.

Inspection of both Table III and autoradiograms like Fig. 3 shows that the level of intracellular ethionine was appreciable but in contrast to the medium, did not represent the major proportion of the radioactivity. Furthermore, the radioactivity in ethionine, relative to the total of the alcohol-soluble radioactivity, actually decreased with time, suggesting preferential loss to the medium, decreased synthesis, or preferential utilization as the supply of available sulfur diminished. On the other hand, ethionine became increasingly predominant in the medium, again suggesting a preferential elimination from the cells.

Autoradiograms (*e.g.* Fig. 3) of soluble intracellular materials revealed levels of radioactivity higher in methionine than in ethionine at all the times studied. They also showed that inorganic sulfate did not accumulate appreciably inside the cells under the conditions of this study. Either sulfate is converted to some other form for transport into the cell, or the intracellular utilization of sulfate is fast relative to the transport system.

Apparently *E. coli* possesses a high enough degree of specificity in incorporation of amino acids to avoid inhibition by the ethionine normally synthesized. Added ethionine is reported (2) to inhibit growth of *E. coli* and other bacteria. This effect has been attributed (10) to competition with methionine. Presence

of relatively high normal intracellular levels of methionine may add to the competitive advantage of methionine during protein synthesis in the absence of exogenous ethionine.

Although the only four bacterial species examined all produced ethionine, further study will be needed to delimit the occurrence of this amino acid. However, at present ethionine appears to be quantitatively more important in the metabolism of bacteria than in that of some other taxonomic groups.

Use of ethanol as the extraction solvent does not produce ethionine from methionine or homocysteine by ethyl exchange since application of the same extraction procedure to the yeast, alga, and animal tissue failed to yield a detectable quantity of ethionine-S^{35}. Nor was methionine in a protein hydrolysate converted to ethionine by exposure to ethanol.

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