cells of the respective experiments. The cultures of the second experiment were naturally contaminated, and thus in contact with SV-40 for a longer period than those of the first experiment. The third experiment was similar to the first, only with a prolonged incubation period prior to embedding. The infected cells, in contrast to those of the first experiment, were shown to contain virus particles. The spread of infection through a cell sheet is thus rather slow, and depends on the length of time that virus is in contact with the cells, as well as on the titer of the supernatant. Direct cell-to-cell transfer may play a role in the spread of infection.

These results may be compared with those of other investigators(6), who made use of the fluorescent antibody technique, and found that only 5 to 10 rhesus kidney cells per culture showed evidence for SV-40 antigen 48 hours after addition of virus, and that only 5-10% of the cultured cells did so after 5 days. Assuming that some of this latter antigen has not yet been assembled into a form visible in the electron microscope, and that some infected cells are bound to be missed because the virus does not happen to be present in the particular thin sections cut, these previously described results agree with those of the present investigation.

Summary. SV-40 viral particles were seen in cultures of infected rhesus kidney. SV-40 spreads slowly through the cell sheet, and thus viral particles were not visualized until some time after infection. The SV-40 appears exclusively in cell nuclei, in contrast to its presence in the nucleus and cytoplasm of cell types lysed by the virus.

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Studies on the Mechanism of *Escherichia coli* Resistance to Ethionine.* (28764)

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Ethionine, the ethyl analogue of methionine, inhibits protein synthesis in rats(1) in bacteria(2,3) and protozoa(4). The site of action of ethionine in mammalian and microbial systems has been studied extensively but to our knowledge little attention has been given to mechanisms whereby living systems may become resistant to its toxic or inhibitory effects.

A strain of *Escherichia coli* made resistant to ethionine showed an extended lag period on subculture in a simple medium in the absence of the inhibitor. The present report is concerned with the basis of this lag period and its implications in relation to the nature of ethionine resistance in this microorganism.

Methods. The microorganism used throughout this study was *E. coli* A.T.C.C. 9637. A glucose-salts medium of the following composition was used for growth and maintenance of the organism: NH_4Cl, 1.0 g; K_2HPO_4, 7.3 g; KH_2PO_4, 3.0 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; Glucose, 4.0 g; H_2O, 1.0 liter. Glucose was autoclaved separately before addition to the salts mixture. The final pH of the medium was 7.0.

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Ethionine-resistant organisms were obtained by adding the methionine analogue in increasing concentration to a series of plates containing the basal medium plus 2% agar. Plates were inoculated with 0.1 ml of a suspension containing approximately 2×10^3 bacteria per ml, incubated at 37°C for 24 hours. Bacteria growing on the highest ethionine concentration tested were transferred to a glucose-salts medium containing the same ethionine concentration, incubated at 37°C for 24 hours, and used as inocula on still higher concentrations of DL-ethionine. This procedure was repeated until cells, resistant to 5.0 mg ethionine per ml, were eventually obtained. The original strain was inhibited by 0.5 mg ethionine per ml.

Ethyl-1-C^{14}-ethionine (Volk Radiochemical Corp.) and methyl-C^{14}-methionine (California Biochemical Corp.) were used for incorporation studies. Radioactivity was measured with a windowless, gas flow, Geiger-Muller counter at a constant geometry. Appropriate corrections for background and self-adsorption were made for all experiments. Acids were isolated from the growth medium by continuous ether extraction and purified by developing on a Celite (Johns Manville diatomaceous earth) column with 5% butanol in chloroform(5). Acetic and formic acids were identified by gas chromatography.

Inocula for growth rate studies were prepared from fresh liquid cultures incubated for 24 hours at 37°C. In the case of the resistant strain, the medium contained 5 mg DL-ethionine per ml. The cells were washed 3 times in sterile saline and diluted to an optical density of 0.600. Two ml of this suspension were used to inoculate 80 ml basal medium in 500 ml Ehrlenmeyer flasks. Samples were taken hourly for optical density readings (660 mμ) and plate counts.

Results. Growth curves for sensitive and resistant *E. coli* strains are shown in Fig. 1. Ethionine-resistant cells grown under these conditions require an 8 to 10 hour longer lag phase than the sensitive parent strain. When the basal medium was supplemented with methionine, homocysteine, cystathionine, or ethionine, the extended lag phase was not observed and the growth patterns were similar to sensitive cells(1). In addition, after one passage through the basal medium without ethionine the extended lag phase characteristic of the resistant strain was abolished and concomitantly these bacteria were again sensitive to ethionine.

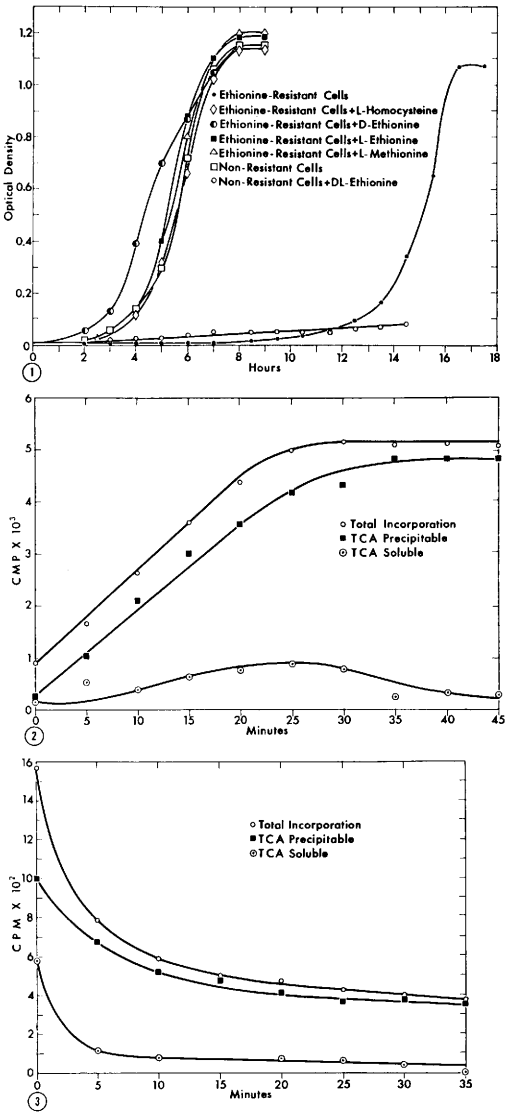

FIG. 1. Extended lag exhibited by ethionine-resistant *E. coli* in the absence of ethionine and its reversal by ethionine, methionine, and homocysteine.

FIG. 2. C^{14}-methionine incorporation by ethionine-resistant cells.

FIG. 3. C^{14}-ethionine incorporation by ethionine-resistant cells.

Labeled amino acid incorporation by sensitive and resistant cells was determined by the method of Roberts *et al*(6,7). Methyl-C^{14}-methionine or ethyl-1-C^{14}-ethionine was added to actively growing (mid-log phase) resistant cells. L-methionine or L-ethionine was present in the basal medium at a concentration of 2.5 mg per ml depending on the tracer used. Samples were withdrawn at 5 minute intervals for amino acid uptake determinations. The incorporation of labeled methionine by resistant cells is shown in Fig. 2. The radioactivity of the TCA-precipitable fraction paralleled the total incorporation of tracer into these cells indicating that there was an apparent rapid transfer of label from the amino acid pool to protein. Similar results were observed with ethionine-sensitive cells.

When ethyl-1-C^{14}-ethionine was added to the medium there was a very rapid uptake of label by ethionine-resistant cells into both the amino acid pool and the protein after which there was a rapid decrease of label in both fractions (Fig. 3). The rapid decrease of label in both the pool and protein indicated that resistant cells could degrade ethionine. Ethionine resistant and sensitive cells were accordingly exposed to ethyl-1-C^{14}-ethionine for 50 minutes in basal medium containing 2.5 mg L-ethionine per ml. Products of ethionine degradation by resistant *E. coli* are shown in Table I. The highest activity was found to be in formic acid, although CO_2 and acetic acid contained significant labeling. Results also shown in Table I were obtained with sensitive cells. There was some decomposition of ethionine by these cells:

TABLE I. Specific Activity of Products of C^{14}-Ethionine* Degradation by Nonresistant and Resistant Cells.

Products	Specific activity (cpm/μM)
	Resistant cells
CO_2	3
Acetic acid	198
Formic "	10,000
	Nonresistant cells
CO_2	4
Acetic acid	164

* Specific activity of substrate ethionine was 14,000 cpm/μM.

Acetic acid and CO_2 were labeled but no formic acid was detected in the supernatant from these cells. The amount of degradation by sensitive cells was relatively low.

Discussion. The extended lag phase observed when ethionine-resistant *E. coli* was grown in the absence of ethionine was reversed by ethionine and also by methionine. The similarity of reversal patterns of methionine and the corresponding analogue appears to indicate that the latter is being converted to methionine. Tracer studies demonstrate that the alkyl group of ethionine was degraded. Removal of this group could lead to the formation of homocysteine, a known methionine precursor. This possibility is supported by the demonstration that homocysteine also led to the complete reversal of the long lag period observed with ethionine-resistant cells.

Ethionine-sensitive cells are able to synthesize methionine from glucose and ammonia; however, they still contain a low but detectable ethionine decomposing activity. Ethionine resistant cells on the other hand are able to decompose ethionine rapidly, convert the products to methionine but apparently lack a portion of the pathway of methionine synthesis normally occurring in *E. coli*. In addition, the lag shown by resistant cells is abolished after one passage through basal medium without the analogue; the cells again becoming ethionine sensitive. These data suggest that the full expression of the methionine forming pathway from ethionine resulted in a suppression of enzymes in part of the pathway of methionine synthesis from glucose and ammonia. The period required for enzyme synthesis of the suppressed portion of this pathway may explain the extended lag phase observed in the growth of resistant bacteria in the absence of ethionine.

There is little known about the pathway involved in ethionine decomposition by resistant *E. coli* cells. Stekol(8) demonstrated the de-ethylation of ethionine in the rat and a transfer of the methylene carbon of the ethyl group to creatine and choline. In *E. coli*, ethionine appears to be de-ethylated; the label (methylene carbon of the ethyl

group) appearing in CO_2, acetate, and formate. A methionine activating enzyme was shown to activate ethionine to form S-adenosyl-L-ethionine in yeast and rat liver mitochondria(9,10,11,12). Conceivably, a reaction of this type may occur prior to ethionine de-ethylation in resistant *E. coli*. Assuming an initial de-ethylation of ethionine, these labeled products may be explained on the basis of known pathways of 2-carbon metabolism.

Studies on the incorporation of C^{14}-ethionine by resistant cells indicate the methionine analogue is incorporated rapidly into protein (TCA precipitable) followed by a rapid decrease in radioactivity of this fraction and paper chromatographic analysis of the TCA insoluble fraction showed the presence of ethionine. These results indicate that ethionine is transferred to protein prior to its degradation. A de-alkylation of ethionine in peptide bond sequence followed by the formation of methionine, if true, would represent a unique process.

Summary. *E. coli* resistant to ethionine shows a 6 to 8 hour longer lag period than sensitive cells when grown on a glucose salts medium without ethionine. The lag period was reduced to that of sensitive cells when methionine, ethionine, cystathionine or homocysteine was added to the growth medium. When ethyl-1-C^{14}-ethionine was added to resistant cells there was a rapid increase of the tracer into both the amino acid pool and protein followed by a rapid decrease of label in both fractions. Formate, acetate, and CO_2 isolated from culture filtrates of resistant cells metabolizing ethyl-1-C^{14}-ethionine were labeled. No significant differences were noted between the incorporation of C^{14}-methionine by ethionine-resistant and sensitive cells. On the basis of these observations, it is concluded that ethionine resistance in *E. coli* is the result of the synthesis of enzymes capable of converting ethionine to methionine. The data indicate that ethionine is de-ethylated to the level of homocysteine followed by transmethylation to methionine.

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Comparative Studies in the Characterization of Monoamine Oxidases.* (28765)

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Since the early survey studies on amine oxidases(1,2) there have been diverse investigations on monoamine oxidases in various preparations from a number of tissues and species, but many aspects of monoamine oxi-

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