type used for vitamin A chromatography will not separate dimethyltocols from momomethyltocols, and the effect of the unsaponifiable matter may be a joint effect of γ- and δ-tocopherols. This is indicated by the fact that soy bean oil containing 5 times as much totally of these tocopherols than ground nut oil, is effective in one-fifth of the concentration.

The effect of these tocopherols is not one of simple displacement, as γ-tocopherol failed to elute further vitamin A from a column where vitamin A had passed through without protection. It may be a pure antioxidant effect protecting against oxidative substances in solvents and adsorbent. A more likely theory is that the tocopherols take the place of vitamin A in irreversible adsorption on the alumina. This is indicated by the recovery of anhydrovitamin A by ethanol elution of a column where vitamin A had passed with a loss.

The last 1—2 % loss of vitamin A during chromatography may arise from the diffusion of the vitamin A-band on the column. It was possible to prove the presence of vitamin A by concentration of the fractions leaving the column before and after the vitamin A-fraction. Carr-Price spot tests or fluorescence test with U.V.-light had given no positive reaction of vitamin A in these fractions.

Tocopherol-fractions useful for the purpose of protection against losses of vitamin A in chromatography can be produced easily from a proper vegetable oil, and be stored in hexane in the freezer for a couple of months. It is recommended to add a suitable aliquot of such a solution to the vitamin A fraction before chromatography of medium and high potent oils.

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The Isolation of O-Phosphoethanolamine from the Rest Protein Fraction of *Escherichia coli* B

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In earlier communications [1,2] it has been shown that acid hydrolysates of the Schneider rest protein fractions from mammalian organs contain O-phosphoserine and O-phosphothreonine. Phosphoserine has also been isolated from the corresponding protein fraction of yeast [3] but in other microorganisms phosphorylated hydroxyamino acids of different composition seem to dominate in the acid hydrolysates [4,5].

It has now been possible to isolate O-phosphoethanolamine in crystalline form from the acid hydrolysate of the rest protein fraction of *E. coli*. Previously published methods for isolation have been followed. The bacteria were harvested at the end of the lag phase. They had been cultivated for about 2 h on a medium containing 1 mC radioactive phosphate per liter [5] to facilitate the identification of phosphorus compounds. Fig. 1 shows the elution curve of the hydrolysed cell walls in the first step of purification through a Dowex-50 column. Of the three main peaks (I, III, V) No. I consists of inorganic phosphate, No. III is a mixture of at least three ninhydrin positive compounds which are not identical with phosphoserine or phosphothreonine. No. V is practically pure phosphoethanolamine. Peak No. II is probably a nucleotide since it gives UV-absorption at 260 mμ and no ninhydrin reaction. A hydrolysate of peak No. IV shows on a paper chromatogram mainly ethanolamine and a few other weaker ninhydrin spots. Sufficient amounts of material for the identification of peak No. V was obtained by mixing radioactive bacteria with large amounts of unlabelled cells. For large scale cultivation the microorganisms were incubated in a 1 000 liter tank on the same medium for about 20 h. The usual yield of fresh bacteria from a single cultivation was about 2.5 kg. Material from two cultivations was used in this study.

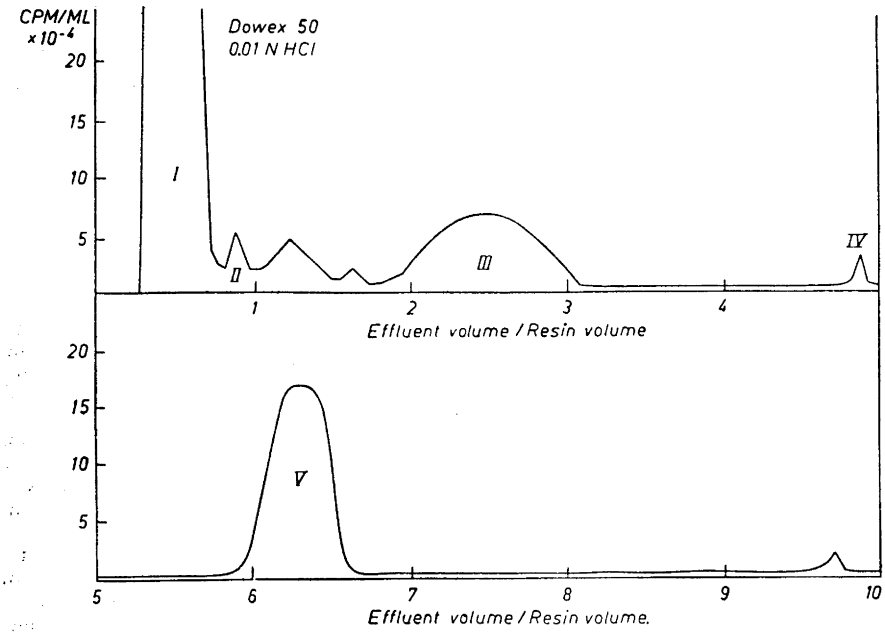

Fig. 1. Radioactivity curve from a hydrolysate of membranes from *E. coli*. incubated for 2½h in a ^{32}P-containing medium. The curve is obtained by plotting the number of impulses per min (cpm) in 1 ml aliquots of each tube.

The identity of peak No. V was verified by elementary analysis (Found: C 17.4; H 5.9; N 9.6; P 23.8. Calc. for $C_2H_8O_4NP$ (141): (C 17.0; H 5.7; N 9.9; P 22.0) and by paper chromatography and ionophoresis. The Debye-Scherrer powder photograph of material from peak No. V was identical with the pattern of a purified commercial preparation of phosphoethanolamine *. The spacings obtained are given in Table 1.

Free phosphoethanolamine has been reported to occur both in normal and neoplastic animal tissues [6]. Ethanolamine has also recently been found in an acid hydrolysate of a peptide-phospholipide-polysaccharide complex from *E. coli* [7]. Recent reports also describe the natural occurrence of phosphatides where the nitrogen moiety consists of a polypeptide [8]. To our knowledge protein-bound phosphoethanolamine has previously not been desribed. We have not found this substance in chromatographic purification of acid hydrolysates from mammalian tissues or in other microcrganisms such as baker's yeast or *L. casei*. The Schneider protein fraction of *E. coli* contained between 0.3—0.5 % of fatty acids. Calculated only as phosphatidylethanolamine the acid hydrolysate of this

Table 1. Values of spacings obtained from phosphoethanolamine.

Intensity	Å
s	5.79
m	4.78
s	4.41
s	4.25
s	3.87
vw	3.77
m	3.57
vw	3.36
w	3.28

vw = very weak, w = weak, m = medium, s = strong.

* Supplied by California Foundation for Biochemical Research.

amount of phospholipid would have yielded less phosphoethanolamine than actually isolated. On the other hand after extraction of lipids in the bacteria the remaining fraction of nucleic acids and protein still contained about 3 % of fatty acids. By the subsequent extraction of nucleic acids with hot 5 % trichloroacetic acid a cephalin-protein compound may have been hydrolyzed giving a part of the phosphoethanolamine bound to the protein part. At present time it is not possible to decide whether the compound is a constituent of a phosphoprotein or represents a link in a lipoprotein complex. In this connection it must be reminded about the fact that *E. coli* as a Gramnegative bacterium contains more phospholipids than a Grampositive microorganism as *L. casei* [9,10]. However, phosphoethanolamine cannot only be isolated from the rest proteins of the walls but also from water soluble fractions of proteins obtained after centrifugation at 105 000 *g* for 30 min at 0°C and precipitation with ethanol or acetone [5].

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Note on the Mutual Solubility of Molybdenum Trioxide and Wolfram Trioxide

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Previous studies on the molybdenum trioxide-wolfram trioxide system have shown that the mutual solubility of these two substances, if there is any at all, is very low [1] (experiments performed at room temperature using samples prepared at 700°C). Access to a Guinier focusing camera with strictly monochromatized $CuK\alpha_1$ radiation has made possible a quantitative estimation of the limits of solubility.

The samples studied were prepared by prolonged heating of intimate mixtures of the two trioxides (both of reagent grade) in sealed, evacuated silica tubes at 700°C. Precautions as described elsewhere [2] were taken to ensure a constant temperature during the annealing time (two periods, of about one week each with intervenient regrinding of the specimen). The samples were quenched in water from the heating temperature. Potassium chloride (Analar, British Drug Houses, $a = 6.2919$ Å at 20°C [3]) was added to the powder specimens as an internal standard [4].

For wolfram trioxide the following unit cell dimensions were obtained:

$a = 7.306 \pm 0.001$ Å, $b = 7.541 \pm 0.001$ Å,
$c = 3.845 \pm 0.001$ Å, $\beta = 90.85° \pm 0.05°$.

The axial lengths exceed by about 0.3 % those derived previously from photographs taken without an internal standard [5,6]. The axial ratios and monoclinic angles obtained from the two experiments are, however, in fair agreement.

Addition of molybdenum trioxide caused slight, continuous modifications of the wolfram trioxide powder pattern up to the approximate composition $W_{0.96}Mo_{0.04}O_3$, which evidently represents the limit of solubility under the conditions mentioned above. These changes are exclusively attributable to a decrease of the length of the a axis, which for the latter composition was found to reach the limiting value of 7.301 ± 0.001 Å, while the other unit cell dimensions were not perceptibly altered.