The gas chromatographic analyses were carried out on a two meter O column (Model Perkin Elmer 116). Column temp.: 60°C; carrier gas: He; flow rate of the carrier gas: 120 ml/min. Retention times: 2.8 min for ethyl isocyanate and 6.8 min for ethyl cyanate.

Reaction of ethyl cyanate with hydrogen sulfide. Hydrogen sulfide was bubbled through a solution of 1 g of ethyl cyanate in 5 ml of ether, cooled in an ice bath, for 4 h. After addition of 10 ml of ether the solution was filtered, washed successively with 2 ml portions of 2 N hydrochloric acid and water, dried over sodium sulfate and evaporated to dryness. The semi-solid residue (0.99 g) was crystallised from 20 ml of heptane and yielded 0.61 g (39 %) of O-ethyl thiocarbamate (xanthogenamide) with m.p. 40–41°C (lit. 40–41°C). (Found: N 13.25. Calc. for C_3H_7NOS: N 13.32). The infrared spectrum was identical in all details with the infrared spectrum of an authentic sample of O-ethyl thiocarbamate.

Reaction of ethyl cyanate with aniline. (*a*) 5-Ethoxythiatriazole (1 g) was dissolved in dry ether (5 ml) and 1 ml of aniline was added. An exothermic reaction took place and the solution had to be cooled to keep the temperature near 20°C. After 24 h the precipitate was isolated by centrifugation and washed with ether and with carbon disulfide (to remove sulfur). The infrared spectrum of the substance was identical with the infrared spectrum of phenylurea. M.p. after recrystallisation from ethanol 146–147°C (lit. 147°C). Yield 0.267 g = 26 %.

(*b*) Aniline (0.5 g) was added to ethyl cyanate (0.5 g) which had been kept for 48 h at room temperature. A reaction took place with heat evolution. After 24 h the reaction mixture was extracted with boiling hexane and the residue recrystallised from 20 % ethanol. Yield 0.25 g of N-ethyl-N′-phenylurea with m.p. 98–98.5°C (lit. 99°C), corresponding to 22 % of ethyl isocyanate in the starting material. The infrared spectrum of the product was identical with the infrared spectrum of an authentic sample of N-ethyl-N′-phenylurea.

Addition of aqueous ammonia to the same sample of ethyl cyanate yielded ethylurea, whose identity was proved by m.p. (91°C) and infrared spectrum.

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Formation of Serine and Threonine Dehydratases during the Growth Cycle of *Escherichia coli*. Evidence of two Different Enzymes

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Previous investigations carried out in this laboratory have shown that the total activities of tryptophan synthase [1] and certain transaminases [2] decrease sharply towards the end of the active cell growth phase to nearly the levels at the beginning of the growth cycle. This decrease in total activity has been demonstrated in many earlier papers that were briefly reviewed in one of ours.[2]

We have now studied the formation of serine and threonine dehydratases (EC 4.2.1.13 and EC 4.2.1.16) during the active growth phases of *Escherichia coli*. The effect of the pH of the growth medium on the formation of the enzymes was also estimated. Evidence for the existence of two separate enzymes is presented below.

Experimental. The activities of the serine and threonine dehydratases were determined by the hydrazone method as modified by Walker.[3] The organism and its cultivation have been described earlier.[4,5] The frozen and thawed cells were used as enzyme sources.[1] The amino acid medium (abbreviated AM) contained L-amino acids in the same ratios as these are present in the protein of *E. coli*.[6] This AM medium is the same as the glucose-mineral salt medium [5] except that the ammonium salts are replaced by amino acids (5.27 g per litre of growth medium).

Results and discussion. Fig. 1 shows the formation of threonine dehydratase by *E. coli* cells in the AM and MM (glucose-mineral salt medium) media. It will be

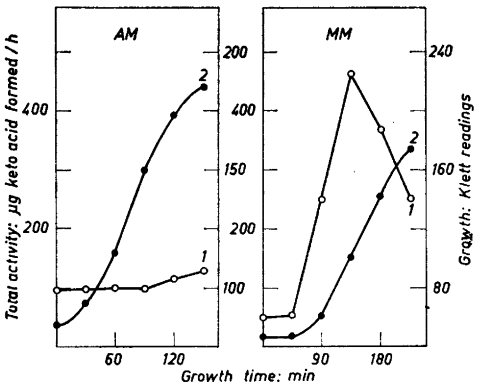

Fig. 1. Formation of threonine dehydratase during the growth cycle of *Escherichia coli*. 1, Enzyme concentration; 2, growth curves (right-hand scales); AM, amino acid medium; MM, glucose-mineral salt medium.

seen that the rate of formation of threonine dehydratase is low in the amino acid medium but high in the MM medium, the lag phase value for the latter medium being 50 µg of keto acid formed per hour and the maximal value 465 µg keto acid formed per hour. Here again the total activity of threonine dehydratase is low in the exponential phase when the pH of the medium is about 6.5. In Fig. 2 the formation of serine dehydratase is shown. As seen from this figure, the increase in total activity is very marked in the AM medium

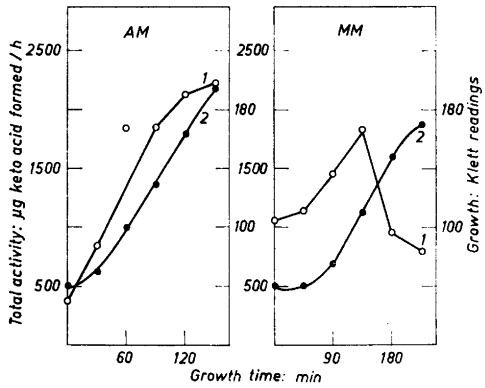

Fig. 2. Formation of serine dehydratase during the growth cycle of *Escherichia coli*. Notation as in Fig. 1.

(approximately 4-fold), but less marked in the MM medium (approximately 2-fold). If L-serine is omitted from the AM medium, the rate of formation is not so marked as when L-serine is present; this result supports the hypothesis that L-serine dehydratase is an inducible enzyme. The formation of serine dehydratase is not repressed in the AM medium as is the formation of threonine dehydratase. When the total activities of these two enzymes in cells growing in the MM media are followed, the decrease is not prevented by keeping the pH constant (at pH 6.7—6.8) by adding sodium hydroxide during the growth cycle

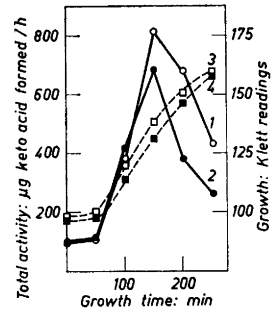

Fig. 3. Formation of serine dehydratase during the growth cycle of *Escherichia coli*. 1, Enzyme concentration (left-hand scale) when the pH of the medium was kept constant (pH 6.7—6.8) during the cultivation; 2, enzyme concentration when the pH changed from the starting value 6.7 to the value 6.0 during the cultivation; 3, growth curve (pH changed); 4, growth curve (pH constant).

(Fig. 3). This observation (a similar one was made with threonine dehydratase) supports the hypothesis that the increasing acidity is not the cause of the decrease in total activity during active cell growth. The decrease has been detected even in the early part of the growth cycle.[7,8] Results similar to those shown in Fig. 3 have also been obtained for all the B_6 enzymes previously studied in this laboratory [1,2] and for tryptophan synthase, whose formation at constant pH was also investigated in connection with the present work.

The formation curves in Figs. 1 and 2 clearly show that the threonine and serine dehydratases are two different enzymes. This is a very interesting observation because the contrary view has also been expressed.[9-11]

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The Detection of Phorbic Acid in *Eurphorbia palustris* L.

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Phorbic acid, a new dilactone acid, was recently isolated from Euphorbium,[1,2] which is the dried up latex isolated from the North African stem succulent *Euphorbia resinifera* Berg. Phorbic acid has now been detected also in the Norwegian species *Euphorbia palustris L.*, which is an herbaceous, thinleaved perennial. This seems to indicate that phorbic acid is widely distributed within the genus *Euphorbia*.

While phorbic acid is the predominating part of the non volatile organic acids of Euphorbium, it represents only a small percentage of the mixture of non volatile organic acids that could be isolated from *Euphorbia palustris*. For this reason the acid might easily have been overlooked in this material if we had not been specially on the look-out for it.

For the purposes of this investigation 1.90 kg of airdried *Euphorbia palustris* was used, and from this material 155 g of a crude acid-mixture was isolated over the lead salts.

Our first attempt to demonstrate phorbic acid in *Euphorbia palustris* failed: 30 g of the 155 g acid-mixture just mentioned was esterified with *diazoethane* and the esters fractionated *in vacuo* at 10^{-1} mm Hg. When the acids from Euphorbium are treated under such conditions, the ethyl ester of dilactophorbic acid crystallizes from the fractions that boil between 180—220°C especially when the fractions are diluted with ethanol and left in the refrigerator for a couple of days. But this time no crystalline ethylester could be obtained.

An examination of the two upper fractions (180—215°C and 215—220°C) showed that these contained an admixture of phenolic substances that were not found in the acid-mixture from Euphorbium. A closer investigation of an I. R. spectrum of the same fractions revealed an absorption peak at 1800 cm^{-1}. This is close to the absorption peak at 1808 cm^{-1} which is given by pure dilactophorbic acid monoethylester.

Encouraged by the last mentioned finding, we worked up a new samle of the crude acid-mixture. This time 70 g was used and the acids were esterified with anhydrous ethanol, using dry HCl gas (2 %) as a catalyst. The phenolic substances were removed from the ester-mixture by washing with sodium carbonate solution and water, whereupon the esters were fractionated in the same way as in the first experiment. The two upper fractions were diluted with equal volumes of ethanol, and this time the expected ester crystallized out after a few hours. The yield after recrystallization from ethanol was 40 mg.

The substance melted at 91—92°C, and showed no melting point depression when mixed with monoethylester of dilactophorbic acid. Also the I.R. spectrum of the isolated substance was identical with the monoethylester of dilactophorbic acid.

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