Although the majority of the organisms recorded are not human pathogens and the concentration of penicillin used is very much higher than blood-level concentrations achieved clinically it is interesting to find a further unification and extension of this fundamental biochemical action of penicillin. The above experiments have in the case of *E. coli* NCTC 5934, *Serratia marcescens* and *Ps. hydrophila* been repeated on the 250 ml. scale and by careful centrifugation followed by resuspension in 0·33M sucrose a washed suspension of protoplasts was obtained suitable for further study.

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On the Quantitative Estimation of Amino Acids by Paper Chromatography

SIR,—The quantitative estimation of amino acids as their dinitrophenyl (DNP) derivatives by Levy [1] was made by two dimensional paper chromatography using a toluene:chloroethanol:0·8N ammonium hydroxide system in the first direction followed by 1·5M phosphate buffer in the second direction. Chloroethanol is poisonous[2] and it is desirable to replace it. The method of Rockland and Dunn[3] was used for screening various organic liquids (hydrocarbons, ketones, alcohols, ethers, esters) as developers in the chromatography of DNP-amino acids. Low R_F values were obtained using the hydrocarbons, ethers and chloroform whilst higher values were obtained with the alcohols.

A system referred to as "Ethyl benzene" was devised and consists of ethyl benzene:*tert.*-amyl alcohol:1·6N ammonium hydroxide 1:3:2 (v/v/v), it was used in the first direction, followed, after drying the paper, by 1·5M phosphate buffer in the second direction. Figure 1 shows the separation obtained with a mixture of DNP-amino acids.

The factors given by Levy were found not to be applicable to the analysis of β-lactoglobulin under the conditions of this experiment. The reactions of amino acid with dinitrofluorobenzene (DNFB) by Sanger's[4] method in 66 per cent ethanol and in aqueous solution[1,5] was investigated. A mixture of amino acids containing 0·00002M of each was reacted with DNFB, the DNP-amino acids were subjected to quantitative paper chromatography using the ethyl benzene system in the first direction followed by 1·5M phosphate buffer. From the optical density reading of each DNP-amino acid and the concentration of the amino acid, a factor "F" was deduced which gives the concentration of the amino acid in moles for an optical density reading of 1. The factors obtained by reaction in 66 per cent ethanol for 1½ and 2 hours differed and when the factors for 1½ hours reaction was applied to a protein hydrolysate, the values for valine, leucines, lysine and phenylalanine were low. The results were consistent and reproducible $\pm$ 5 per cent when the reaction was carried out in 0·1N potassium chloride for 1½ and 2 hours. The factors "F" $\times 10^7$ are, Asp and Glu 0·635; Gly 0·784; Ala 0·713; Val 0·65; Leu's 0·692; Ser 0·765; Thr 0·695; CyS 0·383;

CySH 0·766; Met 1·0; Arg 0·744; His 1·55; Lys 0·392; Tyr 0·9; Pro 0·56; Phe 0·785; Orn 0·421; Hypro 0·6.

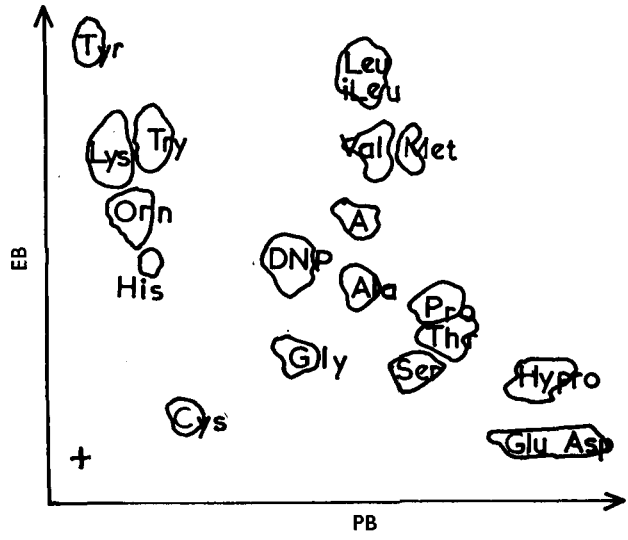

FIG. 1. Two-dimensional chromatogram of a known mixture of DNP-amino acids. EB, direction of flow of ethyl benzene system developer. PB, direction of flow of phosphate buffer developer. Spots (amino acids as DNP derivatives): A, α-aminobutyric acid; Ala, alanine; Asp, aspartic acid; Cys, cystine; DNP, dinitrophenol; Gly, glycine; Glu, glutamic acid; His, histidine; Hypro, hydroxyproline; Leu, leucine; iLeu, *iso*leucine; Lys, lysine; Met, methionine; Orn, ornithine; Pro, proline; Ser, serine; Tyr, tyrosine; Thr, threonine; Try, tryptophane; Val, valine.
+, Point of application of initial mixture.

Histidine gives rise to Di-DNP histidine in 66 per cent ethanol while in aqueous solution both the mono and di DNP-histidine are obtained.

Quantitative paper chromatography of DNP-amino acids has been applied to the analysis of β-lactoglobulin using the system and the factors reported above; the results obtained compared favourably with the reported data[6,7].

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