These results are of interest since indole derivatives and noradrenaline-like substances have not been detected in extracts of strawberries, cherries, rhubarb, raspberries, blackcurrants, gooseberries, lemons, oranges, apples, figs, prunes or potatoes.

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Penicillin-induced Round Bodies in Gram-negative Bacteria

SIR,—Lederburg[1,2] has recently shown that if penicillin is allowed to act upon growing cultures of *Escherichia coli* and *Salmonella typhimurium* in hypertonic medium in the presence of Mg^{++}, spherical forms or round bodies are generated, due it is postulated, to complete or partial inhibition of cell-wall synthesis during cell division. Similar results are reported for *Pr. vulgaris*[3] and *Alcaligenes faecalis*[4]. The term round body rather than protoplast has been retained in describing these forms in accordance with the suggestion of Brenner and others[5] that the term protoplast should be applied only when there is additional evidence to show that the round bodies contain no cell wall residues.

It is obviously of interest to apply this elegant but simple experiment to other Gram-negative organisms. In our experiments 0·15 ml. of a 17 hour culture of the organism containing about 10^7 viable cells was inoculated into 10 ml. of a medium containing in each litre: sucrose 114, $MgSO_4.7H_2O$ 2·5, NaCl 5, Lab Lemco 10 and peptone (Oxoid) 10 g., and varying quantities of the potassium salt of benzylpenicillin. Growth was allowed to proceed at 37° for 4–5 hours and the cultures examined by interference microscopy.

The results for 12 organisms are summarised in Table I.

TABLE I

Organism	Concentration of penicillin to induce round bodies (units/ml.)	Diameter of round body (μ)	Size of organism in hypertonic medium without penicillin (μ)
E. coli[1]	4000	3·5–6·0	0·52 × 2·6
E. coli[2]	25–100	4·0–5·0	0·52 × 1·7
Cloaca cloacae[3]	100–200	6·2–6·8	0·78 × 1·7
Citrobacter freundii[4]	3000	4·8–5·2	0·52 × 1·7
Klebsiella aerogenese[5]	1500–2000	4·3–5·2	0·78 × 2·1
Serratia marcescens[6]	1000–5000	3·5–5·5	0·52 × 0·78
Proteus vulgaris[7]	1000	3·4–4·3	0·78 × 2·6
Proteus morganii[8]	5000	5·2–6·0	0·52 × 1·7
Pseudomonas aeruginosa[9]	250–2000	6·0–6·5	0·52 × 2·1
Pseudomonas hydrophila[10]	4000–5000	5·5–6·5	0·52 × 1·7
Vibrio cyclosites[11]	100	2·6	0·78 × 2·6
Vibrio neocistes[12]	100	2·6	0·78 × 2·6

[1]*E. coli* NCTC 86. [2]*E. coli* originally NCTC 5934. [3]*Cloaca cloacae* NCTC 8155. [4]*Citrobacter freundii* NCTC 8165. [5]*Aerobacter aerogenese* NCTC 8197. [6]*Serratia marcescens* isolated in the laboratory. [7]*Proteus vulgaris*—Constantinople OX19 NCTC 7052. [8]*Proteus morganii*—692 NCTC 417. [9]*Pseudomonas pyocyanea* NCTC 7244. [10]*Pseudomonas hydrophila*—Kulp NCTC 7810. [11]*Vibrio cyclosites* NCIB 2581. [12]*Vibrio neocistes* NCIB 2582.

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;