Table 1. R_F AND SPECTRAL PROPERTIES OF PRODUCTS FROM *E. coli*

Experiment No.	Spot designation	R_F	*A* ratio (265/355)
1	*A*	0·68	–
	B	0·78	–
2	*C*	0·66	3·83
	D	0·78	3·31
3	*E*	0·95	–
	F	0·69	–
	G	0·75	–
Standard	OTC	0·76	1·24
Standard	Tetracycline	0·78	1·11
Standard	Desdimethylamino-oxytetracycline	–	1·32

The experiments were run as follows: Cells from 50 ml. of nutrient broth culture incubated for 15 h at 37° C were collected by centrifugation and washed twice with water. They were resuspended in 50 ml. of a salts and glucose medium containing OTC (50 μg/ml.), and incubated for 105 min at 37° C with shaking. The cells were recovered by centrifugation (yield about 100 mg wet weight). The cells were extracted with 10 per cent TCA at 0° C for 1 h. The TCA was removed by three extractions with ether. The extract was freeze dried to concentrate the material, then resuspended in a small volume of water, and analysed by ascending paper chromatography in a solvent of isobutyric acid—0·5 N ammonium hydroxide (5 : 3). Chromatography was extended over 15 h at room temperature (about 25° C).

Except for experiment No. 2, it has not been possible to elute the spots from the paper after chromatography; this has greatly limited the scope of the investigations so far. The results given in Table 1 would seem to demonstrate that three new compounds are formed from OTC during the incubation period. These are, respectively, a material of slower R_F, faster R_F, and the same R_F as OTC. The spectral properties of the R_F 0·78 material suggest that it is not the parent compound. A control exposure of 5 mg/ml. of OTC to 9 per cent TCA for 1 h at 0° C resulted in only one spot on paper chromatography, of R_F 0·78 and an absorbancy ratio (265/355) of about 0·96. It was apparently unchanged OTC. Another control experiment showed that no fluorescent spots were extracted from *E. coli* carried through this procedure when OTC was omitted from the incubation mixture. Immersion of cells in a boiling water bath for 15 min before exposure to OTC completely eliminated their ability to convert the drug to these new compounds. The ability of the *E. coli* to concentrate OTC from the external medium[2] was destroyed concomitantly. Although alternative explanations are possible for these observations, it would appear that bacterial viability is essential for the observed changes in the OTC to occur. These changes are presumably the result of enzyme catalysed reactions within the bacteria.

When bioautographs were prepared from the paper chromatograms (experiment No. 3), using *Staphylococcus aureus* as the test organism, clearly defined zones of inhibition were apparent for all three spots. Thus these compounds retain antibacterial properties.

Note added in proof. After the submission of this manuscript, Izaki and Arima (*J. Bacteriol.*, **89**, 1335; 1965) reported further details of the uptake of OTC by *E. coli*.

JEROLD A. LAST
J. F. SNELL

Agricultural Biochemistry Department,
Ohio State University,
Columbus, Ohio.

[1] Meyers, E., and Smith, D. A., *J. Bacteriol.*, **84**, 797 (1962).
[2] Arima, K., and Izaki, K., *Nature*, **200**, 192 (1963).
[3] Izaki, K., and Arima, K., *Nature*, **200**, 384 (1963).

Specific Activity of Phenylalanine Deaminase in Extracts of the *Proteus–Providence* Group

THE possession of a phenylalanine deaminase which converts phenylalanine to phenylpyruvic acid is one of a combination of five properties unique for the *Proteus–Providence* group of organisms[1]. Of a total of 185 *P. hauseri* (*P. mirabilis* + *P. vulgaris*), 155 *P. morganii*, 29 *P. rettgeri* and 239 *Providence* strains qualitatively examined for the presence of this enzyme[2–4], all but two *Providence* strains[3] were positive, although *P. morganii* strains have been reported to yield on occasions weak or negative results[5].

As part of an investigation of this enzyme, its specific activity in extracts of the *P. hauseri*, *P. morganii* and *P. rettgeri* strains previously used[6] was investigated. An additional fifty-seven strains of *P. morganii* were also examined. The strains were grown overnight on Difco nutrient agar supplemented with 0·3 per cent extracts of yeast. Organisms were collected and placed in 0·8 per cent saline and sonicated at 9 kc/s for 20 min before they were centrifuged in the cold at 198,000*g* for 30 min. Protein concentration in the cell-free supernatant was measured by the biuret method and crystalline bovine albumin was used as the standard. The specific activity of phenylalanine deaminase in the supernatant was measured by incubating 1·0 ml. with 0·2 M *tris*-hydrochloric acid buffer at *p*H 7·4 (1·0 ml.) and 0·08 M D,L-phenylalanine (1·0 ml.) for 60 min at 37° C after which the reaction was stopped by the addition of 40 per cent trichloroacetic acid (1·0 ml.). A blank was included for each sample with the trichloroacetic acid added and mixed before addition of the phenylalanine. After centrifugation, 1·0 ml. samples of the supernatant were adjusted to *p*H 2 and assayed with ferric chloride for phenylpyruvate content by the method of Rowsell[7].

Table 1. PHENYLALANINE DEAMINASE ACTIVITY OF *Proteus* AND *Providence* ORGANISMS

	No. strains	< 50	50–2,000	2,000–4,000	4,000–10,000
P. mirabilis	19	—	—	—	19
P. vulgaris	9	—	—	—	9
P. rettgeri	22	4	18	—	—
P. morganii	74	—	5	69	—
Providence	24	—	6	18	—

Overnight nutrient agar growths were collected and placed in 0·8 per cent saline and sonicated in the cold. After centrifugation at 4° C, the phenylalanine deaminase activity of the clear supernatants was determined and expressed as μμmoles phenylpyruvate formed/mg of protein/min.

Results are presented in Table 1. Results were not affected by the incorporation of 0·2 per cent D,L-phenylalanine in the growth medium and the striking feature is the high deaminase activity of *P. hauseri* extracts. *P. hauseri* differs from the other groups of organisms investigated here in its pronounced swarming capacity and the ability to ferment xylose, to produce hydrogen sulphide and to liquefy gelatine. The quantitative difference in phenylalanine deaminase activity demonstrated may support claims[1,8] that biochemical differences among these groups of organisms are at generic level within a tribe Proteae.

J. A. SMIT

South African Atomic Energy Board and
Department of Microbiology,
University of Pretoria.

[1] Rauss, K., *Intern. Bull. Bact. Nom. Tax.*, **12**, 53 (1962).
[2] Henriksen, S. D., *J. Bact.*, **60**, 225 (1950).
[3] Shaw, C., and Clarke, P. H., *J. Gen. Microbiol.*, **13**, 155 (1955).
[4] Singer, J., and Bar-Chay, J., *J. Hyg., Camb.*, **54**, 1 (1954).
[5] Carpenter, K. P., *Rec. Adv. Clin. Pathol.*, **4**, 13 (J. and A. Churchill, London, 1964).
[6] Coetzee, J. N., *J. Gen. Microbiol.*, **31**, 219 (1963).
[7] Rowsell, E. V., *Methods in Enzymol.*, **5**, 685 (Academic Press, London, 1962).
[8] Kauffmann, F., *Zentral. Bakt. I. Orig.*, **165**, 344 (1956).

GENERAL

Origin of the Theory of Errors

J. H. LAMBERT should be given precedence over Gauss as the originator of the theory of errors. Galle[1] stated that Gauss arrived at the idea of the principle of least squares while reading Lambert, but he did not mention the essence of Lambert's work on the subject. Furthermore, no mention has been made of this in the various works on Lambert[2–4].