The dependence on *p*H of the stability of the toxin has also been determined accurately; so far, it has only been stated[3] that above *p*H 7·0 the toxin is rapidly destroyed. It has been found that a *p*H of 7·5 causes a loss of activity of 100,000 *MLD* in 10 min., and at *p*H 8·2 a corresponding decrease by 250,000 *MLD* (at room temperature).

In contradistinction to Lamanna's[3] results on crystalline toxin, no influence of cupric ions (5 per cent cupric sulphate) on the activity of a concentrate was observed; also silver ions were without any adverse effect. Only mercuric salts (potassium iodomercurate) caused inactivation, when applied in concentrations of 1·2 per cent.

Pepsin does not decrease the activity of a toxin concentrate, as was to be expected from the well-known high oral toxicity of the toxin. A considerable increase in amino-nitrogen, however, was observed in the pepsin experiments; this may be due to the attack of the enzyme on impurities of protein character, which accompanied the toxin in the preparation used.

URIEL LITTAUER

Weizmann Institute of Science,
Rehovoth, Israel.
Feb. 8.

[1] Wentzel, Sterne and Polson, *Nature*, **166**, 739 (1950).
[2] Abrams, Kegeles and Hottle, *J. Biol. Chem.*, **164**, 63 (1946).
[3] Lamanna, Eklund and McElroy, *J. Bact.*, **52**, 1 (1946).

Identity of Frequentic Acid and Citromycetin

A WEAKLY antibacterial metabolic product of the moulds *Penicillium frequentans* Westling and *P. vesiculosum* Bainier has recently been described[1] and named frequentic acid. It was said to be yellow in colour and to give a deep green colour with ferric chloride; in these properties it is similar to citromycetin[2], $C_{14}H_{10}O_7.2H_2O$ (m.p. 285° C., dec.), a well-known metabolic product of *P. frequentans*. Though apparently differing in other published physical and chemical properties, for example, empirical formula $((C_5H_5O_3)_n)$, melting point (155° C.), and stability towards hydrolytic reagents, it seemed possible to us that frequentic acid and citromycetin were identical. Further investigation has confirmed that this is so.

The Sir William Dunn School of Pathology, Oxford, kindly supplied us with a sample of frequentic acid and with cultures of moulds from which it had been obtained. These included two strains attributed to *P. frequentans* and one attributed to *P. vesiculosum* (believed to be NCTC 3567).

Following recrystallization from ethanol, the crystals of frequentic acid, when dried at 100° C., no longer melted with effervescence at 155° C., but decomposed at 290–300° C. with considerable previous blackening; found (on a sample dried *in vacuo* over phosphorus pentoxide for two days at 100° C.) C = 57·6, H = 3·6 per cent; (on a sample dried *in vacuo* at 150° C.) C = 57·9, H = 3·6 per cent; calculated for $C_{14}H_{10}O_7$, C = 57·9, H = 3·5 per cent. The equivalent weight of an air-dried sample of frequentic acid was found by potentiometric titration to be 162; calculated for $C_{14}H_{10}O_7.2H_2O$, 163 (dibasic acid).

An authentic specimen of citromycetin obtained from Dr. G. G. Freeman darkened at 260° C. and decomposed at 290–300° C. A mixed melting point determination with frequentic acid showed the same behaviour, although this cannot be taken as a reliable indication of the identity of the two specimens in view of the extensive decomposition which takes place. However, the infra-red absorption spectra of the two materials in the 3–14 μ region were identical.

Both frequentic acid and citromycetin gave an orange solution showing a green fluorescence with concentrated sulphuric acid, and both reduced ammoniacal silver nitrate rapidly in the cold. On boiling frequentic acid with 2*N* sulphuric acid for eight hours, it was converted into citromycin (m.p. 290° C., with previous blackening), giving an intense brown colour with ferric chloride. Frequentic acid was rapidly decomposed on boiling with 3*N* sodium hydroxide solution.

Dr. G. H. Spray (Department of Clinical Medicine, Oxford) has compared for us the antibacterial activities of citromycetin and a sample of frequentic acid which he had prepared in the autumn of 1946. In a cylinder plate assay with *Staphylococcus aureus*, 0·5 per cent solutions in *p*H 7·3 phosphate buffer gave inhibition zones of 25 mm. and 26 mm. mean diameter respectively.

The three moulds from which frequentic acid has been obtained all proved to be typical strains of *P. frequentans* Westling. The strain attributed to *P. vesiculosum* in no way agreed with Bainier's original description; in fact, the status of this specific name has recently been questioned[3]. All these citromycetin-producing strains of *P. frequentans*, together with others we have examined, are quite distinct in colony characteristics from those strains of the species from which another antibiotic, frequentin, has recently been obtained[4].

The foregoing evidence shows that frequentic acid and citromycetin are identical in their chemical and biological properties.

We are indebted to Drs. Weiler and Strauss, Oxford, and to Mr. W. Brown for the microanalyses, and to Mr. H. A. Willis and the directors of I.C.I. Plastics Division for the use of infra-red facilities.

JOHN FREDERICK GROVE
P. W. BRIAN

Butterwick Research Laboratories,
Imperial Chemical Industries, Ltd.,
Welwyn, Herts.
Feb. 8.

[1] Florey, H. W., *et al.*, "Antibiotics" (Oxford, 1949).
[2] Hetherington, A. C., and Raistrick, H., *Phil. Trans.*, B, **220**, 209 (1931).
[3] Raper, K. B., and Thom, C., "A Manual of the Penicillia" (London, 1949).
[4] Curtis, P. J., Hemming, H. G., and Smith, W. K., *Nature*, **167**, 557 (1951).

A Cross-Reaction in Resistance to Antibiotics

IN the course of routine laboratory work on sensitivity to antibiotics, a type of 'cross-reaction' in the development of resistance to them has been observed.

Case 1. A boy aged two years with an obstruction of the right tear duct was treated with distaquane-penicillin, 300,000 u./day for fifteen days. An eye swab was then taken and a blood-agar culture made from it. Three colonies grew and were identified as a *Staphylococcus aureus*. One of these was sub-cultured on blood agar and examined for antibiotic sensitivity by the paper disk method[1]. Four standard anti-