

The molecular weights of the films have been estimated by end-group methods; the nitrogen liberated in a Van Slyke amino-nitrogen apparatus, and the absorption of free carboxyl groups by methylene blue, both point to a figure in the neighbourhood of 15,000, very much lower than the "several million" quoted by Woodward and Schramm<sup>2</sup>.

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<sup>1</sup> Astbury, W. T., and Dalglish, C. E., *Nature*, **162**, 596 (1948).

<sup>2</sup> Woodward, R. B., and Schramm, C. H., *J. Amer. Chem. Soc.*, **69**, 1551 (1947).

<sup>3</sup> Leuchs *et al.*, *Berichte*, **39**, 857, etc. (1906).

### Organisms Producing Antibiotics in Tropical Soils

EXCEPT for some experiments by Meredith<sup>1</sup> with Jamaican soils, little information is available on the presence in tropical habitats of antibiotic-producing micro-organisms. Using a technique similar to that of Stokes and Woodward<sup>2</sup>, we are finding increasing evidence that tropical soils harbour such organisms, sometimes types which have not yet been identified.

Actinomycetes, as was to be expected, are widely represented, but in our opinion are of much less interest than certain spore-forming and non-spore-forming aerobic bacteria which occur there. These produce a surprisingly selective inhibition of the growth of other organisms.

A short rod, for example, discovered by one of us (I. T.) in Trinidad air, and presumably carried there with soil particles, produces a water-soluble thermostable and apparently non-toxic antibiotic which arrests the growth of all the fungi and yeast tested—so far more than forty species. Its action is often a positive one in concentrations of less than one part in a million. Against Gram-positive and Gram-negative bacteria this antibiotic is inactive.

Another organism which has been isolated is active against fungi, bacteria (*Bact. coli commune*) and aerobic bacilli (*Bact. mycoides*), while others are effective against fungi and bacteria.

In one of the investigations now proceeding the presence of peptone in the culture medium is essential for the production of an antibiotic. In another series glucose and peptone are required. It has not yet been ascertained whether peptone can be replaced by amino-acids, and, if so, by which.

It is proposed to extend the study now in progress to cover tropical habitats other than soils, and to alter the technique employed so that organisms may be collected—if they should exist—which may require materials other than peptone and glucose for antibiotic production.

In his review of bacteria in soil, Conn<sup>3</sup> refers to the isolation of antibiotic organisms from soils. He admits the interest of such work, which he classifies as both important and practical, though not agriculturally significant. We suggest that the existence of organisms producing antibiotics may play a part in maintaining a balanced microflora in soil. In this

we are in full agreement with the views of Brian expressed recently.

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<sup>1</sup> Meredith, C. H., *Phytopath.*, **34**, 426 (1944).

<sup>2</sup> Stokes, J. L., and Woodward, C. R., jun., *J. Bact.*, **43**, 253 (1942).

<sup>3</sup> Conn, H. J., *Bact. Rev.*, **12**, 247 (1948).

<sup>4</sup> Brian, D. W., *J. Gen. Microbiol.*, **2**, xvii (1948).

### Concentration of the Toxic Substance from 'Agenized' Flour

IT was shown by Sir Edward Mellanby<sup>1</sup> that flour, treated with nitrogen trichloride, as it is in the commercial 'Agens' process, is capable of causing fits in dogs, when fed in fairly large quantities. Further investigations have proved that the toxicity is associated with the gluten of the flour, and that the toxicity survives digestion with pepsin and with trypsin<sup>2</sup>. The recent publication in abstract by Reiner *et al.*<sup>3</sup> of particulars of the fractionation and concentration of the toxic substance (mainly prepared by the action of nitrogen trichloride on zein), and the publication by Moran *et al.*<sup>4</sup> of a method of concentration of the toxic substance from zein, make it desirable for us to give our own results.

The process employed involves pancreatic digestion of the gluten followed by passage through resin columns and precipitation with mercury acetate. The gluten was not previously separated from the flour. 70 lb. of flour 'Agenized' at the rate of 125 gm./sack was mixed with 14 gallons distilled water, 500 gm. pancreatin and 500 ml. chloroform. The mixture was adjusted to pH 8 with sodium hydroxide and disodium hydrogen phosphate and digested at 37° for 24 hr. The sludge was centrifuged. The recovered gluten was re-suspended in about 3 gallons of water, brought to pH 8, and a further 500 gm. pancreatin added. This mixture was digested for a further 48 hr. at 37° and centrifuged. The supernatant from this digestion was usually more toxic than that from the first. It was treated with 2 vol. of absolute alcohol, and the precipitate formed was centrifuged off. The alcoholic liquor was passed through a column containing 2½ lb. of activated 'Zeocarb 215'. When the adsorbed material was eluted with dilute sodium hydroxide, the toxicity was found in the neutral 'fraction' associated with some residual quantities of the acidic and basic amino-acids.

The concentrate so obtained was treated with acid-activated alumina, which removed the acidic amino-acids. After extraction with butanol, a product was obtained which showed four spots on two-dimensional paper chromatography, three of which could be identified as glycine, serine, alanine, and the fourth as glutamine running with an almost indistinguishable unknown substance.

In order to confirm the possibility that this unknown spot was associated with the toxic substance, a quantity of the concentrate was placed in a line along the edge of a large sheet of filter paper and developed with phenol in a direction at right angles to the line. The unknown spot was then located in a long streak parallel to the original position. This part of the chromatogram was eluted with water, and