discovery of 'Pamaquin', the first synthetic antimalarial. Later, Ehrlich and Hata[3] found that *in vitro* methylene blue was very toxic to the spirochætes of relapsing fever, but that *in vivo* it had no effect on the course of the disease in mice.

It seemed of interest to try the effect of methylene blue on a different genus of spirochætes, the leptospires, which have different growth requirements and a different distribution in the body. The test organism was *Leptospira icterohæmorrhagiæ*. *In vitro*, the organisms were immobilized and killed by very low concentrations of methylene blue (2 μgm./ml.); but *in vivo* doses of 10 μgm./gm. intraperitoneally on two successive days failed even to prolong the life of the test guinea pigs. The strain of *L. icterohæmorrhagiæ* used was highly virulent, and killed guinea pigs in four or five days. It is still possible that methylene blue might be effective in clearing the leptospires from the kidney tubules in cases of chronic infection; but this remains to be tested.

J. J. Lawrence

School of Public Health and Tropical Medicine,
University of Sydney.
May 26.

[1] Yanif, H., and Avi-Dor, J., *Nature*, **169**, 201 (1952).
[2] Guttmann, P., and Ehrlich, P., *Berl. klin. Wschr.*, **28**, 953 (1891).
[3] Ehrlich, P., and Hata, S., "The Experimental Chemotherapy of the Spirilloses" (1911).

Phosphorus as a Factor Preventing DDT-Dehydrochlorination

Dehydrochlorination of DDT by certain chemicals concerns not only agriculture[1] but is also of great importance in anti-mosquito campaigns. A major factor here is the time during which DDT, sprayed on walls of houses, retains its insecticidal power. It has been shown[2] that great differences may exist between apparently identical walls, and that iron and aluminium ions are responsible for rapid dehydrochlorination. The difference in the percentage of mosquitoes killed on wooden and *pisé* walls, as reported by Wilkinson[3], might perhaps be explained on these lines.

In the Katanga Province of the Belgian Congo, I. Vincke also observed a rapid loss of insecticidal power of DDT on the walls of certain African houses. He asked us to investigate the problem. Using a modification of the method of Fleck and Haller, we also found that dehydrochlorination of DDT is related to the presence of iron and aluminium ions.

Analyses of soils from different parts of the Katanga have established the following facts: (1) in positive soils (causing rapid dehydrochlorination) there are ferric ions and few, if any, ferrous ions. Occasionally some slight traces of phosphate are found. (2) Negative soils contain both ferrous and ferric ions; the total iron content is generally higher than in positive soils. There is always a certain amount of phosphate.

Owing to a possible action of phosphate in neutralizing ferric ions by complex building, we have tried to use phosphorus to diminish the action of positive soils.

Laboratory experiments with water-soluble phosphorus salts, such as potassium hydrogen phosphates and ammonium phosphate, have given excellent results. Soils which dehydrochlorinized DDT completely after 12, 15, 17 and 20 min. respectively did not liberate any chlorine after 90 min. when previously treated with a normal solution of such phosphates. Similar results were obtained with less positive soils which, before treatment, dehydrochlorinized 20 per cent of the DDT after 90 min.

Tests on the coating of positive walls also showed that a phosphate spray delayed the dehydrochlorination of DDT for an appreciable time.

Using the biological tests of Downs *et al.*[2], we can conclude that in almost all cases the insecticidal power of DDT sprayed on walls may be prolonged by previous spraying with a normal solution of a phosphate.

This method has already been applied on a large scale by Vincke in the Tanganyika district of the Congo.

H. Maes

Station de Recherches Piscicoles,
Elisabethville,
Congo Belge.
May 9.

[1] Carne, P. B., *Nature*, **162**, 743 (1948).
[2] Downs, W. G., *et al.*, private communication from the Rockefeller Foundation.
[3] Wilkinson, P. R., *Nature*, **169**, 421 (1952).

Counting Soil Algæ by Direct Fluorescence Microscopy

The dilution technique is not entirely satisfactory in studying soil algæ. There is no universal medium for their growth, nor can we assume that all species will grow on any artificial medium now in use. The slow growth of algal colonies involves delays of up to two or three months, causing inconvenience in routine work. Filamentous and colonial algæ introduce further difficulties. A single filament or colony can give one or many colonies according to the number of viable pieces formed from it during dispersion. Available data on the number of algæ in the soil cannot therefore be regarded as absolutely certain.

Only direct microscopy can resolve these difficulties. Its use has been limited by the small numbers of algæ present (sometimes only a few thousands per gram of soil). This involves counting many microscopic fields, and the difficulties are increased by the small size of some algal cells and by their frequent intimate association with soil particles. A new fluorescence technique is proposed to overcome these difficulties.

All active algal cells are believed to contain at least one of the different chlorophylls. (We reserve the question of resting stages which can lose their chlorophyll.) The red fluorescence of chlorophylls illuminated with blue or ultra-violet light can be used for detecting and counting algal cells by the following technique. The soil suspension is illuminated, through a condenser (1·4 aperture), by a 12-volt or 6-volt lamp, the light of which passes through a solution of copper sulphate in aqueous ammonia. A yellow filter below the eyepiece is used to absorb blue light. Objectives of 10× or 20× are used with a 5× eyepiece. Algæ appear as red spots or lines against a black background. Soil particles are usually invisible, but sometimes give a yellow or green fluorescence readily distinguishable from that of chlorophyll. Using a special slide with a depression of 0·1 mm. deep, the amount of soil examined is a function of the area of the field, and the number of algæ per gram of soil can easily be calculated.