the amount of bacteriophage, effective against any particular organism, present in various soils.

A 100 g. sample of each soil is added to 50 ml. of sterile sauerkraut-glycerophosphate medium in a 250 ml. Erlenmeyer flask. This is inoculated with 1 ml. of a 24-hour-old liquid culture of the organism to be tested and incubated overnight at 30°C. The mixture is then filtered, first through paper and then through a sterile Berkefeld N or W filter. An estimation of the quantity of bacteriophage present is made by pouring plates with 10 ml. of an agar medium (suitable for the growth of the organism under investigation) mixed with 1 ml. of properly diluted filtrate and 1 ml. of a 24-hour culture of the organism in question. In pouring these plates care should be taken to mix the filtrate with the agar more thoroughly than in the case of plates for counting bacteria. After 48 hours incubation at 30°C., the plaques are counted and an estimate made of the number of bacteriophage units (whatever they may be) they represent in the original soil.

The agar medium for plating can well be standard nutrient agar (containing tryptone, glucose and beef extract) in the case of soil bacteria, of ordinary nutrient requirements. For the pea nodule organism the following yeast-extract modification of Ashby's mannitol medium is recommended: Mannitol, 8 g., yeast extract, 1.5 g.; $MgSO_4$, 0.2 g.; K_2HPO_4, 0.2 g.; NaCl, 0.2 g.; $CaSO_4$, 0.1 g.; $CaCO_3$, 1 g.; agar, 15 g.; distilled water, 1000 ml.; reaction adjusted to pH 7.6.

The procedure is more sensitive than the test-tube method to the presence of small amounts of bacteriophage in soil. For obvious reasons, the results are not as accurate as plate counts of bacteria; but they do permit a semi-quantitative comparison of the bacteriophage content of various soils.

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QUANTITATIVE ESTIMATION OF STREPTOTHRICIN

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Preliminary purification experiments on streptothricin indicated the need for an accurate quantitative determination of this antibacterial substance. A very satisfactory method is described here, and the concept of a standard streptothricin unit as a measure of streptothricin concentration is introduced. The principle of the cup method for penicillín estimation (Abraham, *et al.* 1941) is employed. A recent review of penicillin assay methods (Foster and Woodruff, 1943) also applies directly to the determination of streptothricin.

Until pure streptothricin is available, uncontrollable biological fluctuations make it imperative to employ for its accurate quantitative measurement a standard preparation of known potency against which unknown samples are compared. For several months a stable streptothricin standard has given excellent results in this laboratory. This primary standard contains 2000 streptothricin units per ml. and is designed to standardize results obtained in different laboratories. The unit was arrived at arbitrarily and has no relation to the Florey penicillin unit.

For reasons of sensitivity, stability and spore formation, *Bacillus subtilis* is the test organism.[1] Heavy spore suspensions are obtained by cultivation for 10 to 14 days with forced aeration followed by pasteurization to destroy vegetative cells. This spore culture is stored cold and may be used as an inoculum indefinitely. Besides eliminating an important biological variable, use of a stock spore inoculum permits the streptothricin to diffuse out into the agar during the lag in growth due to spore germination, thus producing larger zones. Spore cultures are titrated to determine the optimum amount for seeding purposes. The lowest level giving a continuous growth under the assay conditions (usually 0.1 to 0.2 ml. per 100 ml. agar) is selected as optimum.

Setting up the assay parallels the Oxford penicillin cup method except that the agar is seeded before plating, and glass tubing rings can replace porcelain cups. Warming the glass rings slightly by passage through a flame effects a seal between the cup and the agar. Thirteen ml. of seeded agar are apportioned into plates with wide mouth pipettes.

The standard curve is obtained by filling cups in quadruplicate (on different plates) with dilutions of the standard containing 10, 20, 40, 60, 80 and 100 streptothricin units per ml., respectively. Unknowns require only one dilution which is also assayed in quadruplicate cups. This dilution should contain approximately 50 units per ml. on the basis of predicted potency. After overnight incubation at 30°C. the clear inhibition zones around the cups are measured and plotted against unitage to give a standard curve. Unitages of the unknowns are read off the standard curve by projecting the value of the inhibition zone. A new standard curve is made with each run of assays.

One practised person can assay about 50 samples per day. Variability between replicate samples compared on any one run is about 5 to 15 per cent whereas on different days it may be up to 15 to 25 per cent.

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[1] Any sensitive strain of *B. subtilis* is satisfactory. In this laboratory a strain previously found to be very sensitive to streptothricin is used (Waksman and Woodruff, Proc. Soc. Exptl. Biol. Med., **49**, 207–210 (1942)).