Rapid Method for Measurement of Rate of Sorption of DDT by Mud Surfaces

Malaria control by means of residual applications of DDT and related insecticides to the interior surfaces of homes is now an established practice in many countries of the world. This method of control receives extensive support from the World Health Organization as well as from agencies of the United States Government and other governments. It is now well recognized that in those areas where mud is the predominant building material, residual treatments frequently lose their insecticidal activity at a rate which may seriously jeopardize the malaria control program. When dry mud surfaces are treated with DDT or related insecticides, the adsorption of the insecticide which occurs can result in a loss of most of the biological activity of the deposit in a relatively short time.

Bordas, Downs, and Navarro (*1*), Barlow and Hadaway (*2*), and others have made extensive investigations of this phenomenon. To date, all measurements of loss of activity on mud surfaces have been based on biological methods supplemented with chemical analyses. Such methods are time-consuming and difficult to evaluate.

A rapid method based on the measurement of the loss of radioactivity of mud surfaces dusted with C^{14}-labeled DDT is described in this report. Because the C^{14}-labeled compound migrates to a depth only slightly beneath the surface, the weak beta rays are shielded; thus it is possible to follow the loss from the surface by measuring the loss in radioactivity. This technique lends itself to the rapid screening of substances which may deter or prevent adsorption.

Samples of mud for this study were obtained from Savannah River deposits, Guatemala, and Colorado. The samples were air-dried, ground in a mortar, and passed through a 30-mesh sieve. This powder, which was made into a thick paste by adding water, was pressed into cylindrical molds 24 mm in diameter and 6 mm deep. After drying in air for several days, the resulting mud cakes were removed from the molds.

One-third of the mud cakes were placed over silica gel in a desiccator; another third were kept over sulfuric acid solution (specific gravity, 1.35; atmosphere of 47 percent relative humidity); and the balance were kept over sulfuric acid solution (specific gravity, 1.10; atmosphere of 94 percent relative humidity). The cakes were allowed to equilibrate in the humidity-controlled atmospheres for several days.

The powdered DDT used for dusting the mud cakes was prepared by mixing 990 mg of pure *p,p'*-DDT with 9.8 mg of radioactive *p,p'*-DDT (specific activity 0.48 mc/g). The mixture was made homogeneous by adding sufficient CCl_4 to dissolve all the DDT and evaporating the solvent. After drying over silica gel, the residue was ground to a fine powder in a glass mortar. The powdered DDT was placed in the bottom of a glass tower especially designed and constructed so that dry nitrogen could be blown over the powder. The mud cakes were placed in the upper part of this apparatus. When the nitrogen gas was introduced, fine particles of radioactive DDT fell on the mud samples. The particles were measured under a microscope and were found to range from 1 to 25 μ in size, with an average diameter of 6.2 μ. About 1 mg of DDT was dusted onto each cake.

Immediately after dusting, the radioactivity of each cake was counted in a conventional gas flow counter, and the cake was returned to one of the humidity-controlled desiccators. The cakes were recounted at intervals of 1 to 5 days. Samples were counted from 1 to 3 minutes, depending on the counting rate, and were taken from the desiccators only long enough to be counted. In all cases, the count was repeated until an agreement within 3 percent was reached. The average initial count was 4220 count/min.

Within 24 hours, a measurable decrease in radioactivity was observed in all samples. The count for samples kept in the dry atmosphere dropped sharply, with an average loss of 15.2 percent after 1 day. Samples kept in an atmosphere with 47 percent relative humidity had an average loss of 7.6 percent for the same period, while those kept in the high humidity lost an average of only 4.2 percent. Samples of mud from the three sources were observed for approximately 2 weeks. The rate of loss was about the same for all types of mud tested under identical humidity conditions. The decrease in radioactivity with time for a typical series of samples (Savannah River mud) is shown graphically in Fig. 1. Here the effect of humidity is clearly shown.

On the tenth day, sample 22, which had previously been kept in a dry desiccator, and sample 24, which had been kept at 47 percent relative humidity, were transferred to the high-humidity desiccator. Subsequent measurements of these samples showed increased activity. This was repeated on many other samples with the same result. These observations seem to confirm those of Bordas, Downs, and Navarro (*1*) who claim that desorption occurs in periods of high humidity and that insecticidal activity then returns to surfaces which have previously lost their activity because of adsorption.

After the DDT-dusted cakes had stood for approximately 30 days, the top layers were carefully scraped off, 0.5 mm at a time. These scrapings were extracted with acetone and analyzed for DDT by the Schechter-Haller (*4*) method. Analyses of scrapings from samples maintained at 94 percent relative humidity showed an average of 98.0 percent of the DDT to be in the first 0.5-mm layer; 1.4 percent in the second 0.5-mm layer; and 0.6 percent in the third 0.5-mm layer. In similar analyses of scrapings from samples kept at 47 percent relative humidity, 90.7 percent of the DDT was found in the top layer; 7.1 percent in the second layer; and 2.2 percent in the third layer. Scrapings from samples from the dry desiccator averaged 81.9 percent, 15.3 percent, and

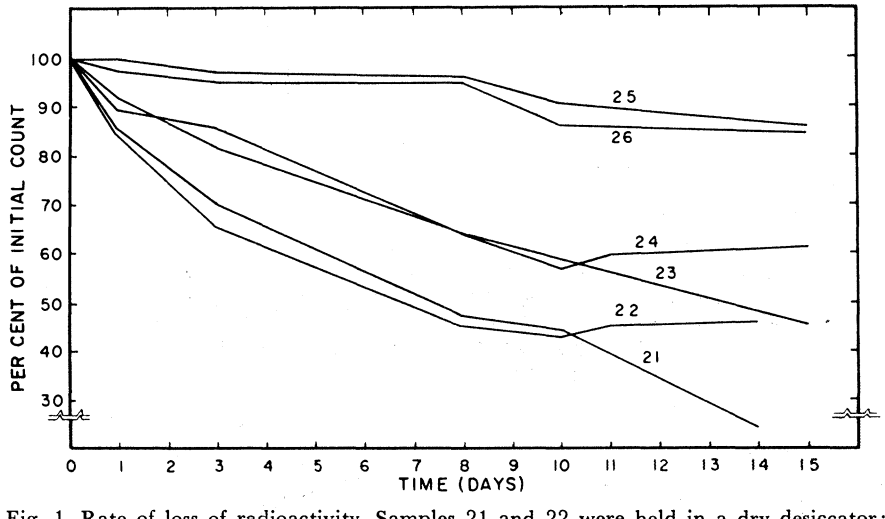

Fig. 1. Rate of loss of radioactivity. Samples 21 and 22 were held in a dry desiccator; samples 23 and 24 were kept at a relative humidity of 47 percent; samples 25 and 26 were kept at a relative humidity of 94 percent. On the tenth day samples 22 and 24 were transferred to a desiccator with a relative humidity of 94 percent.

3.5 percent in the three layers, respectively. Apparently the maximum penetration into the mud cakes was only slightly more than 1 mm even in a dry atmosphere. No DDT was found in layers below a depth of 1.5 mm.

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Selection of Auxotrophic Bacterial Mutants through Diaminopimelic Acid or Thymine Deprival

The significance of unbalanced growth as a cause of cell death has been brought out by Cohen and Barner (*1*), who worked with a thymine-requiring mutant of *Escherichia coli*. When this organism was incubated in a culture medium that lacked thymine, it could not form deoxyribonucleic acid but continued to synthesize its other cell constituents, and, as a result, the viable count fell rapidly. Recently, Lederberg (*2*) found that unbalanced growth similarly underlies the bactericidal action of penicillin. When a suitable protective agent (such as hypertonic sucrose) was added to the growth medium, penicillin no longer caused lysis but, instead, led to protoplast formation. It could be concluded from this observation, as from the independent biochemical findings of Park and Strominger (*3*), that penicillin interferes selectively with bacterial cell-wall formation.

These developments suggested the possibility of replacing penicillin in the selection of auxotrophic mutants of bacteria. In the penicillin method (*4*), a mixed population is exposed to this drug in minimal medium, whereupon the wild-type cells grow and are lysed, while auxotrophic cells, unable to grow, are spared. We now wish to report two modifications of this procedure which utilize a genetic block rather than penicillin to make growth fatally unbalanced.

The first method closely resembles the use of penicillin in that it also involves the cell wall. The method is based on the availability of a mutant (173-25 of the W strain of *E. coli*) that is blocked in the synthesis of *meso*-α,ε-diaminopimelic acid (DAP) (*5*). In certain bacterial species this compound is a cell constituent (*6*) as well as a precursor of lysine (*5, 7*), and analyses of bacterial fractions have suggested that its incorporation as a constituent may possibly be limited to the cell wall (*8*). This suggestion is supported by the results of incubating mutant 173-25 in media that contained lysine but no diaminopimelic acid: the cells lyse in a medium of ordinary tonicity (*9*) but form protoplasts when 20 percent sucrose is also present (*10*). Deprival of diaminopimelic acid thus results in selective interference with cell-wall formation.

To use this "suicidal" property of mutant 173-25 in the selection of mutants with additional requirements, a procedure similar to the penicillin method (*4*) was followed. The bacteria were irradiated with ultraviolet light to about 1 percent survival. For phenotypic expression of the resulting induced mutations, large inocula were cultivated overnight in minimal medium A (*11*) enriched with 10 μg of diaminopimelic acid (*12*) per milliliter and with 0.2 percent tryptic casein hydrolysate (Sheffield NZ–Case) and 0.2 percent yeast extract (Difco). For selection of auxotrophic mutants, the cells were then washed and incubated (10^5 to 10^7 cells per milliliter) for 14 hours at 37°C in medium A supplemented with 20 μg of L-lysine per milliliter (*13*). Survivors were recovered by plating in the enriched medium described, solidified with 1.5 percent agar. Mutant colonies were recognized by the inability of subinocula to grow on minimal medium supplemented with only the compounds required by the parental strain (*14*).

The second method involves the metabolic imbalance originally described by Cohen and Barner and utilizes the same thymine auxotroph (15T⁻). The procedure used was the same as that described in the preceding paragraph except for appropriate changes in the composition of the media. Thus, selection was carried out in minimal medium A, and all other steps were carried out in this medium supplemented with thymine (20 μg/ml), together with other supplements as needed.

In a small-scale experiment, strain 173-25 yielded mutants with various additional requirements: cystine, methionine, *p*-aminobenzoic acid, arginine plus uracil, and an unidentified factor. Strain 15T⁻ yielded offspring with additional requirements for arginine, methionine, phenylalanine, and a mixture of aromatic metabolites.

The alternative methods that have been described may have advantages, in certain circumstances, over the use of penicillin. In particular, it is known that "thymineless death" can be produced by a method that is capable of quite general application: by using a sulfonamide antagonist of *p*-aminobenzoic acid to prevent the synthesis of a group of products of one-carbon metabolism and simultaneously providing all these products except thymine (*1, 15*). The present work (*16*) suggests that production of thymine deficiency in this way might be useful for selecting mutants of organisms (such as yeasts) that are susceptible to sulfonamides but indifferent to penicillin.

The use of penicillin for the selection of auxotrophic mutants of bacteria can be replaced by taking advantage of the fact that a DAP-requiring or a thymine-requiring strain is suicidal when it grows in media that lack the required compound (*17*).

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12. For samples of *meso*-α,ε-diaminopimelic acid we are indebted to Charles Gilvarg, to Lemuel Wright of Merck, Sharp and Dohme, and to Chas. Pfizer and Co. This supplement is necessary since the complex enrichments used do not contain detectable amounts of DAP.
13. Presumably, as in the penicillin method [see J. Lederberg, *Methods Med. Research* 3, 5 (1950)], longer or shorter periods of incubation would also be satisfactory.
14. The supplement contained L-lysine (20 μg/ml) as well as DAP (10 μg/ml), since strain 173-25 has a relative requirement for lysine as well as an absolute requirement for DAP (*5*).
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16. This work was aided by research grant RG-4235 from the U.S. Public Health Service.
17. A similar method has proved useful in the isolation of mutants of the molds *Ophiostoma* sp. and *Aspergillus* sp., since the survival of certain auxotrophs of these species, when incubated in minimal medium, is significantly prolonged by the presence of various additional metabolic blocks. [See N. Fries, *Hereditas* 34, 338 (1948); G. Pontecorvo, *Advances in Genet.* 5, 141 (1953)].

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