

### TOXICITY OF DICHLORO-DIPHENYL-TRICHLORETHANE (DDT) TO GOLDFISH AND FROGS

In the course of pharmacological studies of 2,2, bis (p-chlorophenyl) 1,1,1 trichlorethane<sup>1</sup> (DDT) the writers have noted that this substance is more toxic to goldfish and frogs than rats, cats and rabbits in terms of the lethal doses recently reported by Smith and Stohlman<sup>2</sup> (150 mgs/Kg for rats; 200 to 300 mgs/Kg for cats; and 300 mgs/Kg for rabbits, when given intragastrically in olive oil).

Single doses of DDT dissolved in olive oil and incorporated in food pellets, when swallowed by 6 to 10 gm goldfish, were lethal in amounts ranging from 63 to 200 mgs/Kg. Within this range the total mortality was approximately 55 per cent., the number of deaths being correlated roughly with the size of the dose. Death followed these single ingestions of DDT in 24 hours to 6½ days, the onset of the symptoms of poisoning being delayed in some cases for more than four days. The fish became hyperirritable at first and subsequently developed muscular incoordination, muscu-

lar spasms and finally marked prostration, during which phase the fish lay on its side, breathing irregularly and at times making convulsive movements. The incoordination and prostration in some cases persisted for 3 days or more before death. The gross picture of the DDT poisoning resembled that produced by phenol or picrotoxin.

All frogs receiving DDT dissolved in olive oil, by injections into the dorsal lymph sac were all killed in 4 to 72 hours by single doses of 150 mgs/Kg. Some frogs died following injection of quantities as small as 10 mgs/Kg.

These findings that these two cold-blooded aquatic vertebrates are even more sensitive to single doses of DDT than the common laboratory mammals are of interest in connection with the proposed use of DDT in regions where malaria is endemic against the larvae of the mosquito vectors of that disease.

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## SCIENTIFIC APPARATUS AND LABORATORY METHODS

### INHIBITION OF *E. COLI* BY PENICILLIN<sup>1</sup>

*E. coli* possesses a marked resistance to penicillin.<sup>2</sup> In the course of investigations on the effect of certain amino acids upon this resistance the following observations were made.

A laboratory strain of *E. coli*, the most susceptible to penicillin<sup>3</sup> among the few strains tested, was employed for the work. Suitably diluted 5-6 hour old cultures in plain broth served as inoculum. All assays were done in the total volume of 8 ml. After 16 hours at 37° C, the optical density of the growth was determined in the Lumetron photoelectric colorimeter using red filter 650 with uninoculated broth as the blank.

#### *Effect of penicillin upon various concentrations of*

<sup>1</sup> The writers are indebted to the Geigy Company of New York City for samples of DDT.

<sup>2</sup> M. I. Smith and E. F. Stohlman, *Public Health Reports*, 59: 984, 1944.

<sup>3</sup> From the Laboratories of Bacteriology, The Mount Sinai Hospital, New York, N. Y.

<sup>4</sup> E. P. Abraham, *et al.*, *Lancet*, 2: 177, 1941; J. Florey, *Brit. Jour. Exp. Path.*, 23: 120, 1942; C. L. Hobby, R. Meyer and E. Chaffee, *Proc. Soc. Exp. Biol. and Med.*, 50: 281, 1942.

<sup>5</sup> Sodium salt of penicillin, Merck and Co., was routinely used for assaying of susceptibility of bacteria isolated from patients under treatment with this drug. Solutions remaining from these tests were employed on the day of their preparation for the work described in this article. The potency was ascertained at the time of each experiment by control titration against the strain H of *Staphylococcus* under standard conditions. (C. M. McKee, G. Rake and A. E. O. Menzel, *Jour. Immun. Virus Res. and Exp. Chemoth.*, 48: 259, 1944).

*E. coli* at zero hours: On repeated tests with the same batch of broth, a linear relationship was obtained between the log of bacterial concentration (horizontal axis, x) and the amount of penicillin necessary to produce complete inhibition (vertical axis, y). When abscissas were log 3 to log 8 of bacterial concentration per ml at zero hours; and the ordinates were 4 to 36.5 O. U. per ml, a straight line was obtained which could be expressed fairly accurately by a two-point equation  $y = 6.5x - 15.5$ . The slope varied with the nutritive value of the medium. The line was curved between the point of origin and log 3. Thus, there was a definite and possibly greater susceptibility of *E. coli* to penicillin than hitherto assumed. Within the range studied the linear relationship shown for *E. coli* did not hold with the standard strain H of *Staphylococcus*.

*Effect of dl-methionine and mixture of dl-methionine<sup>4</sup> and penicillin upon growth of E. coli in plain broth:* As previously shown,<sup>5,6</sup> methionine was toxic to *E. coli*, the degree of inhibition depending on the concentration of the substance and the number of cells per ml at zero hours. With  $1.5 \times 10^6$  cells, 1.25 and 2.5 mg gave less than 15 per cent. of inhibition, while

<sup>4</sup> dl-Methionine, S. M. A. Corporation, was dissolved in plain broth in initial concentration of 2 per cent. and sterilized by filtration through Berkefeld N candle.

<sup>5</sup> E. A. Bliss and P. H. Long, *Bull. Johns Hopkins Hosp.*, 69: 14, 1941.

<sup>6</sup> H. I. Kohn and J. S. Harris, *Jour. Pharmacol.*, 73: 343, 1941.