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[1] (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[2] (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, muscular 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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INHIBITION OF E. COLI BY PENICILLIN[1]

E. coli possesses a marked resistance to penicillin.[2] 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[3] 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 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[4] and penicillin upon growth of E. coli in plain broth: As previously shown,[5,6] 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 1.5×10^5 cells, 1.25 and 2.5 mg gave less than 15 per cent. of inhibition, while

[1] The writers are indebted to the Geigy Company of New York City for samples of DDT.

[2] M. I. Smith and E. F. Stohlman, *Public Health Reports,* 59: 984, 1944.

[1] From the Laboratories of Bacteriology, The Mount Sinai Hospital, New York, N. Y.

[2] 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.

[3] 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).

[4] 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.

[5] E. A. Bliss and P. H. Long, *Bull. Johns Hopkins Hosp.,* 69: 14, 1941.

[6] H. I. Kohn and J. S. Harris, *Jour. Pharmacol.,* 73: 343, 1941.

5 and 7.5 mg per ml brought about 30 per cent. and 69 per cent. inhibition, respectively. Inhibition exerted by a mixture of methionine and penicillin was roughly the sum of inhibition obtained with each ingredient separately. Thus, 7.5 mg of methionine and 3.5 O. U. of penicillin giving each separately 69 and 40 per cent. inhibition, respectively, induced 100 per cent. inhibition when used together. The finding is of interest for the following reason. It was previously demonstrated that penicillin produced its effect during the stage of active bacterial multiplication. Agents inhibiting growth interfered with penicillin activity (*i.e.*, cold, saline, phenol, sulfadiazine, etc.).[7] In contrast, the combined effect of methionine and penicillin was clearly synergistic.[8] Since methionine is non-toxic *in vivo*, its use in conjunction with penicillin therapy of *E. coli* infections seems worthy of consideration. However, prior to these attempts it was deemed advisable to determine the effect of blood serum upon the concerted action of the agents, as follows:

Combined effects of blood serum, methionine and penicillin upon growth of E. coli: Preliminary tests demonstrated the well-known inhibitory effect of normal blood serum upon *E. coli*. The concentration of inhibitory factors varied considerably in fresh rabbit sera tested. Greatest inhibition obtained with filtered sera stored for 24 hours at 4° C prior to use was 20 and 40 per cent. when they were diluted 1:10 and 1:5, respectively, in broth containing 1.5×10^5 cells per ml.[9] Most of studies described below were carried out with sera of lower bactericidal potency than just mentioned. Penicillin, 3.5 O. U. per ml combined with some batches of sera diluted 1:6.67 gave 100 per cent. inhibition, while each ingredient separately gave 40 and 30 per cent., respectively. It appeared that the inhibition caused by the mixture exceeded the sum of inhibition produced with each ingredient separately. Suggestively, one of the ingredients may be capable of enhancing the susceptibility of *E. coli* to the effect of the other. This observation awaits further investigation.

The toxic effect of methionine alone upon *E. coli* described above was abolished by blood serum reducing up to 80 per cent. the inhibitory effect of 7.5 mg of methionine per ml. The neutralizing property varied in concentration. It could be clearly demonstrated in sera of low bactericidal titer.[10]

The most interesting fact in these studies is that serum-methionine mixtures, by themselves of low inhibitory potency, may enhance greatly the susceptibility of *E. coli* to penicillin. Thus, penicillin in concentration of 0.5 to 1.5 O. U. per ml gave 100 per cent. inhibition of 1.5×10^5 cells per ml at zero hours in broth containing 7.5 mg methionine and sera diluted 1:8. The mixture of the same ingredients without penicillin gave only 12–25 per cent. inhibition. In the absence of the serum-methionine mixtures, 0.5 O. U. of penicillin gave no inhibition and 1 to 1.5 O. U. only slight inhibition; 18 O. U. being required for complete inhibition of the above number of cells at zero hours. Obviously, serum-methionine mixtures were capable of increasing the susceptibility of *E. coli* to penicillin as many as 12 to 36 times.

Summary: Within a certain range, there exists a linear relationship between the log of *E. coli* cells at zero hours and the concentration of penicillin in O. U. per ml. required to produce complete inhibition. Methionine inhibits the growth of *E. coli*. Methionine and penicillin exert together a synergistic inhibitory effect upon the microorganism. Inhibition of growth obtained with a mixture of rabbit blood serum and penicillin may exceed somewhat the sum of inhibition induced by each ingredient, separately. The inhibitory effect of methionine alone may be abolished in considerable part by blood serum.

"Neutralized" mixtures of methionine and serum producing by themselves only incomplete inhibition may greatly increase the susceptibility of *E. coli* to penicillin.

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[7] M. H. Dawson, *et al.*, *Jour. Clin. Invest.*, 1941, 20: 434, 1941; G. L. Hobby and M. H. Dawson, *Proc. Soc. Exp. Biol. and Med.*, 56: 178 and 181, 1944; C. P. Miller and Foster A. Zimmerman, *Proc. Soc. Exp. Biol. and Med.*, 56: 205, 1944.

[8] There are no additive effects of sulfanilamide and methionine. On the contrary, sulfanilamide appears to neutralize the antibacterial action of methionine (see footnote 5).

[9] Sera separated from coagulated heart blood were sterilized by filtration through Berkefeld N candle, stored in the refrigerator and used 24 hours following preparation.

[10] J. Gordon and J. W. M'Leod reported that the inhibitory effect of a number of amino acids may be abolished by serum. Methionine was yet unavailable. J. Gordon and J. W. M'Leod, *Jour. Path. and Bact.*, 1926, 29: 13, 1926.

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