16951

Effect of DDT on Functional Development of Larvae of *Rana pipiens* and *Fundulus heteroclitus.*

EVELYN SCHREIMAN† AND ROBERTS RUGH.*

From the Biology Department, Washington Square College.

The effect of DDT (1-trichlor-2, 2-bis-p-chlorophenyl ethane) on adult fish, frogs,[1] and mammals,[2] has been reported recently. The general results indicate that DDT specifically affects the nervous system, a fact previously demonstrated in studies on insects. It seemed desirable to determine the stage in early embryonic development when DDT first affects the central nervous system. For this study poikilothermous and aquatic forms were chosen, namely the embryos of the frog *Rana pipiens* and the fish *Fundulus heteroclitus.*

Materials and methods. A total of 400 larvae of *Rana pipiens* and 250 of *Fundulus heteroclitus* were used in this study. The larvae were introduced into the experimental medium at various stages of development, for varying periods. The stages of *Rana pipiens* are those established by Shumway[3] and for *Fundulus heteroclitus* by Oppenheimer.[4]

The experimental medium consisted of a saturated solution of DDT in spring water for Rana and in distilled water for Fundulus (the latter being able to survive well in distilled water). DDT is quite insoluble but it is estimated that when 4 parts per million are used, that at least 1 part per million goes into solution.

Rana pipiens larvae were provided with 50 cc of medium for 25 larvae in a 250 cc finger bowl while *Fundulus heteroclitus* larvae were placed 50 to a finger bowl of 75 cc of medium. The experimental media were changed daily to keep the concentration at the experimental level and to avoid chemical deterioration. The controls were treated in an identical manner except for the use of DDT. Laboratory temperatures of 23°C to 25°C were used for all larvae.

The response to stimulation was measured by the Detwiler[5] "race-track" method. The photographs were taken with a Mifilmca Adapter, through the low magnification microscope, on Microfile film and were developed in Microdol developer.

Experimental data. A. *Growth Rate. Rana pipiens*: Frog larvae placed in the experimental medium at stage 11 and left for 7 days showed no appreciable change in growth rate. By the 9th day of exposure, however, the experimentals showed an average length 2 mm less than that of the controls and on the 11th day, the experimentals had shown no further growth while the controls had continued to grow to an average of about 15 mm. Growth, therefore, was completely inhibited by 9 days after the initial exposure of the larvae to DDT.

Fundulus heteroclitus: Fish larvae were subjected to the experimental medium at stage 8 and at stage 29, were allowed to hatch and survive for 6 days beyond hatching and showed no evidence of any effect on their growth.

B. *Deformities. Rana pipiens*: Beginning the 9th day after subjection to the experimental medium of DDT, the larvae showed a narrowing of the trunk region and on the 11th day there developed a constriction just posterior to the operculum and a lateral acute bending of the tail at the point of junction with the body (Plate I, Fig. 1 to 3). These deformities were observed in the experimental larvae almost without exception. There developed a degree of respiratory paralysis in

* Now at Radiological Research Laboratory, Columbia University.

† Submitted in partial fulfillment of the requirements for the M.A. Degree at Washington Square College of New York University.

1 Ellis, M. M. *et al., Science* 1944, **100**, 477.

2 Nelson, A., *et al., Public Health Rep.*, 1944, **59**, 1009.

3 Shumway, W., *Anat. Rec.*, 1940, **78**, 139.

4 Oppenheimer, J., *Anat. Rec.*, 1937, **68**, 1.

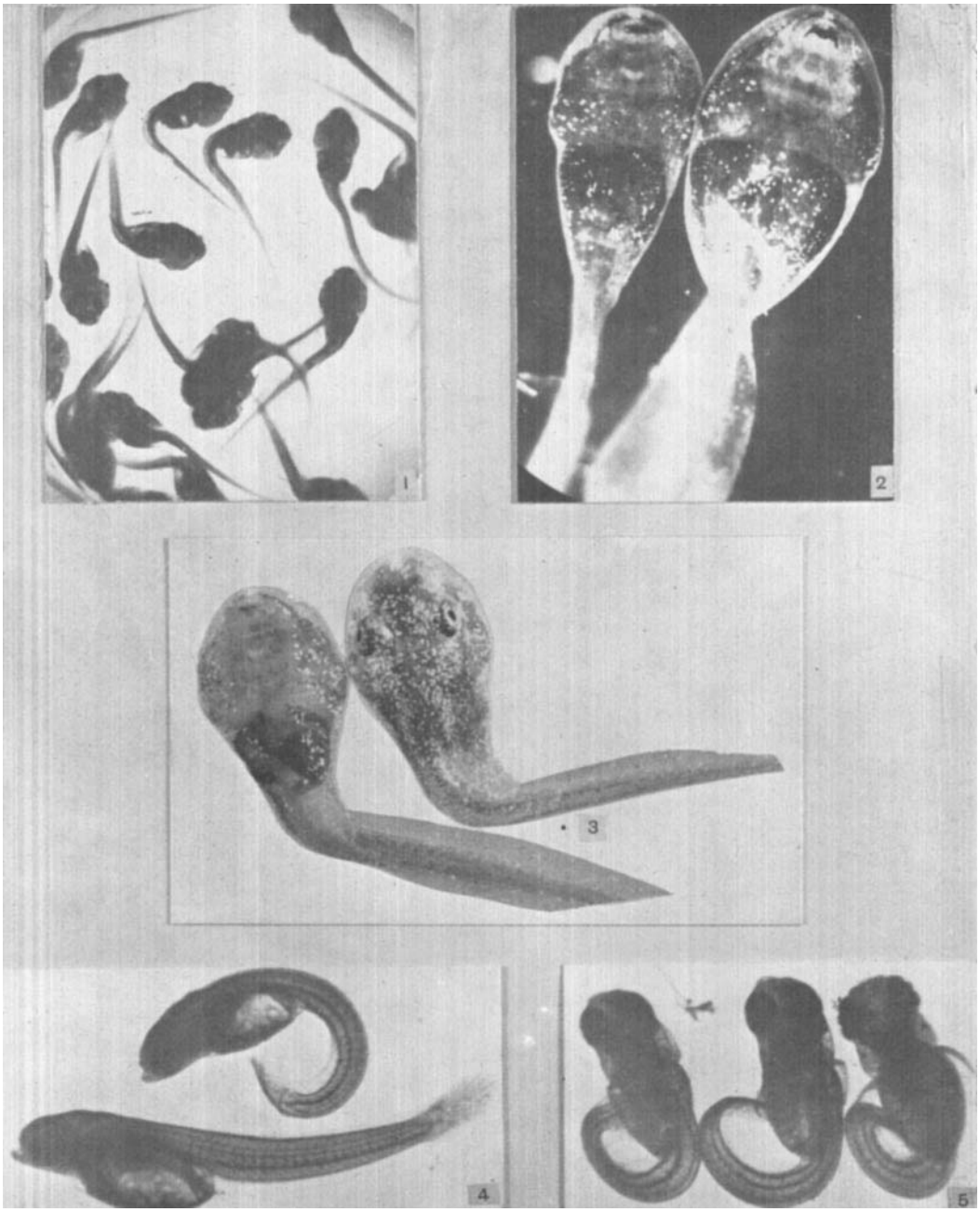

PLATE 1.

Tail curvature of *Rana pipiens* and *Fundulus heteroclitus* larvae following exposure to saturated solutions of DDT.

FIG. 1. Larvae of *Rana pipiens*, placed in a saturated solution of DDT at stages 11 and 12, after 11 days of treatment.

FIG. 2. Larvae of *Rana pipiens*, placed in a saturated DDT solution at stages 11 and 12, after 9 days of treatment. The experimental animal on the left shows an impairment of growth as compared with the control animal on the right.

FIG. 3. Higher magnification of larvae from some experimental group as larvae in Fig. 1.

FIG. 4. Larva of *Fundulus heteroclitus* (above) showing a ventral curvature of the tail upon hatching from a saturated DDT solution, in which it was immersed for 19 days, treatment beginning at stage 8; control (below) untreated.

FIG. 5. Larvae of *Fundulus heteroclitus* showing lateral curvature of tail upon hatching from a saturated DDT solution in which they were immersed for 19 days, treatment beginning at stage 8.

which the mouth was held open but the larvae showed none of the usual respiratory movements.

Larvae introduced into the experimental medium at stages 16, 18, 19 and 25 also showed these deformities but their onset was slower, the older the larvae.

Fundulus heteroclitus: Larvae introduced into the experimental medium at stage 8 hatched at the same time as the controls but upon hatching the experimentals exhibited an acute ventral bending of the tail in more than 80% of the larvae, (Plate I Fig. 4 and 5). There was also a slight constriction just below the operculum, and the mouth was held open (as with *Rana pipiens larvae*) without related respiratory movements.

When Fundulus larvae were introduced into the experimental medium at stage 29, they exhibited mild cases of lordosis and kyphosis by the 6th day post hatchings but there was no bending of the tail shown by larvae exposed earlier and for longer periods.

C. *Mortality*. The mortality rate of the experimentals was more than twice that of the controls for both *Rana pipiens* and for *Fundulus heteroclitus* larvae. Only 75 out of 400 *Rana pipiens* larvae and 35 out of 150 *Fundulus* larvae survived the treatment.

D. *Neuro-Muscular responses. Rana pipiens*: Using the race-track method of Detwiler[5] the degree of response to tactile stimulation at different stages in DDT treatment was determined. A decrease in motor activity was first noted on the 7th day of exposure, was considerably more pronounced on the 9th day and almost extinct by the 11th day (see Fig. 1). On the 11th day the responses to tactile stimulation consisted of generalized fine tremors and an occasional initial sharp single contraction of the tail musculature. There was a definite decrease in muscle tonus and in the ability to assume a normal position.

Fundulus heteroclitus: Larvae exposed to

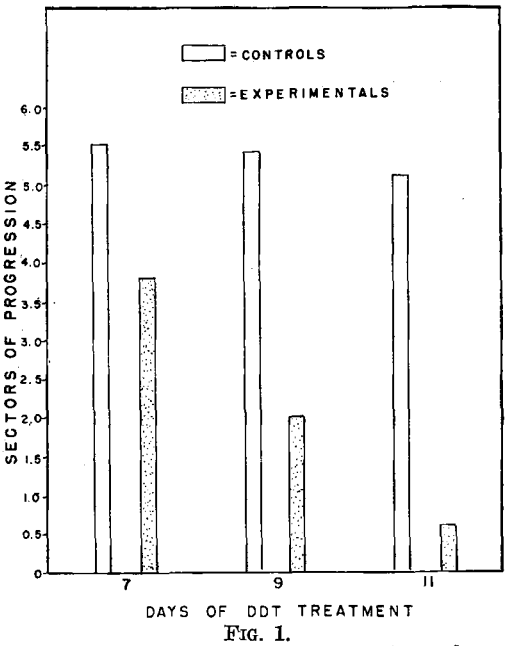

FIG. 1.

Neuro-muscular responses of *Rana pipiens* larvae to tactile stimulation following exposure to saturated solutions of DDT.

the experimental medium showed a slight decrease in motor responses to tactile stimuli as early as the fourth day after hatching, even though there was no morphological evidence of damage. There was marked decrease in the response by the 6th day, the larvae showing very much reduced activity and occasionally developing spasmodic contradictions of the trunk and tail musculature, and general fine tremors throughout the body without appreciable forward progression. There was no swimming activity.

E. *Recovery. Rana pipiens*: Larvae subjected to the DDT medium at stage 11 were removed at daily intervals from the 2nd to the 11th day after initiation of the treatment, and placed in the normal control medium. Complete recovery was effected in those larvae which were returned to the normal medium up to and including the 6th day of treatment. Beyond the 6th day of exposure, the larvae

[5] Detwiler S. R., *J. Exp. Zool.*, 1946, **102**, 321.

retained all of the morphological and physiological symptoms present at the time of removal from the experimental medium, but without further effects.

Fundulus heteroclitus: Larvae which were removed from the experimental DDT medium on the 16th day after immersion at stage 8 retained the same deformities and abnormalities in behavior as the experimentals which were allowed to hatch in the DDT medium. Prior to the 16th day there was little or no retention of damage effects.

Discussion. The exact mechanism of DDT action on the living system is still unknown, except that the findings of this investigation corroborate earlier findings on higher forms that the effect is primarily on the neuro-muscular mechanism.

Ellis *et al.*,[1] and Odum and Sumerford,[6] found that cold-blooded animals are more easily affected than are the warm-blooded animals. Ellis *et al.*[1] fed DDT to goldfish in pellet form and the total mortality was approximately 55%. The fish were hyper-irritable, and then developed muscular incoordination, muscular spasms, and finally marked prostration during which time the fish lay on its side, breathing irregularly and manifesting convulsive movements. These symptoms were also found in the present investigation in the larvae of *Fundulus heteroclitus*.

The dose required to produce symptoms of toxicity varies with different animals. Odum and Sumerford,[6] found that the median lethal dose for goldfish was 0.1 ppm, for Gambusia was 0.01 ppm and for Culex larvae was 0.0001 ppm. Ginsburg,[7] stated that mosquito larvae fed on DDT were not poisonous to goldfish, but that DDT in solution was definitely toxic.[7] Concentrations of DDT at 1 part in 10,000,000 caused 40% mortality while 1/20,000,000 seemed to be non-toxic.

The concentration of DDT in solution is very difficult to regulate or determine. It was found possible to increase the concentration to 4 ppm by treating the mixture ultrasonically, but the control medium, also treated ultrasonically, was somewhat toxic to the larvae. Based upon rough calculations in relation to the amount introduced and the amount undissolved, and to observations of others, it was estimated that the concentrations used amounted to at least 1 ppm. For purposes of duplication of experimental procedures, however, it can be stated that the concentrations used were saturated at laboratory temperatures of 23° to 25°C, and that this probably represented 1 ppm.

Recently Oppenheimer[8] found similar damaging effects on the larvae of *Fundulus heteroclitus* following subjection to dilute solutions of metrazol. The sharply angled tails appeared as identical morphological effects of DDT and metrazol, and it is entirely possible that the two compounds effect the neuromuscular apparatus of the larvae in much the same manner.

Conclusions. 1. A saturated solution of DDT (estimated at 1 ppm) in spring water for *Rana pipiens* larvae and in distilled water for *Fundulus heteroclitus* larvae, was sufficiently toxic to produce morphological deformities of a similar nature, the most obvious of which was the sharply bent tail and the very much reduced neuro-muscular response to tactile stimulation.

2. The initial evidence of damage by DDT appeared after 7 days of treatment of *Rana pipiens* larvae (beginning at stage 11) and after hatching of *Fundulus heteroclitus* larvae (treatment beginning at stage 8). There was a considerable lag in the effect, due, in all probability, to the fact that DDT is relatively insoluble and therefore difficult to assimilate by the larvae.

3. In addition to the bent tail, there was a gradual decrease in neuro-muscular responses as determined by the Detwiler race-track method following tactile stimulation, in both Rana and Fundulus larvae. Ultimately the larvae responded with only muscular tremors.

4. The toxic symptoms were not manifest in Rana larvae if they were returned to the control medium after 6 days of subjection to

[6] Odum, E. P., and Sumerford, W. T., *Science*, 1946, **104**, 480.

[7] Ginsburg, J. M., *J. Econ. Ento.*, 1945, **40**, 475.

[8] Oppenheimer, J. M., PROC. SOC. EXP. BIOL. AND MED., 1946, **66**, 49.

the DDT or in Fundulus as late as 16 days in the medium if subjected at stage 8.

5. There was no effect of DDT on the growth rate of Fundulus larvae, but after 9 days of exposure of Rana larvae they show a definite inhibition of growth.

6. The older the larvae at the time of DDT exposure, the longer the period before the onset of deleterious symptoms.

16952

Biochemical Studies on Livers of Chicks Receiving Graded Levels of Pteroylglutamic Acid.*

JOHN R. TOTTER, WILLIAM E. MARTINDALE, MARION MCKEE, CECELIA K. KEITH, AND PAUL L. DAY.

From the Department of Biochemistry, School of Medicine, University of Arkansas, Little Rock, Ark.

In a recent communication[1] it was shown that the apparent xanthine oxidase activities of livers from chicks fed a purified diet were inversely related to the pteroylglutamic acid (PGA) content of the diet. The livers from the same experimental chicks were also studied for their nucleic acid and PGA content and for their apparent conjugase activity; the values so obtained are reported here.

Experimental. The diets and the methods of handling and care of the chicks are given fully in the previous report.[1] PGA was determined microbiologically by use of *Streptococcus faecalis*.[2] Conjugase was determined by the method of Laskowski, Mims, and Day[3] using Difco yeast extract as the substrate. Since there may be several conjugase inhibitors[4-7] in a mixture such as that used in these tests the absolute values may not be valid, but there seems to be no reason why such a method is not satisfactory for comparative purposes when using a single batch of substrate and the same organ from each bird.

Pentose nucleic acid and desoxypentose nucleic acid were determined on aliquots of 1:5 liver brei employing the methods outlined by Schneider.[8,9]

Results and discussion. The results of the nucleic acid determinations are given in Table I. As indicated earlier,[1] the livers of chicks were relatively larger when the diet was PGA-deficient. The data are arranged to show the nucleic acid content per gram of liver, the total nucleic acid per chick, and the amount per 100 g of chick. The values ob-

* Research paper No. 883, Journal Series, University of Arkansas. This investigation was supported by research grants from the Division of Research Grants and Fellowships of the National Institute of Health, U. S. Public Health Service, the National Live Stock and Meat Board, and the Nutrition Foundation, Inc. A part of the data contained in this report is taken from a thesis submitted by William E. Martindale to the Graduate School of the University of Arkansas in partial fulfilment of the requirements for the degree of Master of Science. The pteroylglutamic acid used was furnished by the Lederle Laboratories Division of the American Cyanamid Company.

1 Keith, C. K., Broach, W. J., Warren, D., Day, P. L., and Totter, J. R., *J. Biol. Chem.*, 1948, **176**, 1095.

2 Mitchell, H. K., and Snell, E. E., Univ. of Texas Pub., 1941, No. 4137, 36.

3 Laskowski, M., Mims, V., and Day, P. L., *J. Biol. Chem.*, 1945, **157**, 731.

4 Bird, O. D., Robbins, M., Vandenbelt, J. M., and Pfiffner, J. J., *J. Biol. Chem.*, 1946, **163**, 649.

5 Sims, E. S., and Totter, J. R., *Fed. Proc.*, 1947, **6**, 291.

6 Mims, V., Swenseid, M. E., and Bird, O. D., *J. Biol. Chem.*, 1947, **170**, 367.

7 Hodson, A. Z., *Arch. Biochem.*, 1948, **16**, 309.

8 Schneider, W. C., *J. Biol. Chem.*, 1945, **161**, 293.

9 Schneider, W. C., *Cold Spring Harbor Symposia on Quantitative Biology*, 1947, **12**, 169.