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Uptake of DDT from the Yolk Sac into the Early Chick Embryo as Measured by Gas Chromatography

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Numerous studies can be found throughout the literature concerning the effects of pesticides on the developing avian embryo (MCLAUGHLIN et al. 1963; WALKER 1967, 1968; DUNACHIE & FLETCHER 1969). The results of these previous investigations have been obtained by examining embryos for abnormalities late in development following long-term exposure to the pesticide. Since more and more yolk is incorporated into the embryo with time, it stands to reason that when the pesticide is injected into the yolk sac more of the pesticide will become available to the embryo with time. However, many critical events in avian embryonic development occur within the first few days of embryonic life (development of the extra- and intra-embryonic circulatory systems, development of the neural tube, migration of primoridal germ cells, etc.). Can an agent such as DDT, when administered into the yolk sac prior to incubation, become incorporated into the embryo within a few days and affect some of these early developmental processes or does such a method of chemical exposure require more time for the embryo to incorporate the agent, thus limiting its effect to later developmental phases of organogenesis?

In the study described here, we examined the ability of the chick embryo to incorporate DDT within five days when exposed via the yolk sac and found a significant amount of DDT uptake within this short time. This indicates that DDT would be present in sufficient amounts to affect early developmental processes, if such processes were susceptible to this agent.

MATERIALS AND METHODS

Fertile white Leghorn chicken eggs (Truslow Farms, Inc., Chestertown, MD) were used. The insecticide DDT was obtained from Aldrich Chemical Company, Inc., Milwaukee, WI. The pesticide was dissolved in dimethylsulfoxide (DMSO) and a 0.05 mL volume containing either 5, 10 or 20 mg of DDT was administered into the yolk sac of unincubated eggs. Eggs receiving only the DMSO vehicle served as controls. A detailed description of the injection technique was previously described (SWARTZ 1980).

Following injection the eggs were placed in a forced-draft incubator maintained at 37°C and 60% relative humidity. After

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five days of incubation (Stages 24-27; HAMBURGER & HAMILTON 1951) the embryos were removed from the eggs and cleaned of all extra-embryonic membranes and yolk. Samples of yolk were also removed from the eggs to be analyzed separately. The embryos and yolks from the 50 eggs in each of the dosage groups and the control group were then frozen separately in preparation for subsequent gas chromatographic assay. The experiment was repeated three times in the case of embryos receiving 5 and 10 mg of DDT and twice in that of those receiving 20 mg of DDT. Preparations for analysis of DDT incorporation began with extracting 5 g samples of yolk or embryonic material with acetone for 3 min in a sonic blender. The material was then partitioned and cleaned up using the modified method devised by the Environmental Protection Agency (1980). Hexane was used to elute the Florisil column (MILLS et al. 1972).

Two separate gas chromatographic columns, one packed with 3% OV-1 on 80-100 mesh Supelcoport and the other with 3% OV-210 on 100-120 mesh Gas Chrom Q were used to compare results on polar versus non-polar materials. The temperature of the two columns (1.8 m x 2mm ID) was 180 and 170°C, respectively. The temperature of the injector was 250°C while that of the ^{63}Ni detector was 300 °C. The carrier gas (argon/methane) flow rate was 50 mL/min.

Levels of DDT were read in samples of yolk and embryos from each of the three dosage groups and the control group. A control yolk sample was fortified with DDT to check for recovery in this system. The one-sided Student's t test was employed to determine whether the uptake of DDT into the embryo was significant.

RESULTS AND DISCUSSION

Intravitelline injections of DDT into eggs from white Leghorn chickens, prior to incubation, did not affect the ability of the embryos to survive to five days of incubation. Mortality rates for embryos receiving, 5, 10, and 20 mg of DDT were 4.3, 6.3 and 5.7%, respectively. These compared favorably with the 5.8% rates in the controls.

Five days after the intravitelline injection of DDT, the majority of the pesticide was still found within the confines of the yolk sac at all dosage levels employed. Averages of 40, 253, and 301 ppm were found in the yolk of five day embryos, injected with 5, 10 and 20 mg of DDT, respectively, prior to incubation. All eight groups of embryos receiving DDT in their yolk sac prior to incubation contained DDT after five days. Gas chromatographic assay revealed averages of 2.1, 1.7 and 2.8 ppm in the groups of embryos receiving 5, 10 amd 20 mg, respectively, of DDT (Fig. 1). Each point and its corresponding interval in Figure 1 represents a mean $\pm$ 2 SEM and, thus, a range of values indicating a 95% confidence level of DDT uptake for each dose group. Both control embryos and yolk exposed only to the DMSO vehicle demonstrated an absence of DDT when examined chromatographically.

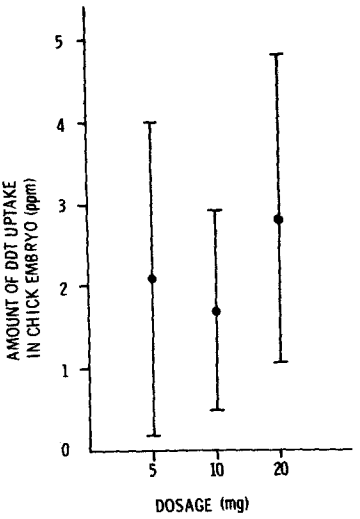

Fig. 1 Amount of DDT uptake in five-day chick embryos following intravitelline injection of different dosages of DDT prior to incubation.

The three dose group means were all significantly greater than zero ($p < 0.05$) applying the one-sided Student's t test with five degrees of freedom. The differences among the different dose-groups were not statistically significant at the $p < 0.05$ level (Fig. 1).

Several investigators (MCLAUGHLIN et al. 1963; WALKER 1967, 1968) have discussed the value of the avian embryo as a toxicological model. Owing to the specific gravity of the yolk medium into which a chemical agent is placed, the specific gravity of the vehicle used to deliver the agent could determine the degree to which the developing embryo will be exposed. Dissimilar results have been reported with respect to the role of specific vehicles in the degree of exposure to developing avian embryos. A four-fold increase in mortality was reported in chick embryos exposed to acetone for 20 days as opposed to corn oil (WALKER 1967). On the other hand, a much higher incidence of mortality was reported when acetone was used as the vehicle when compared to sesame oil (DUNACHIE & FLETCHER 1969; SWARTZ 1980). None of these studies, however, determined what amount of pesticide, if any, became incorporated into the embryo and thus was available to exert an effect.

The present study is the first to demonstrate that DDT can be incorporated from the yolk sac into the chick embryo within five days following pre-incubation exposure. The amount of incorporation does not differ significantly with increases in dosage.

Most previous studies with DDT and other pesticides have dealt with the effects of the agents after long-term exposure (15-20 days) to the chick embryo. Little is known as to whether

pesticides injected into the yolk sac can become available to the embryo in sufficient amounts early in development to possibly alter early stages of embryogenesis. DAVID (1975) has recently shown that exposure to DDT prior to incubation results in a decrease in the number of primordial germ cells colonizing the gonads in six-day quail and chick embryos. A different method of exposure was employed which involved dipping the eggs in an aqueous solution of DDT for 30 s prior to incubation. Gas chromatographic assays performed by this same investigator (DAVID 1979) showed that the DDT passed through the shell; however, to what degree it became available to the embryo was not specifically determined, since the assayed material consisted of the entire contents of the egg (yolk, albumen and embryo).

Longer exposure times do certainly increase the possibility of toxic effects of an agent; however, it makes the elucidation of the mechanism of action of such an agent difficult. Since DDT does become incorporated into the embryo within five days, studies of the effects of the agent on developmental processes occurring during this shorter time span may assist in better portraying the mechanism of action of this pesticide.

This study emphasizes the need to assess the rate of incorporation of not only pesticides but also other agents which are used in toxicity studies employing chick embryos. The knowledge of the time of incorporation of sufficient amounts of specific chemical agents can serve to better elucidate just what stage of organogenesis is affected by these agents.

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