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Dissimilarities in the Toxic Response of Early Chick Embryos to DDT Administered in Different Vehicles

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The widespread use of pesticides and their reported effects on the animal and human populations have stimulated much research to determine what role, if any, such agents play in abnormal embryonic development. Results obtained from such studies have resulted in many pesticides being banned from usage and others being placed on a restricted list. An example of the former is DDT.

However, conflicting reports exist as to the effects of DDT exposure on embryonic development of avian species. No consistency prevails as to the method of administration and the vehicle used to dispense the pesticide. DAVID (1976a) reported a 42% mortality in chick and quail embryos exposed to a yolk sac injection of DDT dissolved in olive oil, whereas immersing the eggs in water containing the pesticide caused only a 22% mortality. GLICK and WHATLEY (1966) demonstrated a 58% mortality in chick embryos exposed to DDT in sesame oil. DUNACHIE and FLETCHER (1966) found yolk sac injections of 10 mg of DDT dissolved in acetone to be harmless.

The present study is designed to determine if different vehicles play dissimiliar roles in mediating the effects of DDT in the early chick embryo. This will be assessed by determining the incidence of mortality and anomalous conditions following five days of exposure to DDT in different vehicles deposited within the yolk sac.

MATERIALS AND METHODS

Fertile white Leghorn chicken eggs (Truslow Farms, Inc., Chestertown, MD) were used in this study. The pesticide 1,1-Bis-(p-chlorophenyl)-2,2,2-trichloroethane (DDT) was obtained from Aldrich Chemical Company, Inc., Milwaukee, WI. The pesticide was dissolved in three commonly used vehicles: 1) dimethylsulfoxide (DMSO), 2) sesame oil and 3) olive oil. The solution was then administered into the yolk sac of unincubated eggs in either 5 mg or 10 mg dosages.

Preparation for injection consisted of placing a small opening in the blunt end of the egg with an egg punch. A 21 gauge needle attached to a syringe containing the substance to be injected was placed into this opening and passed through the air sac into the yolk sac where the solution was deposited. The volume of injection

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was 0.1 ml. Following injection the opening in the egg was sealed with Scotch tape. The eggs were then placed in a forced-draft incubator maintained at 37°C and 60% relative humidity. Eggs receiving the vehicles only and a group of non-manipulated eggs served as controls. These control groups were incubated under the same environmental conditions in a separate incubator.

After five days of incubation (Stages 24-27; HAMBURGER and HAMILTON 1951) the eggs were removed from the incubator and the embryos examined. Tabulations were made as to the viability of the embryos and as to any defects occurring in the different groups of eggs.

RESULTS

Dosages of 5 and 10 mg of DDT dissolved in DMSO resulted in mortality rates of about 10% for both groups (Table 1). Of the embryos receiving DMSO alone only 2 of 49 failed to survive (4.1%). There was no incidence of developmental deformities in any of the survivors of this group. Five day exposure of embryos to 5 and 10 mg of DDT dissolved in sesame oil resulted in mortality rates of 25% and 20% respectively (Table 1). The 5.9% mortality of the embryos receiving the sesame oil alone very nearly equalled the 7.4% rate of the non-manipulated controls. Only one of the DDT-sesame oil embryos and one of the sesame oil controls displayed any anomalous conditions and in both cases it involved the eyes. In the former embryo exposed to 10 mg of DDT bilateral microophthalmia with pigment only in the left eye was seen. An abnormally large cystic left eye was observed in the sesame oil control embryo.

TABLE 1. Toxicity of DDT administered with different vehicles to early chick embryos

Vehicle	Dosage (mg)	Total No. of embryos	No. Living	Mortality (%)
DMSO	5	49	44	10.2
	10	48	43	10.4
	Vehicle	49	47	4.1
Sesame oil	5	20	15	25.0
	10	20	16	20.0
	Vehicle	17	15	5.9
Olive oil	5	73	47	35.6
	10	57	34	40.3
	Vehicle	79	56	29.1
Non-manipulated controls	--	94	87	7.4

Those embryos exposed to 5 and 10 mg of DDT in olive oil presented a much higher rate of mortality (Table 1) than seen in either the DDT-DMSO or DDT-sesame oil groups. It should be noted that 23 of the 79 embryos receiving olive oil alone (29.1%) failed to survive to five days of incubation. This is approximately a four-fold increase in mortality over the non-manipulated controls. Only one survivor of the DDT-olive oil group showed any abnormality and again it involved the eye. This embryo possessed microophthalmia of the left eye following exposure to 10 mg of DDT.

DISCUSSION

Although it has been shown by others that the route of administration of DDT can vary the intensity of the toxic effects of DDT in the avian embryo (DUNACHIE and FLETCHER 1966, 1969; GLICK and WHATLEY 1966; DAVID 1976a), this study clearly demonstrates that even with a uniform method of injection (into the yolk sac) the toxic effects of DDT can be modified by the vehicle in which it is presented to the embryo. No significant increase in mortality was seen in embryos exposed to the DDT-DMSO injection when compared to controls whereas approximately a three-fold increase in mortality was observed in embryos receiving DDT dissolved in sesame oil. Groups of embryos exposed to 5 and 10 mg of the DDT-olive oil combination demonstrated the lowest percentage of survival to five days. However, in the group receiving olive oil alone a 29% mortality rate was observed, significantly higher than that of the non-manipulated and vehicle control groups.

It would appear that the high mortality rate of embryos receiving the DDT-olive oil combination might be due to the toxic effect expressed by the olive oil itself. In fact DAVID (1976a) reported a 24% mortality rate in chick embryos and 30% in quail embryos treated similarly with olive oil alone. Most studies dealing with the effects of DDT on avian embryos have been concerned with hatching ability of the embryos following exposure (SOMERS et al. 1974; DAVID 1976b). The present study was limited to exposing the embryo to DDT for only 5 days in order to determine whether this pesticide could exert its effect within this short time period, a time span during which many important developmental processes are occurring.

No matter what vehicle was used, the incidence of malformations in the survivors was negligible. Whether this would change with further development is not known.

The differences in toxic response of DDT in the different vehicles to the chick embryo observed in this study could be due to several factors. The higher mortality rate observed with sesame oil as the vehicle for DDT when compared to DMSO could be attributed to the former providing for greater diffusibility of the DDT into the embryo from the yolk sac or the latter exerting an inhibitory effect on the expression of DDT toxicity. It is thus imperative to consider the vehicle employed in the administration of DDT when evaluating the toxicity of such an agent on the early

development of the chick embryo.

Work is currently in progress evaluating the uptake of the DDT in the different vehicles by the embryos for the five day period. Gas chromatographic assays of embryos exposed to DDT for five days will ascertain whether the actual amount of DDT incorporated into the embryos differs depending upon the vehicle employed.

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