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Sensitivity of Chick Embryo to Various Solvents Used in Egg Injection Studies[1] (41784)

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Abstract. Two experiments were conducted to study the sensitivity of developing chicken embryos to various solvents used as vehicles and their effect on hatchability. No significant differences on embryonic mortality were observed between the sham-injected control and corn oil-injected groups. Acetone, ethylene glycol, and ethanol (0.10 ml/egg) significantly reduced the percentage hatchability and showed a high embryonic mortality during the first week of incubation. Levels of cottonseed oil (0.05–0.10 ml) and propylene glycol (0.05–0.15 ml) were well tolerated by the developing embryo but were slightly inferior to corn oil. Levels of 0.05–0.15 ml of corn oil are suitable vehicles for fat soluble compounds in studies involving the injection of eggs. Higher levels of solvent can be injected at later incubation periods. The choice of the best solvent must be made depending upon (1) solubility of test material, (2) amount of test material needed, (3) toxicity of the solvent at the appropriate levels, (4) route of administration, and (5) stage of development.

Extensive use of developing chicken embryos to test the toxicity and the teratogenic effects of food additives (1), insecticides (2), and mycotoxins (3–5) have been reported. Behavioral changes in chicks treated with alcohol during their embryonic development has been studied (6). Investigations (7) on nonspecific malformations in chick embryo have been reported when insoluble compounds were introduced into the amniotic cavity. Because of varying solubilities of different compounds it is difficult to find a suitable solvent for egg injection experiments. Egg injections are used with promising results in certain biological and toxicological studies. It is necessary to select an appropriate dose and suitable solvent without adversely affecting the hatchability of the eggs.

Materials and Methods. *Experiments 1 and 2.* Pullorum-free fertile eggs weighing between 58 and 62 g from New Hampshire × Single Comb White Leghorn hens were candled and selected. The exact location of the air cell was outlined with a pencil, and 30 eggs were randomly assigned to six treatments in the first experiment. Eggs in each treatment group received 0.10 ml of corn oil, cottonseed oil, propylene glycol, acetone, ethylene glycol, or ethanol before incubation. In the second experiment treatment levels were 0.05, 0.10, and 0.15 ml/egg (30 eggs/treatment level). All eggs in the treatment and sham-injected control groups were placed on egg flats with the large end up. The surface was wiped with 70% ethanol, and a small hole was drilled in the shell over the air cell. Care was taken not to tear the shell membrane. Injections were made at a 45° angle through the hole, and the solvents were deposited near the yolk without puncturing the vitelline membrane with a sterile 0.25-ml syringe. The holes were sealed with a small piece of cellophane tape. The eggs used as controls were swabbed, drilled, sham-injected, and sealed as above. All the eggs were placed in the same incubator maintained at recommended temperature and humidity levels. The eggs were turned six times in 24 hr and candled every week. On the 18th day of incubation, the eggs were transferred to a hatcher. Percentage embryonic mortality during the first, second, and third week of incubation and the percentage hatchability of fertile eggs at the end of 22 days were recorded. Data were analyzed for statistical significance by using the analysis of variance (8), and the differences between the treatment means were tested by using Duncan's multiple range test (9).

Results and Discussion. *Experiment 1.* Percentage hatchability and embryonic mortality during the first, second, and third week of incubation are presented in Table I. No sig-

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TABLE I. EFFECT OF INJECTING VARIOUS SOLVENTS ON PERCENTAGE HATCHABILITY AND EMBRYONIC MORTALITY, EXPERIMENT 1

Solvent†	Percentage hatchability	Percentage embryonic mortality		
		1st Week	2nd Week	3rd Week
Control	82.1 ± 6[a]‡	17.8 ± 6[a]	0	0
Corn oil	81.7 ± 3[a]	18.2 ± 3[a]	0	0
Cotton seed oil	76.0 ± 1[a,b]	15.7 ± 3[a]	8.3 ± 4[a]	0
Propylene glycol	67.7 ± 1[b]	25.1 ± 4[a]	7.0 ± 3[a]	0
Acetone	50.0 ± 3[c]	42.1 ± 2[b]	7.8 ± 4[a]	0
Ethylene glycol	47.6 ± 4[c]	43.8 ± 6[b]	0	8.4 ± 4[a]
Ethanol	22.0 ± 5[d]	55.6 ± 5[b]	0	22.3 ± 11[b]

† 0.10 ml/egg.
‡ Values are means (±SD) of 30 eggs.
[a,b,c,d] Means within columns with same superscripts are not significantly different ($P < 0.05$).

nificant difference in percentage hatchability and embryonic mortality during the first week of incubation was observed between the control group and the group which received 0.10 ml corn oil or cottonseed oil. The percentage hatchability of eggs receiving cottonseed oil and propylene glycol was 76 and 67%, respectively, with no significant differences in percentage mortality during the first and second week of incubation. Injection of 0.10 ml acetone and ethylene glycol showed a significant ($P < 0.01$) reduction in percentage hatchability with more than 40% embryonic mortality during the first week of incubation. Lowest hatchability (22%) and maximum percentage embryonic mortality (55%) during the first week and 22% during the third week of incubation was observed in the group which received ethanol at the 0.10 ml/egg level.

Experiment 2. Figure 1 shows the effect on

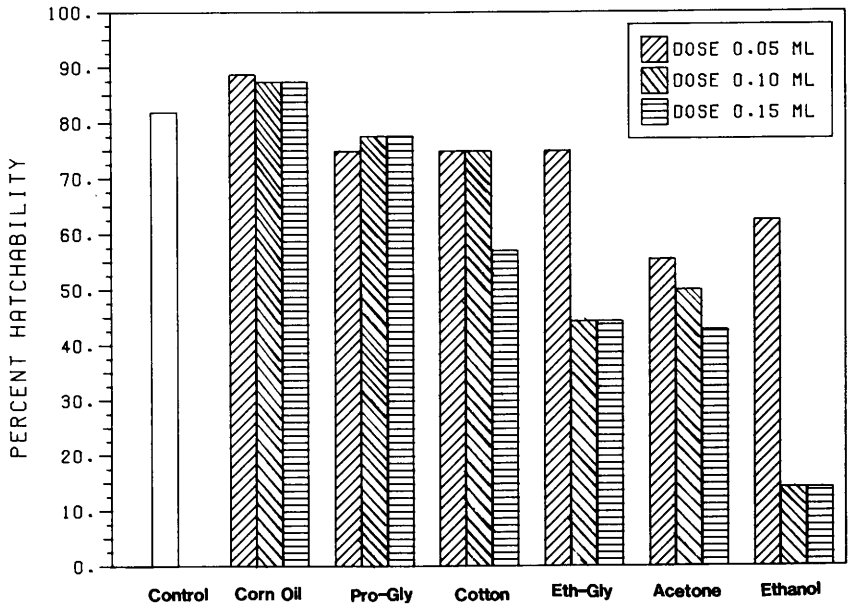

FIG. 1. Effect of corn oil, propylene glycol (PRO-GLY), cotton seed oil (COTTON), ethylene glycol (ETH-GLY), acetone, and ethanol on hatchability of chicken eggs.

hatchability of using several solvents at various dose levels when injected into the eggs before incubation.

No significant differences in percentage hatchability were observed between the control and the groups which received corn oil, propylene glycol, or cottonseed oil except that a significant ($P < 0.05$) reduction in hatchability was observed when 0.15 ml of cottonseed oil was injected. Injecting 0.05 ml of ethylene glycol was well tolerated by developing embryos, and the percentage hatchability was comparable to propylene glycol or low levels of cottonseed oil. However, the higher levels of ethylene glycol appeared toxic and did not support maximum hatchability.

Gebhardt and Van Logten (10) indicated that the chick embryos are made more sensitive to the lethal action of glycols by destroying the extra embryonic blood vessels when injected on the fourth day of incubation. Acetone and ethanol significantly ($P < 0.01$) reduced the percentage hatchability, and 85% of the embryos failed to hatch at 0.10 and 0.15 ml levels of ethanol. These results are in agreement with McLaughlin *et al.* (11) who observed a reduction in percentage hatchability when 234 mg of ethanol or 78 mg of acetone per egg was injected into the eggs. Contrary to our results, lower embryo mortality was observed with acetone than with corn oil, when DDT was injected dissolved in either compound regardless of DDT levels (2).

Although numerous studies have been conducted to test the sensitivity of chicken embryos with a variety of compounds, the choice of solvent must be made depending upon its toxic responses based on the solubility of test compound, its specific gravity, coagulating effect, dose, route of administration, and the stage of embryonic development. Since it is not feasible to introduce more than 0.2 ml of any solvent into a fresh egg without affecting the embryonic survival a volume of 0.05 ml is preferred. However, depending upon the solubility of the test compounds levels higher than 0.05 ml must be used. It is possible to use a level of 0.2 ml when the embryo is 15 days old, unfortunately at this time higher mortality caused by hemorrhage due to improper distribution of solvents affect the percentage hatchability. From the results of these investigations, it can be suggested that corn oil can be used as a suitable vehicle because of its stability, uniform dispersion, and low toxicity for studies involving the injection of eggs.

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