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THE EFFECT OF CITRIC ACID ON THE TERATOGENIC ACTION OF TRYPAN BLUE[1]

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Trypan blue has been shown to be teratogenic when injected into the subgerminal cavity or yolk sac of the developing chicken egg (1,2,3,4). The most common abnormality observed after such treatment is rumplessness. The mechanism of action of this dye is not known, although, it has been proposed that it acts primarily on the mesoderm (3,4).

Landauer has obtained evidence which shows that certain teratogens are active during chick development by interfering with metabolic processes. He has demonstrated that the teratogenic effects of some compounds can be prevented, or lessened, by supplying the embryos with compounds that play a role in metabolism (5).

The present study was undertaken to determine if a metabolic compound could modify the teratogenic effects of trypan blue in the chicken egg. Citric acid was selected for testing because of its effectiveness in preventing induced rumplessness.

Materials and Methods

Fertile eggs of the White Leghorn chicken were obtained from

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three different commercial sources. The eggs were injected into the yolk sac at 36 hours incubation: Hamburger-Hamilton stages 9-11 (6). Trypan blue[2] was prepared as a 0.1% saline solution and 0.1ml was injected. This dose has been shown to be teratogenic in the developing hen's egg (7). Citric acid[3] was prepared in saline so that the injected volume of 0.1ml contained 25 mg of citric acid. Twenty-five milligrams of citric acid has been used successfully by Landauer in preventing insulin induced rumplessness (8). Citric acid was injected first followed immediately by trypan blue. Control eggs were either injected with saline or incubated without injection. The embryos were recovered on the 10th day of incubation, fixed, weighed and examined for gross malformations.

Results

Table 1 summarizes the results of this experiment. It is evident that when citric acid is injected in conjunction with trypan blue there can be a marked reduction in the teratogenic effects of the dye. In the eggs from flocks one and two there was a reduction in mortality from 41% to 27% and from 56% to 30% respectively. The incidence of malformations was reduced by more than one-half in treated eggs from each flock. Statistical analysis by the chi-square method revealed these reductions to be significant (see table for P values). Identical experiments with eggs of the third flock resulted in an increase in mortality and did not reduce the numbers malformed to a significant degree.

Discussion and Conclusions

The results of this experiment show that it is possible to

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[2]Matheson, Coleman & Bell Co. Norwood, Ohio

[3]Nutritional Biochemicals Corp,. Cleveland, Ohio

TABLE 1

The Effect of Citric Acid and Trypan Blue on Chick Development when Injected into Eggs of Three Different Flocks at the 36th Hour of Incubation[1]

	Total treated	Percentage mortality	Percentage malformed survivors	Percentage all eggs affected
Flock 1				
Untreated controls	231	5.6	2.3	7,8
Saline controls	135	17.0	5.4	21.4
Citric acid	182	17.0	1.3	18.1
Trypan blue	226	41.2	30.3	58.8
Trypan blue & Citric acid	462	27.3 (P= 0.001)[2]	10.1 (P= 0.001)	34.6 (P= 0.001)
Flock 2				
Untreated controls	134	11.9	3.4	14.9
Saline controls	141	12.0	2.4	14.2
Citric acid	77	5.2	1.4	6.5
Trypan blue	94	56.5	56.2	71.2
Trypan blue & Citric acid	153	29.8 (P= 0.001)	19.3 (P= 0.001)	43.5 (P= 0.001)
Flock 3				
Untreated controls	117	2.6	0.8	3.4
Saline controls	121	11.5	1.9	13.2
Citric acid	91	8.8	6.0	14.3
Trypan blue	156	49.4	45.6	72.4
Trypan blue & Citric acid	134	61.9	31.4 (P= 0.10)	73.9

[1]Dosage: 0.1mg trypan blue; 25mg citric acid.

[2]Probability determined by chi-square method.

interfere with the teratogenic action of trypan blue in the chicken egg by simultaneous treatment with citric acid. Landauer has shown conclusively that supplementation with certain chemicals of metabolic importance to the chick embryo can protect against the action of certain teratogens. The effectiveness of this protection varies with the teratogenic agent, the supplemental agent and the embryonic period (5). One of the agents Landauer found most effective in preventing insulin induced sumplessness was pyruvic acid. I have used pyruvic acid in combination with trypan blue in 104 eggs of flock two, but no protection was afforded the embryos of these eggs. Citric acid is an effective protecting agent in eggs of the same flock. The action of trypan blue is apparently restricted to the first 96 hours of chick development with a peak in activity at 36 hours (7). It has been shown that the peak in the incidence of rumplessness induced by insulin follows treatment at 31 hours of development and that pyruvic acid and citric acid are effective as protective agents if given within 3 hours of the injection of insulin (8,9). Thus there is similarity in both embryonic period and type of malformation produced between eggs treated with insulin and eggs treated with trypan blue. Further experiments will be necessary to determine the nature of the interaction between trypan blue and citric acid. It is possible that some inactivation of the trypan blue molecule takes place at the injection site, e.g., due to the formation of a complex between dye and acid, or due to a local change in pH.

The use of eggs from three different flocks of White Leghorn chickens points up the importance of genetic variation in teratogenesis. In the present experiment protection against the embryotoxic and teratogenic effects of trypan blue by citric acid was

most marked in eggs from two of the three flocks. In the third flock the protection was lacking, in fact, there was a synergistic action in the lethal effect. All factors of treatment and incubation were the same for each flock, the only apparent difference is genetic. Landauer (10) has shown significant differences in response to the teratogenic effects of boric acid by the eggs of different mothers within the same flock. Also suggestive of genetic variation among the flocks was the observation that malformed embryos from flock 2 had, in addition to rumplessness, many more types of defects than embryos from either flock 1 or 3. In the latter two flocks rumplessness was most often the only defect exhibited by the embryo.

Landauer and Rhoades (8) have proposed that insulin induced rumplessness is due to a disruption in carbohydrate metabolism. It is too early to postulate the same for trypan blue induced rumplessness although the results of this first series of experiments would be in agreement with such a postulate.

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