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Complex Formation and Chemical Specificity of Boric Acid in Production of Chicken Embryo Malformations.* (20200)

WALTER LANDAUER. (Introduced by E. Zwilling.)

From the Storrs Agricultural Experiment Station, University of Connecticut, Storrs.

Boric acid, when injected into the yolk sac of developing chicken eggs, acts as a potent teratogenic substance(1). Following treatment at 4 days of incubation a typical syndrome arises, with features of shortening (often extreme) of the mandible, facial coloboma and cleft palate, shortening and bending of the tarsometatarsus, and poor development of the toes or their complete lack, the fourth toe being affected preferentially. Length of femur and tibiotarsus may also be reduced, depending on the amount of injected boric acid(2). Among hatched chicks the most prominent symptom is curled-toe paralysis.

These effects of boric acid on development assume particular interest on account of varied evidence, also previously discussed, pointing to the conclusion that the malformations are caused by an interference with the embryos' supply of riboflavin. In the experiments to be reported here we sought information on two points: 1. Will substances which are known to form complexes with boric acid, reduce or abolish the teratogenic qualities of the latter when they are dissolved in the boric acid solution prior to treatment or when they are used as supplements? 2. Is the teratogenic action specific for boric acid or is it, more generally, a quality of boron?

Methods. Sterile solutions of the compounds to be tested were made up in saline with 0.25% phenol added. The injections were given into the yolk sac in volume of 0.05 cc using a tuberculin syringe and No. 27 needle. The experiments were carried through to hatching. The results are based on all survivors of the 13th day of incubation.

Results. The results of experiments with compounds which are known to form complexes with boric acid are shown in Tables I and II. In a first series of tests (Table I) the teratogenic effects of boric acid were compared with the consequences of simultaneous but separate injection of boric acid and either D-ribose or pyridoxine hydrochloride. It can be seen that the addition of D-ribose or pyridoxine hydrochloride increased the chances of embryos to develop normally and reduced the incidence of every one of the different types of malformations. Considered individually, many of the differences between the group treated with boric acid and the three other groups are on the borderline of significance; but, if all features are taken into consideration, the differences become highly significant. D-

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TABLE I. Experiments with 2.5 mg/egg Boric Acid Alone and Supplemented by Simultaneous Injection of 2.5 or 10 mg D-ribose or 5 mg Pyridoxine Hydrochloride.

	Boric acid	Boric acid and 2.5 mg D-ribose	Boric acid and 10 mg D-ribose	Boric acid and 5 mg pyridoxine hydrochloride
Survivors of 13th day	60	73	72	112
Normal, %	36.7 ± 6.2*	49.3 ± 6.0	54.2 ± 6.0	50.9 ± 4.8
Simple beak defects, %	15.0 ± 4.6	9.6 ± 3.5	11.1 ± 3.8	4.5 ± 2.0
Complex beak defects, %	43.3 ± 6.4	38.3 ± 5.8	26.3 ± 5.1	27.7 ± 4.3
Tibiotarsus bent, %	6.7 ± 3.2	2.7 ± 1.9	1.4 ± 1.4	0
Abnormal tarsometatarsus, %	11.6 ± 4.1	4.1 ± 2.4	2.8 ± 2.0	2.7 ± 1.6
Abnormal toes, %	20.0 ± 5.2	9.6 ± 3.5	7.0 ± 3.0	3.6 ± 1.8

* Standard errors.

TABLE II. Results of Injecting Solutions Containing 2.5 mg Boric Acid Alone or with Addition of 2.5, 10 or 20 mg D-sorbitol Hydrate.

		Boric acid containing		
	Boric acid alone	2.5 mg D-sorbitol hydrate	10 mg D-sorbitol hydrate	20 mg D-sorbitol hydrate
No. treated	78	93	90	243
Hatched, %	19.2 ± 4.4*	38.7 ± 5.1	61.1 ± 5.1	54.3 ± 3.2
Curled-toe paralysis (among hatched), %	33.3 ± 5.3	19.4 ± 4.1	7.3 ± 2.7	0
Survivors of 13th day	60	86	81	194
Normal, %	36.7 ± 6.2	40.7 ± 5.2	79.0 ± 4.6	99.0 ± .7
Simple beak defects, %	15.0 ± 4.6	7.0 ± 2.7	8.6 ± 3.1	.5 ± .2
Complex beak defects, %	43.3 ± 6.4	47.7 ± 5.3	8.6 ± 3.1	0
Tibiotarsus bent, %	6.7 ± 3.2	2.3 ± 1.6	0	0
Abnormal tarsometatarsus, %	11.6 ± 4.1	12.8 ± 3.5	1.2 ± 1.2	0
Abnormal toes, %	20.0 ± 5.2	9.3 ± 3.1	2.4 ± 1.7	0
Syndactylism, %	1.7 ± 1.7	4.7 ± 2.2	0	0

* Standard errors.

ribose was more effective in preventing defects at the higher than at the lower dosage level.

We obtained still clearer results when, prior to injection, D-sorbitol hydrate was dissolved in the boric acid solution (Table II). At the 2.5 mg level the effect of D-sorbitol was slight and its significance uncertain, except that survival and hatching were definitely improved; at the 10 mg level the incidence of all abnormalities was much decreased and the percentage of normal chicks correspondingly raised; at the 20 mg level the teratogenic consequences of boric-acid treatment had been very nearly abolished. A comparison of the data in Tables I and II shows that at the same dosage levels D-sorbitol was more effective than D-ribose in reducing the teratogenic action of boric acid. This may have been due to the different manner of administration, but it is also known(3) that D-sorbitol is more reactive with boric acid than D-ribose.

In earlier experiments it had been observed that the teratogenic effects of boric acid can be mitigated by the addition of riboflavin. The present tests with other polyhydroxy compounds, known to undergo complex formation with boric acid, confirm the assumption that in the form of complexes boric acid ceases to be teratogenic. Carbohydrates which do not form complexes with boric acid, *e.g.* glucuronic acid lactone, are ineffective. Tests with D-sorbitol illustrate, on the other hand, that with increasing amounts of this acyclic polyol the teratogenic effectiveness of boric acid declined in a quantitative manner.

A study of the chemical specificity of boric acid is made difficult by the dearth of water-soluble boron compounds other than borates. All borates, on the other hand, with one exception to be dealt with presently, liberate boric acid in solution. Tests were made with sodium metaborate (2.5 and 5 mg/egg) and with sodium borate (3.5 mg/egg). At the lower dosage level sodium metaborate was responsible for a few cases of curled-toe paralysis, but did not produce morphological defects;

TABLE III. Experiments with Boric Acid, Triethanolamine Borate* and Sodium Aluminate. Injections at 96 hr incubation.

	Boric acid, 2.5 mg	Triethanolamine borate, 5 mg	Sodium aluminate 2.5 mg	Sodium aluminate 5 mg
Survivors, 13th day	83	132	100	81
Normal, %	1.2 ± 1.2†	34.1 ± 4.1	89.0 ± 3.1	82.7 ± 4.2
Simple beak defects, %	6.0 ± 2.6	12.9 ± 2.9	0	1.2 ± 1.2
Complex beak defects, %	67.5 ± 5.2	49.2 ± 4.4	0	0
Tibiotarsus bent, %	2.4 ± 1.7	0	4.0 ± 2.0	16.0 ± 4.1
Abnormal tarsometatarsus, %	33.7 ± 5.2	20.5 ± 3.5	4.0 ± 2.0	2.5 ± 1.7
Abnormal toes, %	18.1 ± 4.2	3.8 ± 1.7	0	0
Syndactylism, %	4.8 ± 2.3	.8 ± .8	0	0

* Prepared by Sigma Chemical Co., St. Louis. † Standard errors.

at the 5 mg/egg level typical malformations occurred, but with low incidence. Experiments with 3.5 mg/egg sodium borate (about equivalent in boron to 2.5 mg/egg boric acid) yielded, on the other hand, results that were quantitatively and qualitatively very similar to those obtained with the injection of 2.5 mg/egg boric acid. For the reason indicated, it is difficult to know how much of the teratogenic action of these borates was due to boric acid.

Brown and Fletcher(4) have reported the synthesis of triethanolamine borate which is hydrolyzed only very slowly in dilute acid and little, if at all, in neutral water. At the stage of our experimentation the yolk pH is about 6. We have, therefore, made a test comparing the effects of boric acid (2.5 mg/egg) with those of triethanolamine borate (5 mg/egg). The boron content of the borate was somewhat lower than that of the boric acid; higher doses proved to be too toxic. Compared with boric-acid treatment, injection of triethanolamine borate allowed more embryos to complete development normally and the various kinds of malformations occurred with lower frequency (Table III). Yet, the incidence of abnormalities was high and the defects were morphologically identical with those produced by boric acid. Hence, this boron compound shares the teratogenic qualities of boric acid.

It seemed of interest to determine if aluminum, next to boron in the periodic system, interferes with the development of the chicken embryo in a fashion similar to that of boron. The results of experiments with sodium aluminate are reproduced in Table III. The teratogenicity of the aluminum salt was, on the whole, low; but there were some instances of shortened and bent tarsometatarsus, very similar to the characteristic effects of boron compounds. Syndactylism, reduction of toes and complex beak defects were not encountered. Bending of the tibiotarsus, on the other hand, occurred with surprising frequency (χ^2 of difference in incidence after 2.5 mg boric acid and 5 mg sodium aluminate was 7.974, with $P < .01$). Among the hatched chicks we found a few cases of typical curled-toe paralysis. There is little doubt that the action of the aluminum salt is related to, if much weaker than, that of the boron compounds. Our data suggest that in the appendicular skeleton sodium aluminate tended to interfere more proximally with development than do boric acid and other boron compounds.

Summary. Polyhydroxy compounds (D-ribose, pyridoxine hydrochloride, D-sorbitol hydrate), known to form complexes with boric acid, were tested for their effect on the teratogenic action of boric acid during development of the chicken embryo. It was found that complex formation reduces or abolishes the teratogenic qualities of boric acid. Triethanoalmine borate produced the same malformations as did boric acid. Injected sodium aluminate caused few developmental defects, but they seem to be related to those brought about by boron compounds. Our results support the working hypothesis that boric acid interferes with normal development by complex formation in ovo with polyhydroxy compounds, thereby producing symptoms resembling riboflavin deficiency. Additional evidence suggesting that in our material boric acid acted

on coenzymes rather than on enzymes was presented earlier.

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Hepatic Circulation in Hemorrhagic Shock in the Rat.* (20201)

EDWARD W. FRIEDMAN, SAMUEL MILROD, HOWARD A. FRANK, AND JACOB FINE.
From the Kirstein Laboratory for Surgical Research, Beth Israel Hospital, and the Department of Surgery, Harvard Medical School, Boston, Mass.

Intrahepatic vasoconstriction during hemorrhagic shock has been demonstrated in the dog by measurements of portal-caval pressure gradients(1,2) and by x-ray visualization of the intrahepatic veins delineated by thorotrast injection(2). This communication deals with experimental hemorrhagic shock in rats in which the reactions of the sinusoids and the pre-sinusoidal and post-sinusoidal venules were observed by direct microscopy of the transilluminated liver during a prolonged period of hypotension and after transfusion. Data are included on portal venous pressures.

Methods. Pressure in the portal venous bed was measured by a saline manometer connected to a cannula in a mesanteric vein. The ligature on the cannula also secured the accompanying artery to prevent blood loss into the area of obstructed venous return. A segment of intestine about an inch long was thus deprived of blood flow, but the remainder of the intestinal circulation was not disturbed. The incision was closed about the catheter and the rat was allowed to recover from ether anesthesia. After control readings were taken, irreversible hemorrhagic shock was produced as previously described(3). In a number of these rats, *thorotrast* was injected through the catheter to permit x-ray demonstration of the portal vein and its branches. For *direct microscopy,* the liver edge was exposed through a transverse subcostal incision, transilluminated with the aid of a curved quartz rod conducting light from a 200-watt tungsten filament lamp, and examined at a magnification of 150 x. The tip of the rod and the exposed liver were bathed in flowing gelatin-Ringers solution at 37°C. A metal bracket, curved to fit the dome of the liver and notched to avoid vena cava and aorta, was inserted between liver and diaphragm and clamped securely to minimize respiratory motion. Control observations showed that the presence of the bracket did not change vessel size or blood flow. Estimation of change in vessel size was aided by a micrometer scale in the ocular lens (each division representing 7.7 micra in the field). To make these observations, it was necessary to avoid initial heparinization. Hence these rats were bled in accordance with the criteria described(3) but they were not cannulated for continuous measurement of arterial pressure until ready for transfusion.

TABLE I. Portal Venous Pressures in Rats before and after Bleeding and Blood Replacement. 11 experiments.

Portal venous pressure (cm H_2O)			
Before bleeding	During hypotension	After blood replacement	After additional transfusions
17	8.5	15.5	—
15	9	—	—
10.5	7.5	10.4	—
12	7	9	11.5
15	10	16	26
16	10	16	16
17.5	10	18	17.5
14.5	10	15	18
17	11	15	17.2
14.5	9.5	15	22.0
15.1	9.0	15.2	15.9
Mean 15	9.0	14.5	—

Results. Portal venous pressure (Table I). Portal pressures fell from the mean control level of 15 cm H_2O(4) to a mean of 9 cm H_2O

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