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Effect of Boric Acid on Biological Activity of Alloxan.

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In the course of studies on the effect of dietary factors in alloxan diabetes it was found[1] that when rats on a tocopherol deficient diet are injected with alloxan they exhibit marked hemoglobinemia and hemoglobinuria. This reaction has not been observed in animals receiving tocopherol. Because he thought it might be of interest to use in connection with this problem, Professor Richard Kuhn (Heidelberg) kindly sent to us in advance of publication the results of a study by Kuhn and Quadbeck[2] on the effect of simultaneous administration of alloxan and boric acid. These authors found that when boric acid was administered with alloxan, blood sugar values were lower and survival time longer than when alloxan was given alone. When a second injection of alloxan and boric acid was given a week later, however, severe diabetes was produced. Boric acid is known to increase the activity of alloxan in its lactin form[3,4] and they attribute the protective effect of boric acid to increased reactivity of alloxan in other active centers so that less reaches the pancreas; failure of protection on reinjection may indicate saturation or inactivation of these centers. We have carried out experiments similar to those of Kuhn and Quadbeck on animals receiving our special rations, and have studied hemolysis as well as diabetes.

Experimental. The general procedure in this study was the same as that previously reported.[1] Two types of diet were used: a high-lard diet (casein 20%, sugar 36%, lard 38%, cod liver oil 2%, salt mixture 4%) which in the previous experiment had caused marked hemolysis and high mortality, and a low-fat diet (casein 20%, sugar 76%, salt mixture 4%, supplemented with 3 drops of corn oil per day and 3 drops of percomorph oil per week) where hemolysis and mortality were low and there was consequently better opportunity of observing the development of diabetes. All animals received a daily supplement of crystalline B-vitamins and the tocopherol-treated groups received 3 mg of mixed tocopherols* daily. Female rats of the Sprague-Dawley strain weighing 100 to 145 g were used. They were kept on the experimental diet for a month before injection of alloxan.

Alloxan and boric acid (10-16 mg/cc) were injected intraperitoneally at a level of 160 mg/kg each. When both were given they were injected separately but almost simultaneously. Blood sugars were taken the second day after injection and determined by the method of Somogyi[5] as modified by Nelson.[6,†] In some cases blood NPN was also determined. Hemolysis was estimated by hematocrit determinations 15 and 30 minutes after injection and by the amount of hemoglobin observed in the urine which was collected on filter paper.

The study previously reported[1] had furnished a large number of control animals so only a few injections of alloxan alone were made in the present series. These special control animals were used for simultaneous

1 György, P., and Rose, C. S., *Science*, 1948, **108**, 716.

2 Kuhn, R., and Quadbeck, G., unpublished.

3 Kuhn, R., and Weygand, F., *Ber. d. deutsch. chem. Gesellsch.*, 1935, **68**, 1282.

4 Kuhn, R., Reinemund, K., Weygand, F., and Ströbele, R., *Ber. d. deutsch. chem. Gesellsch.*, 1935, **68**, 1765.

* Kindly furnished by Distillation Products, Inc., Rochester, N. Y.

5 Somogyi, M., *J. Biol. Chem.*, 1945, **160**, 61.

6 Nelson, N., *J. Biol. Chem.*, 1944, **153**, 375.

† The blood sugar determinations were made in the George S. Cox Institute through the courtesy of Dr. F. D. W. Lukens.

TABLE I.
Effect of Boric Acid Injected with Alloxan on Survival and Diabetes.

Diet	No. of rats	Treatment*	Survival (%) 2 days	Survival (%) 7 days	Blood sugar, 48 hr (mg %)
High lard	10	A	50	10	405 (350-478)
" "	8	A + B	100	88	151 (114-213)
High lard—tocopherol	10	A	60	10	461 (376-643)
" " "	4	A + B	100	75	142 (111-135)
Low fat	6	A	100	83	467 (230-898)
" "	6	A + B	100	100	123 (111-136)
Low fat—tocopherol	5	A	100	80	612 (479-873)
" " "	5	A + B	100	100	134 (94-200)

* A = Alloxan, 160 mg/kg; B = Boric acid, 160 mg/kg.

comparison of hemolysis caused by alloxan with and without boric acid.

Results. In Table I are given the blood sugar and survival data for rats receiving alloxan and alloxan with boric acid. In every group those animals which received alloxan with boric acid had nearly all normal blood sugars, while all receiving alloxan alone were diabetic. When alloxan was injected survival was much better in the groups receiving the low-fat ration than in those which had the high-fat diet although the diabetes was equally severe in both cases. When boric acid was injected with alloxan there was definite improvement in survival in the groups on the high-lard diet. There was no mortality during the first 2 days following injection, and only 2 animals of the 12 died within 7 days, while with alloxan alone 18 out of 20 had died during this period. All animals on the low-fat diet which had been injected with alloxan and boric acid survived the 7 day period, but with alloxan alone, only 2 of 11 had died. In contrast to the animals which received alloxan alone blood NPN was never elevated in rats treated with the combination of alloxan and boric acid.

We found much less serious effects following a second injection than did Kuhn and Quadbeck. Of the whole series of animals, only 3 died after the second injection, all within the first 2 days. Two had been on the high-lard diet, one of the low-fat diet. None of the survivors showed elevated blood sugar levels and urine sugars determined a week or so after injection were almost all negative, with no more than a trace of sugar found in any sample. In Table II are given values found on animals which received 2 to 4 injections of alloxan with boric acid. In no case was there an elevated sugar, but when alloxan was given a diabetic sugar level was found in those animals which survived for 2 days following the injection.

The primary purpose of Table II is comparison of hemolysis following injection of alloxan with and without boric acid. Hemolysis is never observed in animals receiving tocopherol and is relatively mild in animals on a low-fat diet. Therefore, all of the animals considered here were on the high-lard ration without tocopherol. The first animals observed showed only mild hemoglobinuria, an estimated trace to +, as compared with an almost uniform ++ to ++++ in animals previously observed which had been injected with alloxan alone. However, since the observations were only semi-quantitative and there might be some difference in interpretation and, of greater importance, since we had found that tocopherol intake in the pre-experimental period might have some effect, simultaneous injections of alloxan and alloxan with boric acid were made. Five such pairs were studied, 2 on a first injection, 3 others by injecting one of a pair of animals which had both previously been injected with alloxan plus boric acid with alloxan alone, while the other again received alloxan with boric acid. In every case hemolysis as estimated from hemoglobinemia and hemoglobinuria was ++ or +++++ when alloxan was given

TABLE II. Blood Sugar and Hemolysis in Rats on a High Lard Diet Injected with Alloxan and Alloxan with Boric Acid.

Rat	1st injection			2nd injection			3rd injection			4th injection		
	Date treatment*	Hemolysis	Blood sugar 48 hr (mg %)	Date treatment	Hemolysis	Blood sugar 48 hr (mg %)	Date treatment	Hemolysis	Blood sugar 48 hr (mg %)	Date treatment	Hemolysis	Blood sugar 48 hr (mg %)
1	1/25 A	—	147	2/2 A + B	+	145	2/6 A	++++	†			
2	1/25 A + B	+	213									Dead 1/28
3	1/25 A + B	+	155	2/2 A + B	+	141	2/6 A + B	—	159	2/12 A	+++	380
4	1/26 A	+++	*									
5	1/26 A + B	+	194	2/3 A + B	+	155	2/6 A	++++	†			
6	1/27 A	+++	732									Dead 1/31
7	1/27 A + B	—	125	2/3 A + B	-	134	2/6 A + B	-	139	2/12 A + B	+	119

* A = Alloxan, 160 mg/kg; B = Boric acid, 160 mg/kg. † Dead within 48 hours following injection.

alone, never more than + when boric acid was given simultaneously.

Discussion. Production of diabetes is not the only known biological effect of alloxan. Before this property was known it had been studied as a capillary poison and spasmodic. Labes and Freisburger[7] made a study of the chemical properties of alloxan from this point of view. Kidney damage has been frequently observed in connection with alloxan diabetes. Houssay and Martinez[8] studied the toxicity of alloxan with reference to diet and among other findings noted that low-fat rations protected against early mortality following injection. Data in the present paper confirm this finding but indicate no difference in severity of diabetes with low or high fat in the diet (Table I). The earlier report of the authors[1] indicated another factor in alloxan effect again related to diet: marked hemolysis following alloxan injection in animals deficient in tocopherol. There was no difference in the diabetes produced in the presence or absence of tocopherol (Table I) and little difference in survival except for those tocopherol-deficient animals which died of anemia within a day or so after injection.

When boric acid was given with alloxan there was decreased effect of alloxan in the 3 factors studied: diabetes, mortality, and hemolysis. Kuhn and Quadbeck's suggestion that the increased activity of alloxan in the presence of boric acid results in its dissipation before it reaches the pancreas seems unlikely in view of the decreased effect on mortality and hemolysis. Alloxan disappears from the blood stream very rapidly[9] and the production of diabetes depends on its concentration during the first few minutes following injection. The 160 mg/kg dose of alloxan is not far above the minimum effective dose for intraperitoneal injection. Another possible mode of action of boric acid would be formation of an alloxan-boric acid complex

[7] Labes, R., and Freisburger, H., *Arch. f. exp. Path. u. Pharmakol.*, 1930, **156**, 226.

[8] Houssay, B. A., and Martinez, C., *Science*, 1947, **105**, 548.

[9] Leech, R. S., and Bailey, C. C., *J. Biol. Chem.*, 1945, **157**, 525.

which would reduce the effective concentration of alloxan at any time.

Our results confirmed completely those of Kuhn and Quadbeck with regard to the effect of a first injection of alloxan with boric acid. We are unable to account satisfactorily for the discrepancy between our results and theirs on second injection. They found the second injection much more toxic than the first while we observed this in only a few cases. No effect of alloxan could be seen from most of our blood sugar values, but the mild hemolysis showed that it was not always completely inactivated. A somewhat more serious damage to the pancreas might have occurred in the animals of Kuhn and Quadbeck, not sufficient to cause frank diabetes but leaving them more susceptible to injury by the second dose. This is more probable since their dose of alloxan varied from 150 to 250 mg/kg while ours was always 160 mg/kg.

Summary. When rats on special rations were injected simultaneously with alloxan and boric acid, the deleterious effects of the alloxan were reduced. Only a few animals showed mild diabetes while alloxan alone produced severe diabetes in all cases. Survival for 7 days in animals receiving a high-lard diet was raised from 10% to 80%. Intravascular hemolysis in tocopherol-deficient animals on the high-lard diet, always severe with alloxan alone, was absent or mild when the boric acid was given.

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Transfusion of Leukocytes and Products of Disintegrated Leukocytes.*

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It is a common clinical observation that no demonstrable rise in leukocyte count can be obtained despite repeated transfusions of whole blood, even when given to individuals with marked leukopenia. It has been thought that this failure to successfully increase the leukocyte count by transfusion might be due either to the short life span of leukocytes, to their rapid disappearance from stored blood or possibly to type incompatibilities. This investigation was undertaken to determine whether transfused leukocytes would remain in the blood stream, and if not, to study the mechanism of their removal.

Methods. White blood cells were obtained from the rabbit's peritoneal cavity by a modification of the method of Mudd and coworkers.[1] 300 to 500 cc of physiologic saline were injected into the peritoneal cavity in the evening and a similar amount of saline the next morning about 15 hours later. Overdistension of the rabbit's peritoneum is poorly tolerated and may result in death of the animal. Four hours after the second injection of saline, the fluid was removed from the peritoneal cavity through a 16 gauge needle, using 0.5 cc (5 mg) of heparin as an anticoagulant. The fluid obtained was centrifuged at a low speed for 5 minutes and the sediment resuspended in 10 to 20 cc of Tyrode's solution so that the leukocyte count was in the neighborhood of 50,000 cells per cmm. This cell suspension was then administered intravenously to the same rabbit (autotransfusion) or into other rabbits (heterotransfusion). The cell free peritoneal fluid was also administered intravenously. The cells obtained by this method exhibited apparently normal ameboid movement, were actively phagocytic for *staphylococcus albus*, and took up vital stains. About 90 per cent

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1 Mudd, S., Lucké, B., McCutcheon, M., and Strumia, M., *J. Exp. Med.*, 1929, **49**, 779.