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Effect of Ascorbic Acid on Diabetogenic Action of Alloxan.

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Since it is known that alloxan will react with many substances, and because preliminary unpublished work in this laboratory suggested that ascorbic acid potentiated the action of alloxan, the effect of ascorbic acid on the diabetogenic action of alloxan was investigated.

Experimental. Hooded rats weighing approximately 250 g were used in this study. These animals were fed Purina Dog Chow during the course of the investigation. The animals were fasted 24 hours before the experiment in order to obtain blood samples.

Ascorbic acid was administered intravenously. It consisted of a solution of the free acid made up in distilled water so that 1 cc of solution contained 100 mg of the acid. Most of the animals which received the ascorbic acid, were given a 50 mg dose, while a few received 100 mg. *d*-Isoascorbic acid,* which was used as a control, was given at the same level as ascorbic acid. In all cases the intravenous injections were made in the tail of the rat. Following the immersion of the tail in warm water to dilate the veins, injection was easily accomplished using a 26-gauge needle.

Alloxan was administered intraperitoneally as a 5% aqueous solution in dosage of 100 mg per kg body weight. Previous trials had shown that this amount of alloxan is just below the level necessary to produce diabetes in the test animals.

Mapharsen (3-amino-4-hydroxyphenyl arsinoxide hydrochloride) was administered intravenously to a few rats at a dose level of 2 mg per rat.

* The *d*-isoascorbic acid used in these experiments was supplied, through the courtesy of the Charles Pfizer and Company, Brooklyn, N.Y.

TABLE I.
Effects of Various Substances on Production of Alloxan Diabetes. 100 mg of Alloxan per kg Given Intraperitoneally in All Cases. Other Substances Given Intravenously 1 Minute Before the Alloxan.

Treatment	No. animals	No. diabetic	% diabetic
Alloxan	29	5	17.2
Alloxan and 50 mg ascorbic acid	19	9	47.2
Alloxan and 100 mg ascorbic acid	8	4	50
Alloxan and 50 mg *d*-isoascorbic acid	10	1	10
Alloxan and 2 mg mapharsen	5	0	0
Mixture of alloxan and 50 mg ascorbic acid (intraperitoneally)	5	0	0

The general plan of study was to fast the animals 24 hours, obtain blood samples for sugar analysis; one group was then given ascorbic acid intravenously and at a definite time after the intravenous injection, alloxan was administered. A control group received alloxan without having any preliminary treatment. To a third group mapharsen was given intravenously one minute before the alloxan was administered. Blood sugar estimations were made ort samples obtained from the tails of the animals by the micro Folin-Malmros method[1] as adapted for the photoelectric colorimeter. Three days later the animals were again fasted and blood sugar estimations were made. At this time urine samples were obtained and qualitative sugar and acetone determinations were carried out. The criterion of diabetes used in this work was a fasting blood sugar of 140 mg % or greater, after the alloxan treatment, while the control value was 112 mg % or less.

Results. In preliminary unpublished work 50 mg of ascorbic acid were given 10 to 15 minutes before the alloxan. The doses of alloxan ranged from 50 to 150 mg per kg of body weight. Ascorbic acid under these experimental conditions produced no effect, that is, the lower doses of alloxan produced no diabetes while the higher doses produced severe diabetes equally well in the group that received ascorbic acid and the group that did not. In addition, this work has also shown that ascorbic acid alone does not produce hyperglycemia.

In succeeding work, 50 mg of ascorbic acid were given one minute before the alloxan. This level of alloxan produced diabetes only infrequently in the animals when no previous treatment with ascorbic acid had occurred. Table I is a summary of the work. In these studies, alloxan alone produced diabetes in 17.2% of the animals. On the other hand, if the animals had previously received ascorbic acid the incidence of diabetes rose to 47.3%. *d*-Isoascorbic acid was used as a control and when given under conditions equivalent to ascorbic acid, diabetes was produced in only 10% of the animals. Thus, it appears that ascorbic acid under the conditions used potentiated the diabetogenic effect of alloxan. Though a few animals were used in the study it is of interest that when 100 mg of ascorbic acid was administered, the incidence of diabetes produced by alloxan increased. This high dose of ascorbic acid must be administered cautiously or death ensues.

In order to determine whether ascorbic acid forms a complex with alloxan which is more diabetogenic than alloxan alone, a few animals received intraperitoneally a mixture of alloxan and ascorbic acid. The mixture consisted of 50 mg of ascorbic acid plus an amount of alloxan equivalent to 100 mg per kg of body weight. None of the 5 animals tested showed hyperglycemia 3 days after this combined treatment.

Discussion. There appear to be 2 possible explanations for the action of ascorbic acid on diabetogenic action of alloxan. One possibility is that ascorbic acid acts as an antioxidant and as such prevents the destruction of alloxan. The fact that alloxan is resistant to the action of strong oxidizing agents, particularly in an acid media, makes this ex-

1 Folin, O., and Malmros, H., *J. Biol. Chem.*, 1929, 83, 115.

planation unlikely. Also Yourga, Esselen and Fellers[2] have reported that d-isoascorbic acid may act as an antioxidant even for ascorbic acid, yet *d*-isoascorbic acid does not cause any potentiation of alloxan activity when administered just before the alloxan. Another possible explanation is that ascorbic acid decreases the available SH groups in the blood and thus permits more of the alloxan to reach the pancreas. Lazarow[3] has shown that administration of compounds containing the sulfhydryl group, as cysteine, reduced glutathione, and thioglycolic acid, prevent the diabetogenic action of alloxan. Prunty and Vass[4] have shown that feeding human subjects large doses of ascorbic acid decreases the amount of reduced glutathione in the red blood cells. Sulfhydryl groups are known to reduce alloxan to dialuric acid, a substance which has been reported not to possess diabetogenic action.[5]

An attempt was made in one series of animals to bind the SH groups by injecting the animals with mapharsen one minute before they received the alloxan. The animals appeared listless after the injections and almost on the verge of coma. When they moved they dragged their bodies around the cage, rubbing their bellies on the floor of the cage. This effect lasted about 3 hours. In no case did hyperglycemia develop in these animals. The dose of mapharsen given was low, but the MLD_{50} for this substance is only 16 mg per kg of body weight.

It appears most probable from these studies that the potentiation of the diabetogenic action of alloxan by ascorbic acid is due to the oxidation of SH groups in the animal and as such allows more alloxan to reach the pancreas.

Summary. Administration of ascorbic acid to rats which are given low doses of alloxan increases the diabetogenic action of the alloxan. To obtain this effect the alloxan must be given within a minute after the administration of the ascorbic acid. rf-Isoascorbic acid, on the other hand, does not produce the potentiation of the alloxan diabetes.

2 Yourga, F. ,T., Esselen, W. B., and Fellers, C. B., *Ford Research,* 1944, 2, 188.

3 Lazarow, A., PROC. Soc. EXP. BIOL. AND MED., 1946, 61, 441.

4 Prunty, F. T. G., and Vass, C. C. N., *Biochem. J.,* 1943, **37,** 506.

5 Goldner, M. G., and Gomori, G., *Endocrinology,* 1944, 35, 241.

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