Effect of Dehydroascorbic Acid in Rabbits. (20287)

SACHCHIDANANDA BANERJEE, BHAVANI BELAVADY, AND ACHINTYA KUMAR MUKHERJEE.

From the Department of Physiology, Presidency College, Calcutta, India.

Patterson(1) reported that intravenous injection of dehydroascorbic acid, dehydroisoascorbic acid and dehydroglucoascorbic acid in rats (70-100 mg/100 g) led to hyperglycemia and glycosuria like alloxan. Like alloxan, the diabetogenic action of these substances could be prevented by a prior injection of either cysteine or glutathione(2). Patterson (3) also observed cataract in rats a few weeks after the injection of dehydroascorbic acid and found a linear relationship between the appearance of mature cataract and the blood sugar level of the animal. Profuse lachrymation and salivation were noticed just after the injection of dehydroascorbic acid of which the latter could be prevented by the destruction of the chorda tympani nerve and both by the injection of atropine. The toxic action of dehydroascorbic acid was much reduced by artificial respiration(4). Merlini, however, could not produce diabetes in rats by intraperitoneal injection of dehydroascorbic acid although he could observe lachrymation and salivation (5,6). Scurvy in guinea pigs where there is hypofunction of the islands of Langerhans as evidenced by diminished insulin content(7,8) and degranulation of the beta cells(9) is associated with the presence of dehydroascorbic acid and a decrease in the glutathione content of the tissues, whereas there is no dehydroascorbic acid in the tissues of normal guinea pigs(10). The present investigation was undertaken to study the effect of intravenous administration of dehydroascorbic acid in rabbits.

Experimental. Healthy Himalayan rabbits of weights varying between 1100 and 2000 g were used. Dehydroascorbic acid was prepared according to the directions of Patterson(1) and injected into the marginal ear vein of rabbits (1 g/kg) in 2 doses at intervals of 10 minutes, the initial dose was 250 mg. Repeated intravenous injections were also given in some animals. Immediately after the injection severe salivation and lachrymation were observed. Some of the rabbits died after the first injection while others survived after they were given artificial respiration. Sugar and glutathione values of blood were estimated in samples of blood taken at various intervals after injection of dehydroascorbic acid. Animals which survived the first injection were placed in metabolism cages for the collection of urine under toluene. Blood sugar was estimated by the method of Hagedorn and Jensen(11) and blood glutathione by the method of Woodward and Fry(12). Urinary sugar was estimated by the Benedict reagent. The results are given in Tables I and II. Six rabbits were given an intravenous injection of 0.25 g dehydroascorbic acid, followed after 10 minutes by another injection of the drug (1.25 g/kg). Only 2 of the rabbits survived the second injection and in these rabbits oral glucose tolerance test was performed after feeding a 50% solution of glucose (1 g/kg) on different days after the injection of dehydroascorbic acid (Table III). Blood sugar values of rabbits increased after injection of dehydroascorbic acid. To find out whether this rise was due to secretion of epinephrine, the effect of injection of dihydroergotamine, which inhibits the epinephrine-induced hyperglycemia(13), prior to the injections of epinephrine and dehydroascorbic acid in the rabbit was

TABLE I. Effect of Intravenous Injection of Dehydroascorbic Acid (1 g/kg) on Blood Sugar and Blood Glutathione Values of Rabbits (mg/100 cc Blood).

Wt of rabbit, g		Before inj.	Hr after inj. 1	2	3	24
1600	Blood S*	129	201	221	221	121
	Blood G	42	24	38	31	21
1300	Blood S	98	139	139	98	129
	Blood G	39	22	34	—	36
1500	Blood S	127	197	157	127	121
	Blood G	43	30	29	29	30
1350	Blood S	110	178	157	146	115
	Blood G	29	24	21	15	22

* Blood S = Blood sugar; Blood G = Blood glutathione.

TABLE II. Effect of Two Intravenous Injections of Dehydroascorbic Acid (1 g/kg) Repeated after 24 Hr on Blood Sugar and Blood Glutathione Values of Rabbits (mg/100 cc Blood).

Wt of rabbit, g	Before inj.	24 hr after 1st inj.	24 hr after 2nd inj.
	Blood sugar		
2000	119	112	139
1100	96	128	107
1600	129	121	132
1300	98	129	140
1500	127	121	127
1350	110	115	Dead
1300	110	—	129
	Blood glutathione		
2000	33	31	31
1100	40	34	30
1600	43	21	37
1300	39	36	32
1500	43	30	—
1350	29	28	Dead
1300	37	34	32

TABLE III. Oral Glucose Tolerance Test (1 g Glucose/kg) in Rabbits Injected with Dehydroascorbic Acid (1.5 g/kg).

Days after inj. when test done	Initial blood sugar (mg %)	Blood sugar (mg %) Hr after feeding glucose ½	1	1½	2½
1	107	160	171	171	175
3	107	171	192	181	128
7	128	164	164	171	130
1	133	233	—	270	216
3	123	203	266	308	282
7	131	200	223	212	178
12	135	189	178	178	132

studied. 0.2 mg of dihydroergotamine methanesulfonate* was injected in a rabbit and the blood sugar values at different hours after the injection were estimated. In another rabbit 20 μg of epinephrine was injected intravenously and blood sugar was estimated in different samples of blood. In 2 rabbits, half an hour after the injection of 0.2 mg dihydroergotamine, 10 μg of epinephrine was injected and the same dose of epinephrine was repeated after 45 minutes of the injection of the first dose of epinephrine. Blood sugar values were estimated at different hours after the injection. In another rabbit, half an hour before the intravenous injection of dehydroascorbic acid (1 g/kg), 0.2 mg of dihydroergotamine was injected and the blood sugar changes were recorded. The results are given in Table IV. Rabbits in which the injection of dehydroascorbic acid (1 g/kg) were repeated daily for 3 days died after the third injection. Tissues of these animals were studied histologically. Tissues were fixed in Zenker-formol solution and paraffin sections were cut and stained with azan and Heidenhain's iron-hematoxylin stains.

Results. After the injection of dehydroascorbic acid there was a rise in the blood sugar level for a few hours and the values returned to normal within 24 hours. Along with the rise in the blood sugar level there was a fall in the glutathione values of blood which also became normal after 24 hours. Urine of the animals contained albumin but no sugar was present. Repeated injections of dehydroascorbic acid for 3 days produced death of all animals. Glucose tolerance test carried out in 2 of the rabbits which survived the injection of dehydroascorbic acid (1.5 g/kg) did not show persistent diabetic type of glucose tolerance curve. The rise in the blood sugar level after the injection of dehydroascorbic acid could be prevented by a prior injection of adrenergic blocking drug—dihydroergotamine methanesulfonate. Histological studies showed changes in the kidney and testis and no changes were observed in the pancreas, suprarenal, pituitary and liver. In the kidney hyaline degeneration of some of the glomerulus and proximate tubules were observed. The glomerular tuft was atrophied and the space between the glomerulus and the Bowman's capsule increased. No change in the collecting tubules was observed. In the testis peripheral degeneration of the interstitial cells was observed.

Discussion. After the injection of dehydroascorbic acid there was profuse salivation, lachrymation, dilatation of the pupil and respiratory failure which indicated the stimulation of both the sympathetic and parasympathetic nervous systems. The initial rise in the blood sugar level seemed to be due to the secretion of epinephrine because this rise could be prevented by the adrenergic blocking drug —dihydroergotamine. The slight reduction of the blood glutathione level might be due to the

* Kindly supplied by Sandoz Ltd., Basle, Switzerland.

TABLE IV. Effect of Intravenous Injection of Different Substances on Blood Sugar of Rabbits.

Wt, g	Treatment	Blood sugar (mg/100 cc blood) Hr after inj. of substances 0	½	1	1½	2	3
1500	Inj. with 0.2 mg dihydroergotamine	124	—	115	—	94	99
1500	Inj. with 20 μg epinephrine	135	175	221	240	—	—
1500	Inj. with 0.2 mg dihydroergotamine followed by inj. of 10 μg epinephrine ½ hr and 1¼ hr after inj. of dihydroergotamine	88	88	95	97	85	63
2000	Same as above	83	95	95	104	90	63
1100	Inj. with 0.2 mg dihydroergotamine followed ½ hr later by inj. of dehydroascorbic acid (1.1 g)	96	107	89	—	84	

oxidation of glutathione and reduction of dehydroascorbic acid to ascorbic acid in the body. Persistent hyperglycemia and glycosuria could not be observed in any one of the rabbits injected with dehydroascorbic acid. The rabbits also did not show persistent diabetic type of glucose tolerance curve. No histological changes could be observed in the pancreas. All these findings indicate that dehydroascorbic acid is not diabetogenic in rabbits.

Summary. Intravenous injections of dehydroascorbic acid in rabbits in the dose 1 g/kg or 1.5 g/kg could not produce either persistent hyperglycemia or persistent diabetic type of glucose tolerance curve. Rabbits so treated neither excreted sugar in the urine nor showed any histological changes in the pancreas, suprarenal and pituitary. All these findings indicated that unlike rats dehydroascorbic acid is not diabetogenic in rabbits.

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Unidentified Factors Capable of Reducing Stress in Iodinated Protein-Fed Rats.* (20288)

D. V. TAPPAN, R. E. BOLDT, AND C. A. ELVEHJEM.
From the Department of Biochemistry, University of Wisconsin, Madison, Wisc.

Liver preparations have been shown capable of partially relieving the stress produced in animals by a considerable number of experimental conditions. These studies have been summarized recently by Ershoff(1). The deleterious effects resulting from the administration of thyroid-active materials may be controlled under certain circumstances by yeast(2) and by several semi-purified protein materials(3,4) as well as by liver fractions when fed in conjunction with diets consisting

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