TABLE 1

PROGENY FROM MATINGS OF FEMALES HETEROZYGOUS FOR HEMOPHILIA WITH HEMOPHILIC MALES*

Litter No.	Pups in litters		Females		Males	
	Total No.	No. tested	Hemophiliacs	Non-hemophiliacs	Hemophiliacs	Non-hemophiliacs
1	15	5	2	0	2	1
2	9	8	2	1	1	4
3	11	11	1	4	2	4
4	9	7	0	3	1	3
5	7	6	4	0	1	1
6	7	4	1	1	1	1
Total	58	41	10	9	8	14

* Four dams and two sires were used in these matings. Two dams had two litters each; litters 2 and 5 were from one dam, litters 3 and 6 from another dam. Animals not tested were either stillborn or died during the first 2 days of life. In litter 1, dam suffered from dystocia, and only the first few pups were viable.

ously (*3*). All of them have suffered from many hemorrhagic episodes, particularly hemarthroses and subcutaneous hemorrhages. The joint hemorrhages have recurred frequently, and in the two oldest animals permanent joint deformities have resulted. Repeated transfusions of normal plasma, in amounts varying from 2 to 4 ml/kg body weight, have served to control the hemorrhagic manifestations, and no animal thus treated has died.

TABLE 2

CLOTTING STUDIES ON FEMALE BLEEDER DOGS*

Dog No.	Clotting time (Lee-White)	Bleeding time (mucous membrane)	Prothrombin utilized during 1st	Clotting time 15 min after transfusion
	min	min	%	min
1	70	2½	0	9
2	61	1½	2	..
3	56	2	0	7½
4	60	2	0	6
5	120	2	0	8
6	110	1½	0	7
Normal Control	5½	1¾	>90	..

* Methods used in these tests were described previously (*3*). Transfusions consisted of normal citrated dog plasma given in a dose of 3 ml/kg body weight.

Table 2 shows the results of one group of clotting studies on the female bleeder dogs. All of these animals showed prolonged clotting time, normal bleeding time, delay in prothrombin utilization in shed blood, and a normal or nearly normal clotting time following transfusions of normal plasma. These findings, along with other studies, indicate that the female bleeders differ only in sex from the hemophilic male dogs previously described (*3*). Like human hemophiliacs (*1*), they appear to be deficient in a plasma factor required for platelet utilization and mobilization of thromboplastin.

As far as can be determined, these animals are the first cases of true hemophilia in the female. That the genotype X_hX_h is not a lethal combination, at least in dogs, is of considerable interest. Indeed, the close approximation of the observed incidence to the expected incidence of this genotype suggests that there is no tendency toward prenatal lethality. Our findings suggest that the lack of female hemophilia in humans is due to the paucity of matings between female heterozygotes and hemophilic males.

References

1. BRINKHOUS, K. M. *Proc. Soc. exp. Biol. Med.*, 1947, **66**, 117.
2. FIELD, R. A., RICKARD, C. G., and HUTT, F. B. *Cornell Vet.*, 1946, **36**, 285.
3. GRAHAM, J. B. *et al.* *J. exp. Med.*, 1949, **90**, 97.

Diabetogenic Effect of Dehydroglucoascorbic Acid[1]

John W. Patterson

Department of Anatomy, School of Medicine, Western Reserve University, Cleveland

Dehydroascorbic and dehydroisoascorbic acids have been shown to produce diabetes (*7*, *8*). They are believed to be the only substances of known chemical structure, other than compounds related to alloxan, that will produce lasting diabetes after administration of a few doses. It seemed important to investigate the possible diabetogenic action of related compounds. Therefore, diketogulonic and dehydroglucoascorbic acids were selected for study in rats.

Diketogulonic acid was obtained by permitting dehydroascorbic acid, prepared by the oxidation of L-ascorbic acid with quinone (*8*), to mutarotate at room temperature for 14 days (*10*). Just before injection into the rat, the acid was neutralized to pH 6.7 with 2*N* NaOH. Neutralization required one equivalent of alkali. The product is stable at this pH (*2*). Dehydroglucoascorbic acid was prepared by oxidation of D-glucoascorbic acid[2] with quinone as previously described (*8*).

Six male rats of the Sprague-Dawley strain, ranging in weight between 98 and 130 g, were given intravenously 17.7 millimoles of diketogulonic acid per kg following 48 hr of starvation. The rats showed no hyperactivity, lacrimation, or increased salivation after the injection. Blood sugars were determined on six occasions during the 2 weeks following injection, and there was no hyperglycemia in any of the animals. The rats gained weight normally during this period.

Another series of male rats of the Sprague-Dawley strain were injected intravenously with dehydroglucoascorbic acid following 48 hr of starvation. After the injection there was no hyperactivity, lacrimation, or increased salivation. With a dose of 8.5 millimoles per kg,

[1] Aided in part by a grant from The Permanent Science Fund of the American Academy of Arts and Sciences.

[2] Samples were generously supplied by Dr. H. G. Luther of Chas. Pfizer & Co.; Dr. P. P. Gray of Wallerstein Laboratories; and Dr. A. Welch of the Pharmacology Department, Western Reserve University.

TABLE 1

DIABETOGENIC ACTION OF INTRAVENOUS INJECTIONS OF DEHYDROGLUCOASCORBIC ACID IN MALE RATS

No. of rats	Wt (in g)		Dehydro-gluco-ascorbic acid milli-moles/kg	Avg blood sugar in mg/100 ml blood							
	range	avg		Before injection	After injection						
					2–3 days	3–4 days	1 week	2 weeks	3 weeks	4 weeks	5 weeks
6	110–130	122	8.5*	122	107	114	126	124	—	—	—
5	119–132	123	11.4*	121	119	160	122	121	—	—	—
3	116–126	120	17.0	55	386	219	112	113	—	—	—
1	—	111	17.0	57	430	450	350	358	450	450	395
4	102–128	114	17.9	89	346	217	162	117	—	—	—
2	95–120	108	18.5	98	335	269	291	115	—	—	—
1	—	108	18.5	80	540	450	450	525	900	900	540
1	—	120	18.5	82	450	665	385	340	282	370	352
1	—	128	18.5	99	450	315	410	437	300	437	415
1	—	102	19.3	91	330	146	113	118	—	—	—
1	—	113	19.3	118	463	450	480	425	800	640	565

* Starved 24 hr; all others starved 48 hr before injection.

no hyperglycemia was noted. With a dose of 11.4 millimoles per kg, a slight transient hypergylcemia resulted. With doses between 17.0 and 19.3 millimoles per kg, five of the rats developed a hyperglycemia that persisted for a minimum of 5 weeks. Other rats developed transient hyperglycemias. Doses greater than 20.0 millimoles per kg were fatal. Blood sugars were determined by a micromethod (*1*). The results are summarized in Table 1.

The product resulting from mutarotation of dehydroascorbic acid is thought to be diketogulonic acid (*2, 9, 11*), which results from the opening of the lactone ring. This substance is stable in acid solutions and in slightly alkaline solutions (*2*). In the concentration used in this experiment, which is over twice the diabetogenic dose of dehydroisoascorbic acid, diketogulonic acid did not have any diabetogenic properties. The fact that the ring form, dehydroascorbic acid, has about the same diabetogenic potency as dehydroisoascorbic acid (*8*) would suggest that a ring structure is essential for the production of diabetes. This is in keeping with the finding that derivatives of mesoxalic acid, which is a constituent of the alloxan ring, are also inactive (*3, 4, 6*).

The dehydro derivative of D-glucoascorbic acid differs from the corresponding derivatives of L-ascorbic and D-isoascorbic acids in that the configuration of the asymmetric carbon involved in ring formation is of the opposite type, and in that there is an extra carbon on the side chain. It is also different in that it has no physiological activity in scorbutic animals (*12*). Its ability to produce what appears to be permanent diabetes in rats indicates that the configuration of the asymmetric carbon which is involved in ring formation is not a determining factor for diabetogenic action. The fact that it is necessary to use over twice as much of the glucoascorbic acid derivative as was necessary with the derivative of isoascorbic acid (*8*) may not be related to the relative action of these compounds at the site of diabetes production, but may be only a reflection of some factor such as permeability. It is known that glucoascorbic acid is not concentrated in the fluid of the anterior chamber of the eye, whereas ascorbic and isoascorbic acid are concentrated there equally well (*5*). If this reasoning may be extended to the dehydro forms of these compounds, it would be in keeping with the idea that permeability may be the factor responsible for the larger dose requirement of dehydroglucoascorbic acid.

Since diabetes can be produced from the dehydro derivatives of L-ascorbic, D-isoascorbic, and D-glucoascorbic acids but not from diketogulonic acid, it is evident that the ring is important in the production of diabetes. By analogy with alloxan, the three adjacent carbonyl groups are also important. The configurations of the asymmetric carbons do not appear to be determining factors in the diabetogenic action of these compounds. It is hoped that it will be possible to test some substance such as the dehydro form of reductic acid to determine whether the hydroxyl groups may be of importance in the production of diabetes.

The lack of hyperactivity, lacrimation, and increased salivation following the injection of diketogulonic and dehydroglucoascorbic acids, neither of which has any antiscorbutic activity, again suggests that the finding of these symptoms following the injection of dehydroascorbic and dehydroisoascorbic acids is a reflection of a possible biochemical function of ascorbic acid (*8*).

References

1. FOLIN, O. and MALMROS, H. *J. biol. Chem.*, 1929, **83**, 115.
2. HERBERT, R. W. *et al.* *J. chem. Soc.*, 1933, 1270.
3. HIDY, H. P. *J. biol. Chem.*, 1946, **163**, 307.
4. JACOBS, H. R. *Proc. Soc. exp. Biol. Med.*, 1937, **37**, 407.
5. KINSEY, V. E. *Amer. J. Ophthal.*, 1947, **30**, 1263.
6. LAZAROW, A. and LEVEY, S. *Biol. Bull.*, 1946, **91**, 216.
7. PATTERSON, J. W. *Endrocrinology*, 1949, **45**, 344.
8. ———. *J. biol. Chem.*, 1950, **183**, 81.
9. PENNEY, J. R. and ZILVA, S. S. *Biochem. J.*, 1945, **39**, 1.
10. *Ibid.*, 1943, **37**, 39.
11. *Ibid.*, 403.
12. ZILVA, S. S. *Biochem. J.*, 1935, **29**, 1612.