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On the Rôle of Oxalic Acid in Blood Clotting.

R. H. K. FOSTER. (Introduced by E. Chargaff.)

From the Pharmacology Laboratory, Hoffmann-La Roche, Inc., Nutley, N. J.

Oxalic acid has always been considered an anticoagulant but recently Steinberg and Brown[1] and Schumann[2] reported that *small* doses administered intravenously hasten coagulation. Oxalic acid is a constituent of normal human blood ranging from 2.75 to 4 mg %[3-6] and somewhat higher in rabbit and beef blood. Steinberg and Brown[1] and Steinberg[7] stated that oxalic acid and plant extracts containing oxalic acid were found effective in increasing the coagulability of normal blood and also of blood in hemophilia, purpura, obstructive jaundice, vitamin K deficiency, and in prolonged post-surgical bleeding from other causes. (Reference 7 is a popular article by Hannah Lees describing Steinberg's work and so far is the most extensive source of information.) No data were presented by Steinberg concerning the action in vitamin K-deficient animals. Having available vitamin K-free chicks we decided to make a few tests. The effect in normal rabbits was also studied.

Steinberg stated that a coagulant "unit" was the amount of plant extract causing in a 5 lb rabbit 15 minutes after injection a reduction in the clotting time of 50%. He did not give the dosage of oxalic acid for rabbits, but mentioned the human dosage to be 3 mg.[7] A corresponding dosage in proportion to body weight, with a considerable range on either side, was used in rabbits and chicks.

Coagulation times were determined by a modified Howell method. Blood was drawn into an oiled syringe and transferred to a vaccine tube which was immediately stoppered with a paraffined cork and placed in a water bath. The tube was tilted every half minute until clotting occurred. From chicks 0.2 cc of blood was drawn from the brachial wing vein and from rabbits 1.0 cc by heart puncture.

It is well known that after repeated veni- or heart-punctures increased coagulability may sometimes be observed. Controls were run to rule out this factor and the data are listed in Tables I and II.

1 Steinberg, A., and Brown, W. R., *Am. J. Physiol.*, 1939, **126**, 638.

2 Schumann, E. A., *Am. J. Obst. and Gyn.*, 1939, **38**, 1002.

3 Magerl, J. F., and Rittmann. R., *Klin. Wochschr.*, 1938, **17**, 1078.

4 Merz, K. W., and Maugeri, S., *Z. Physiol. Chem.*, 1931, **201**, 31.

5 Suzuki, S., *Jap. J. Med. Sci., II Biochem.*, 1934, **2**, 291.

6 Kamiya, S., *Jap. J. Med. Sci. II Biochem.*, 1937, **3**, 163.

7 Lees, Hannah, *Collier's*, 1939, Sept. 23, p. 48.

TABLE I.
Clotting Tests on Rabbits.

Animal No.	Dose per kg	Clotting times: Before	Clotting times: 15 min after
303	Control—no injection	2+	3+
306	" " "	3	3+
317	" " "	3	2+
318	" " "	2+	3
319	" " "	3	2+
304	Saline—½ cc	3	3+
307	" ¼ "	2+	4+
325	Oxalic acid— 10 γ	2+	2+
326	" " 20 "	3	2+
328	" " 40 "	3	4+
322	" " 60 "	2	2+
261	" " 80 "	4	2+
280	" " 90 "	3	3+
314	" " 125 "	2+	2+
270	" " 175 "	3	3
315	" " 250 "	3	2+
304	" " 450 "	3	3+
309	" " 600 "	3	> 5 hours

+ indicates half minute.

TABLE II.
Clotting Tests on Chicks.

Group No.	Condition of chicks	Dose γ/chick	N	Clotting times—min
1-3	Normal	Saline 0.02 cc	9	2+, 2+, 3+, 3+, 5+, 4+, 4+, 7, 7
2-4	"	Oxalic acid 2γ	9	1+, 2, 2+, 2+, 4, 5+, 5+, 7, 7+
5-19-22	K-free	Control	12	88, 106, 10 >120
6	"	Venipuncture	4	All >120
7-20-23	"	Saline 0.05 cc	13	31 12 > 120
8	"	Oxalic acid 1 γ	3	All >30
9-10-11	"	" " 2 "	5	50, 92, 105, >30, >180
12	"	" " 3 "	3	All >30
24	"	" " 3.5 "	5	All >30
13-14-15-21	"	" " 5 "	11	18, 25, 99, 8 >120
16-17	"	" " 10 "	4	41, 46, 2 >120
18	"	" " 25 "	2	50, 73

A dose of oxalic acid of 40-50 γ/kg corresponds to a 3 mg human dose. As seen in Table I this dose was without effect in normal rabbits and the only significant effect obtained was with a dose of 600 γ/kg, which caused a great prolongation of the clotting time. Data on 8 other rabbits are omitted from the table to save space. The results were no different. Since the normal value of oxalic acid in rabbits is 6-9 mg %[4] a 600 γ dose would correspond to an increase in the oxalic acid content of the blood of about 15%. The dose

recommended (40-50 γ/kg) as causing a shortening of the clotting time amounts to scarcely a 1% increase in the oxalic acid concentration. It hardly seems logical that so small an increase could affect the clotting time in either direction unless there was *no free oxalate* in the blood to start with. But whether the normal oxalate is combined or free, or whatever may be its function in blood, these data do not support the view that it possesses any *coagulant* effect whatsoever in normal blood.

Table II gives tests on normal and vitamin K-free chicks (Almquist[8] diet). The chicks were 2 weeks old at the time of testing and most of them showed the characteristic hemorrhages caused by prothrombin deficiency resulting from an inadequate vitamin K supply. Doses of oxalic acid varying from 1 γ to 25 γ per chick were used and in no case was the clotting time reduced to normal. A dose of 3.5 γ per chick corresponds to the human dose given by Steinberg. Two chicks showed clotting times of less than 30 minutes, but this is not significant since controls occasionally showed similar results. Although one of the saline controls clotted at 31 minutes, the majority showed no clotting within 2 or 3 hours. Administration of vitamin K returned the clotting time to normal (not included in table). In normal chicks a dose of 2 γ per chick had no effect. The chicks used in the entire series ranged in weight from 50 to 145 g with an average of 98 g for 83 chicks.

Rabbit blood made deficient in coagulability by administering near threshold doses of heparin intravenously was unaffected by small intravenous doses of oxalic acid. With the doses of heparin used the clotting time returned to normal in about one hour. Heparin is generally considered as antiprothrombin and the heparinized animal is therefore comparable to the vitamin K-deficient chick, prothrombin being absent (or greatly diminished) in the latter and immobilized in the former. Only a few tests were run, and while the data were limited, there was no indication of any effect in the doses used nor evidence warranting more extended trials.

The literature describes oxalic acid as being a normal constituent of blood and other body tissues. The concentration changes in various conditions but there is no evident parallelism to changes in coagulation. During narcosis it increases in bile and in the urine.[9, 10] This increase is attributed to anoxemia and Kamiya[11] and others

[8] Almquist, H. J., *J. Biol. Chem.*, 1936, **114**, 241.

[9] Borgstroem, S., *Skand. arch. Physiol.*, 1937, **77**, 16.

[10] Borgstroem, S., *Skand. arch. Physiol.*, 1938, **79**, 1.

[11] Kamiya, S., *Jap. J. Med. Sci. II, Biochem.*, 1937, **3**, 301.

showed that anoxemia in rabbits increased the oxalic acid content of blood by as much as 60%. Kamiya[6] and Marcolongo[12] showed that it rose in high blood pressure, uremia, tuberculosis, syphilis, beri beri, neuralgia, rheumatism, cirrhosis, and in acute and chronic nephritis. Melocchi[13] observed a rise of oxalic acid in the blood during intestinal fermentation of carbohydrates. Olson also made the comment in a letter quoted by Schumann[2] that on the basis of the amount of oxalic acid normally in the blood, it would not seem logical that a small increase could have any effect on the clotting time. The experimental evidence bears out Olson's view.

Conclusions. Oxalic acid injected into animals over a wide range of dosage was found to have no effect on coagulation until a sufficiently high dose level was reached, at which point clotting was delayed. Included in this study were suitable controls, normal and heparinized rabbits and normal and vitamin K-deficient chicks.

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Direct Observations on the Circulation of Blood in Transilluminated Mammalian Spleens.*

DAVID W. MACKENZIE, JR., ALLEN O. WHIPPLE AND MARGARET P. WINTERSTEINER. (Introduced by A. M. Pappenheimer.)

From the Department of Surgery, Columbia University College of Physicians and Surgeons, New York, N. Y.

The spleens of living mice, rats, rabbits, guinea pigs and cats were transilluminated[1] and observed at several magnifications, as high as 600× (water immersion). Each type of spleen was completely delivered through a long paracostal incision and placed in a suitable celluloid chamber, on a light table, above the abdominal wall. By this means, respiratory motions were eliminated in some, and greatly reduced in all specimens. The spleen, thus supported, was totally immersed in rapidly flowing Ringer-Locke solution at 37°-38°C. Anesthesia hypodermoclyses of sodium iso-amyl ethyl barbiturate

12 Marcolongo, F., *Clin. med. ital.*, 1934, **65**, 1068.

13 Melocchi, W., *Giorn. clin. med.*, 1934, **15**, 1669.

* The cooperation of the Department of Pathology, greatly facilitated this work.

1 Knisely, M. H., *Anat. Rec.*, 1938, **71**, 503.