THE EFFECT OF OXALIC ACID

ON

BLOOD COAGULATION TIME AND GROWTH OF ANIMALS

THE EFFECT OF OXALIC ACID
ON
BLOOD COAGULATION TIME AND GROWTH OF ANIMALS

BY

J. C. HORTON

Bachelor of Science

East Central State College

Ada, Oklahoma

1933

Submitted to the Department of Chemistry

Oklahoma Agricultural and Mechanical College

from

The Laboratory of the Department of Agricultural

Chemistry Research

In Partial Fulfillment of the Requirements

For the Degree of

MASTER OF SCIENCE

1941

APPROVED BY:

W G Kelly
Chairman, Thesis Committee

O C Dermer
Member of the Thesis Committee

Otto M. Smith
Head of the Department

L. G. Nelson
Dean of the Graduate School

TABLE OF CONTENTS

ACKNOWLEDGEMENT

The author wishes to express his appreciation to Dr. V. G. Heller for his assistance and supervision, and to the Department of Agricultural Chemistry Research for the use of the laboratories and equipment.

PREFACE

Oxalic acid has always been considered an anti-coagulant, but recently it has been reported that small doses administered intravenously hasten blood clotting and that there appears to be a relationship between the oxalic acid content of the blood and the coagulation time. It has been demonstrated in several thousand clinical cases that small injections of this acid are beneficial in controlling hemorrhage. No instance of the formation of a thrombus has been observed in the treatment of bleeding from hemophilia, purpura, obstructive jaundice, vitamin K deficiency, and prolonged post-surgical bleeding.

No satisfactory explanation has been advanced as to the mechanism of the action of oxalic acid in decreasing the coagulation time. It has been suggested that this hemostatic agent would cause a disintegration of the platelets, with the liberation of thromboplastin. A moderate increase in the viscosity of the blood has been noted, as well as a decrease in the prothrombin time.

Numerous studies have been made with regard to the effect of oxalic acid or an oxalate upon various animals, and it is generally agreed that large quantities injected intravenously or administered orally are fatal, and that smaller doses given over a period of time are toxic.

INTRODUCTION

Since the action of oxalic acid in reducing clotting time is such a new field, there is a scarsity of literature dealing with this topic. In April, 1939, Steinberg and Brown (1) announced that oxalic acid administered intravenously or intramuscularly would lower the coagulation time in man and various animals. This report states that an increase in the clotting time is attended by a fall in the oxalic acid content of the blood. Jackson (2) presents two cases of bleeding ulcer that were treated successfully with this substance, after all other known methods had failed. Page, Russell, and Rosenthal (3) find this drug useful in the treatment of hemophiliacs, although some cases fail to respond. Steinberg, Segal, and Parris (4) report the use of oxalic acid in the treatment of several thousand patients with various types of hemorrhage, with a high incidence of gratifying results. Schumann (5) states this substance is valuable in the treatment of bleeding from a variety of causes and suggests there might be some connection between vitamin K and oxalic acid. According to Miller and Davies (6), oxalic acid, which is poisonous when administered orally, is non-toxic in the proper dosage intravenously. A dose of 7 ml. of 5% solution per 40 pound dog is non-toxic, with clotting time reduction noted. Foster (7) is of the opinion that such small variations in the blood oxalic acid as would be produced by the administration of small doses of this substance would not be expected to alter the clotting time greatly. He finds that

this drug injected into animals over a wide range of dosage had no effect on coagulation time until a sufficiently high level was reached, at which time clotting was delayed.

Gross (8) states that subcutaneous injections of sodium oxalate in dogs produce marked disturbances of blood salts. In whole blood, there is observed a decrease in sodium, chlorine, and calcium, while the total phosphorous and potassium are increased. Jodlbauer (9) observes a fall in plasma calcium after the subcutaneous injection of sodium oxalate in rabbits, reaching a maximum in two hours.

There is a lack of agreement among various workers in the field as to the oxalic acid content of blood. Normal human red blood cells contain slightly more oxalic acid than the serum, according to Mueller (10). The results of Suzuki (11) show that the oxalic acid in human blood plasma varies from 4 to 5 mg. per 100 ml.. Merz and Maugeri (12) determined the content of human blood to be approximately 3 mg. per cent and of rabbit blood to be between 6 and 9 mg. per cent. Barber and Gallimore (13), however, are of the opinion that the above figures are much too high, and report normal human blood contains only 0.4 to 0.6 mg. per cent. Thomsen (14) is in agreement with these smaller figures. On the other hand, Steinberg and Brown (1) state the value as being 5.5 to 7.5 mg. per cent for human blood, and 4.5 to 6.0 mg. per cent for rabbit blood.

According to Doneddu (15), a high concentration of oxalic acid in the blood may result from impaired assimilation of food, defects in the metabolism of tissue carbohy-

drates, and abnormal intestinal conditions. Marcolonga (16) states that the administration of glucose increases the serum oxalic acid in normal humans, and that adrenalin produced a like change. The results of Kamiya (17) show that the blood oxalic acid is normal in most diseases, but increase in high blood pressure, uremia, syphilis, cirrhosis of the liver, beriberi, and rheumatism. Mueller (18) reports an increase of oxalic acid during glycolysis of the blood.

Numerous writers refer to the toxic action of large doses of oxalic acid upon animals. It is usually assumed that this effect is due to the removal of calcium from the organism. Thus, Sarvonat and Roubier (19) observe that guinea pigs, treated subcutaneously with small doses of oxalic acid until death, had a marked reduction of calcium in both bones and soft parts. Giovane (20) is of the opinion that the toxicity of this acid is due to the removal of calcium from the animal. Loew (21) confirms this view, and observes that only the lowest organisms, algae and fungi, which do not require calcium, are not killed by potassium oxalate. Salant and Swanson (22) disagree, however, and find the behavior of animals on oxalates as regards tolerance and cumulation unfavorable to the theory that the effects are due to precipitation of calcium or the transfer of ionic calcium to a non-ionizing form in the cell. Rost (23) reports that oxalic acid and sodium oxalate introduced into the stomachs of dogs causes a gradual

development of local irritation and poisoning. One dog received 490 grams in 253 days before showing signs of poisoning. Hopkins and Gill (24) find that rabbits are killed by stomach doses of 2.5 grams of sodium oxalate, and a post-mortem examination reveals kidney inflammation and oxalate crystals present. Lecoq (25) states that pigeons fed on a normal diet plus two per cent oxalic acid developed polyneuritis, due to the prevention of the utilization of increased vitamin B. Oikawa (26) reports that oral doses of 30 ml. of 3 per cent sodium oxalate causes a great increase in urinary oxalic acid, but that a second dose fails to produce as large an increase, due perhaps to kidney injury. After feeding a dog large quantities of sodium oxalate, Garvonat and Roubier (27) find higher than normal concentrations of oxalic acid in the liver, lungs, kidneys, nerves, and brain, with only a moderate increase in the blood concentration. Mueller (13) states that oxalic acid, injected into rabbits, is absorbed and eliminated gradually. Bahn (28) reports that moderate quantities of this substance are almost completely burned in the organism, but that larger amounts are not so completely used up.

EXPERIMENTAL

The rats used in the experimental feeding of oxalic acid were young, healthy albinos weighing approximately 40 grams each. Following a preliminary test to determine the maximum quantity of oxalic acid that could be tolerated, two males and two females were placed together in each of five cages. The feed consisted of standard breeding ration used by the Experiment Station mixed with finely ground oxalic acid. Another cage was fed a similar diet containing sodium oxalate, and the fifth cage was fed on breeding ration alone, as a control. The rats were weighed once a week and fed and watered daily.

New Zealand white adult rabbits, weighing between 2,500 and 3,500 grams, were used in the blood clotting determinations. The blood was usually obtained by a heart puncture, although in a few instances it was drawn from a vein. Hypodermic syringes were used, and the blood allowed to flow immediately into a special 1 mm. standard bore capillary tube. It was found important to have tubes of uniform size. The clotting time was taken as the interval from the instant the blood started to flow into the capillary tube to the time of the formation of a clot. The tube was tilted back and forth until the fluid failed to move freely, and then the tube was broken at small intervals until a strand of fibrin connected the two broken ends. The first appearance of a strand was considered to be the clot.

In preparing the serum for calcium and phosphorous determinations, 5 ml. of blood was drawn from the heart with a hypodermic syringe and allowed to stand about one hour. During this period the coagulum was stirred occasionally with a glass rod to prevent a large clot from forming. The tubes containing this clotted blood were then centrifuged at 2,500 r.p.m. for approximately 30 minutes. The clear serum was drawn off with a pipette and used as described below.

METHODS

Calcium: A modification of the colorimetric method of Roe and Kahn (29) was used. Briefly, this method was as follows: One ml. of serum was run into 4% trichloroacetic acid, and centrifuged to settle the coagulate. The clear liquid was added to a solution of alkaline sodium phosphate and allowed to stand for 4 hours, after which time it was centrifuged to pack the calcium phosphate precipitate in the tip of the constricted centrifuge tube. The precipitate was drained, washed with an alcohol mixture, and drained again. The analysis was completed by comparing the depth of color produced by adding molybdic acid reagent and dilute stannous chloride solution against that produced by a standard solution treated similarly. This standard solution is prepared by making a solution of NaH_2PO_4 such that one ml. is equivalent to one mg. of calcium measured as $Ca_3(PO_4)_2$. A Genco-Sheard-Sanford photelometer was used in the color comparisons, and the readings taken as early as possible.

Inorganic Phosphorous: The method of Youngburg and Youngburg (30) was used in the determination of both inorganic and total phosphorous. One ml. of serum was added to 10% trichloroacetic acid, thoroughly mixed by rotating the tube, and centrifuged at 2,000 r.p.m. for several minutes. Two ml. of the supernatant liquid were transferred to a graduated test tube, and, since the phosphorous is already in the form of phosphate ions, the color was developed in this tube by the addition of molybdate-sulfuric acid reagent and dilute stannous chloride. A standard was used for comparison by transferring 2 ml. of standard phosphate solution (1 ml. = .02 mg. P) to a test tube and treating in a like manner.

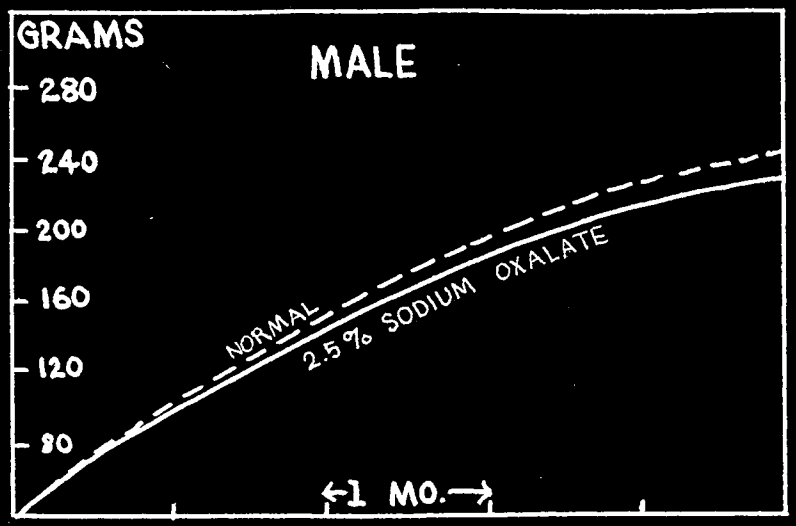
Total Phosphorous: One ml. of serum was treated with 10% trichloroacetic acid as above, and 2 ml. of the supernatant liquid were digested overnight with 0.5 ml. of 10 N sulfuric acid by placing over burning light bulbs. The oxidation of any remaining organic matter was completed by boiling the mixture and dropping in perchloric acid. The tube was then placed on a rack, allowed to cool somewhat, about 2 ml. of water were added, and the contents heated to boiling to insure conversion of any meta or pyro-phosphate to ortho-phosphate. The analysis was completed by developing the blue color as described for inorganic phosphate.

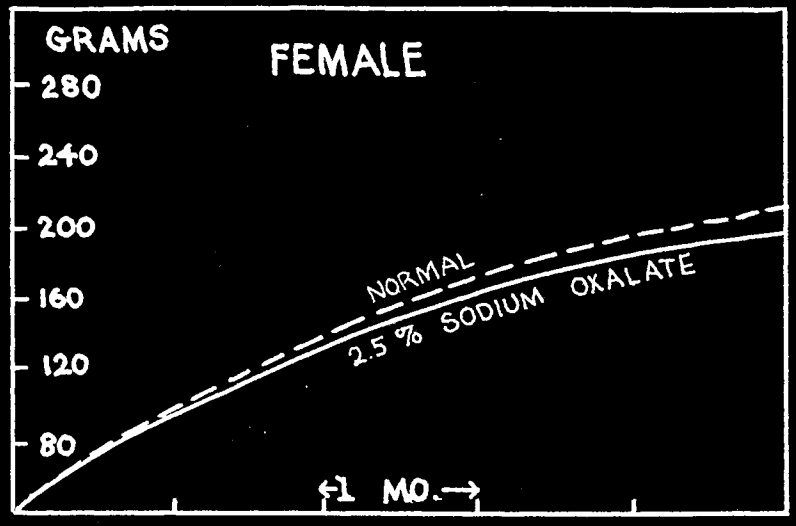
Urinary Oxalic Acid: Two healthy female rabbits from the same litter and weighing approximately the same were placed in metabolism cages which were constructed to collect all

urine.. One animal was fed standard rabbit chow and used as a control, while the other was given this same feed plus 2.5% oxalic acid. The acid was dissolved in water, sprayed on the feed, and the water allowed to evaporate. 150 grams of feed were given every day to each rabbit, and the urine collected at a certain hour each day and placed in an ice-box.. The analysis was performed on 72 hour samples, and the results divided to 3 to obtain the daily output of acid.

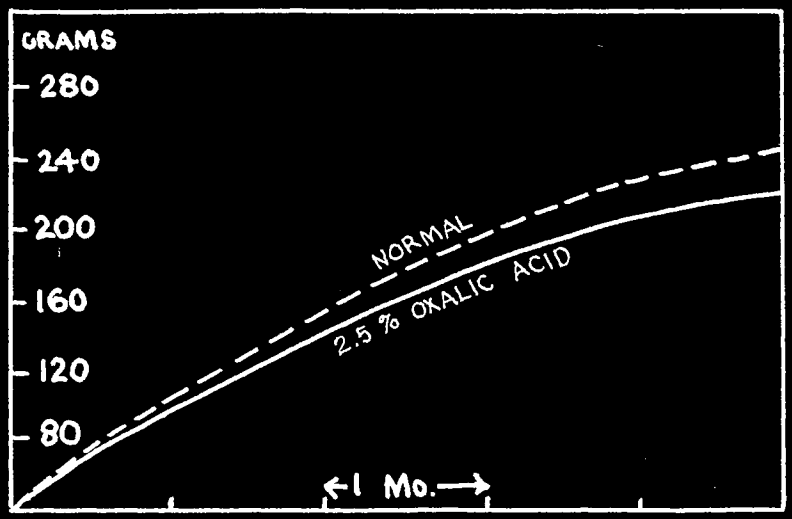
The Dakin (28) method was used in this determination. To the specimen of urine hydrochloric acid was added to make about a 5 per cent solution, and heated on a water bath to break up oxaluric acid. An excess of calcium chloride was added and the mixture made fairly strongly alkaline with ammonium hydroxide. This is allowed to stand over night, then filtered, and the precipitate transferred to a beaker and warmed with a little dilute hydrochloric acid. After filtering, the filtrate was evaporated to approximately 5 ml. and transferred to an extraction tube for continuous extraction with ether for 6 hours. 20 ml. of water were added to the ether extract and the ether distilled off. An excess of calcium chloride and ammonium hydroxide were added and then the mixture was made decidedly acid with acetic acid and allowed to stand in a warm place overnight. The precipitate was filtered on a small paper and washed with small portions of hot water. After treating with an excess of 5% sulfuric acid and warming to 60°, titration was performed with potassium permanganate.

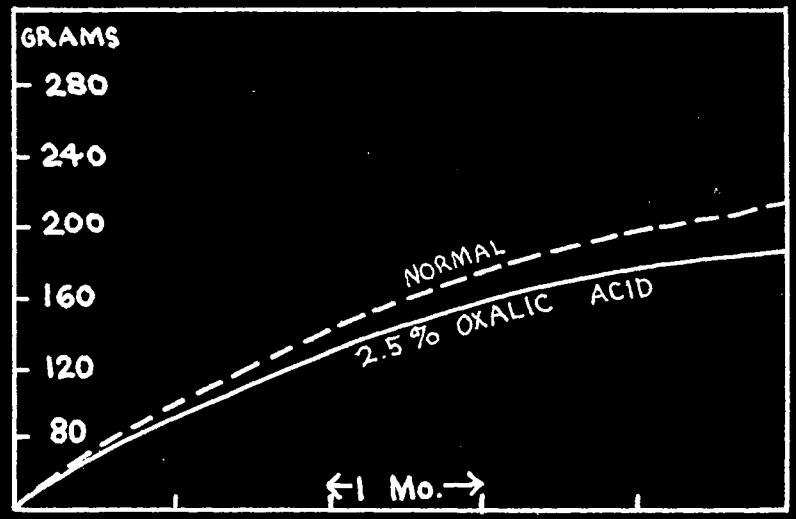
FIGURE I. Graphs showing the growth of rats on a diet containing various percentages of oxalic acid and sodium oxalate.

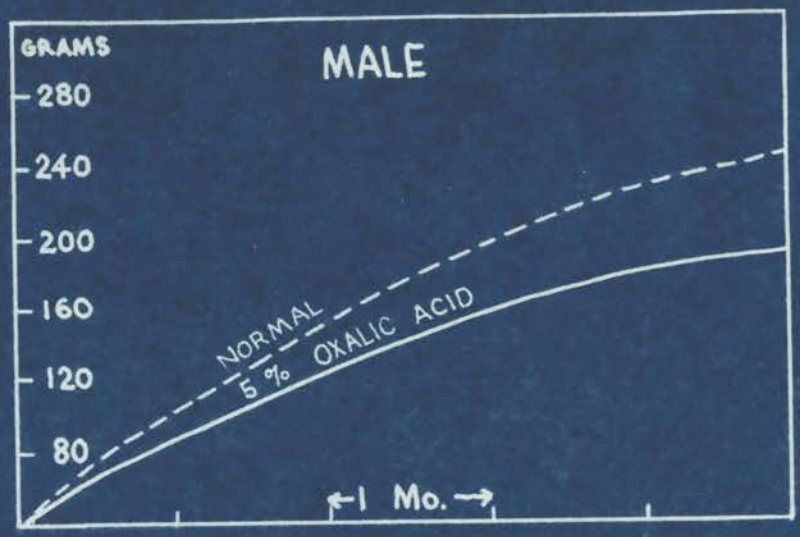


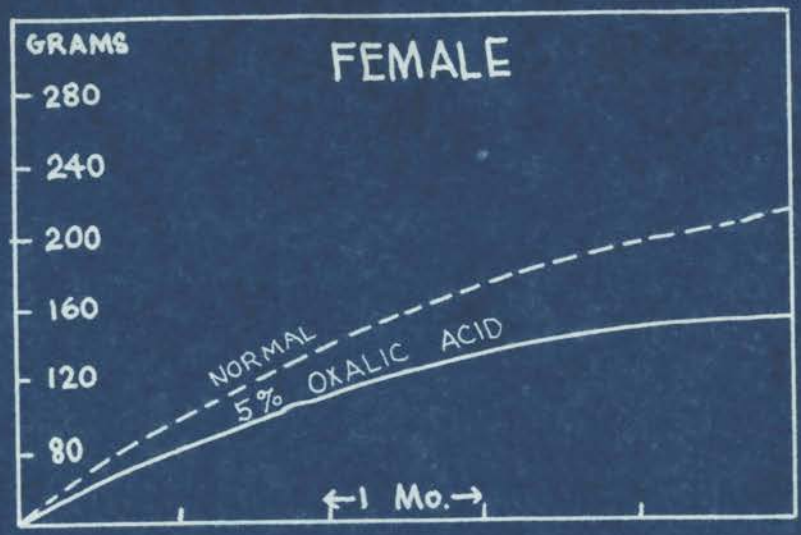


GROWTH CURVES - RATS

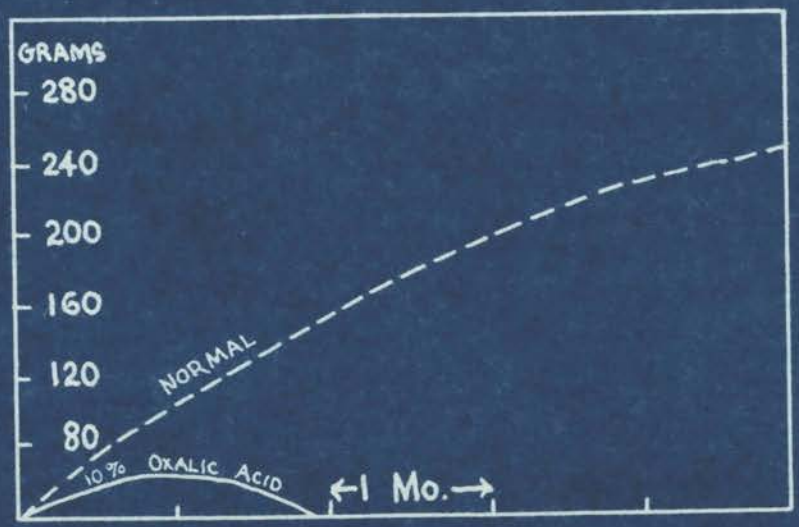








GROWTH CURVES - RATS



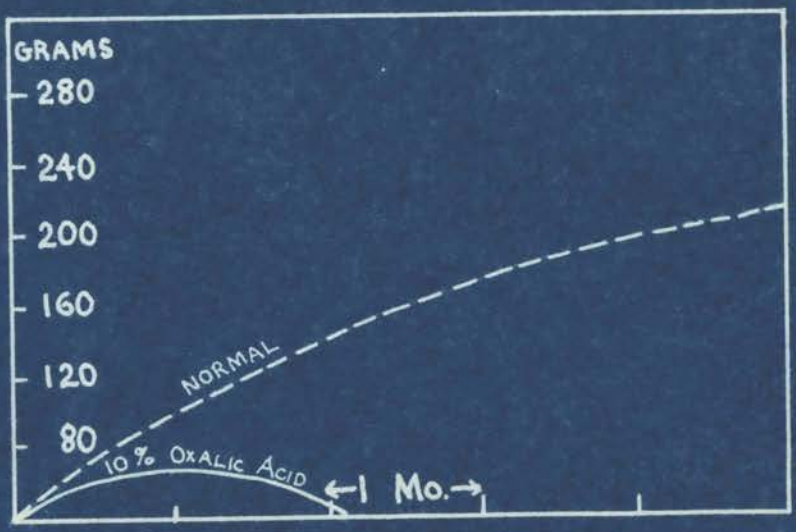


TABLE NO. I. Phosphorous and Calcium Content of Blood Serum One Hour After the Injection of 10 mg. Oxalic Acid.

Animal	Total P		Inorganic P		Total Ca	
	Control	Exper.	Control	Exper.	Control	Exper.
	mg. per 100 ml.					
Rabbit	9.74	10.10	5.26	6.07	10.83	8.92
	8.77	9.52	4.49	5.47	11.16	9.44
	8.75	7.67	4.88	4.35	10.24	10.07
	9.00	9.55	5.73	4.66	10.60	8.78
Aver.	9.09	9.21	5.09	5.14	10.71	9.30
Chicken	10.30	10.10	7.70	7.58	12.61	12.35
	10.77	10.54	6.58	5.82	13.07	12.01
	10.10	9.24	7.15	6.62	11.90	12.13
	9.86	10.24	5.95	4.88	11.50	11.26
	10.94	11.85	6.86	6.99	12.42	12.25
	10.23	10.16	5.85	5.72	13.07	13.39
	10.37	10.35	6.70	6.65	12.43	12.36

TABLE NO. II. Clotting Time After the Injection of 10 mg. Oxalic Acid Intravenously.

Animal	Normal Clotting Time	After 15 min.	After 30 min.	After 60 min.
		Seconds		
Rabbit	210	122	90	102
	165	85	93	90
	132	78	70	67
	126	66	74	50
	187	105	91	93
Average	164	91	85	80
Chicken	200	-	-	105
	160	-	-	88
	245	-	-	113

TABLE NO. III. Oxalic Acid in the Urine of Rabbits Fed A Diet Containing Added Oxalic Acid.

Control	Experimental
Standard Ration	2.5% Oxalic Acid Added
mg. per day	
4.1	72.7*
5.6	23.3
3.8	19.9
5.2	21.9

*Large volumes of urine were present the first few days.

DISCUSSION

An investigation of Figure I reveals that rats can live on a diet containing as much as 5% oxalic acid, but their growth is retarded and they are not able to reproduce. Sodium oxalate does not appear to be as toxic as the free acid, as the growth is more nearly normal and two females became pregnant and gave birth to normal young, some of which survived.

The data in Table I indicate that there is no change in the phosphorous content of the blood serum following the intravenous injection of oxalic acid, either in rabbits or chickens. No alteration in calcium is observed in chickens, but in rabbits it appears that there is a reduction of serum calcium. However, not enough determinations were made to be of statistical significance. Jodlbauer (9) states that in chickens calcium may come from the nucleated red cells, and thus prevent a large change in the serum, whereas in rabbits this could not take place.

Table II shows the reduction in clotting time occurs quickly after the injection of very small amounts of oxalic acid, and continue for one hour or longer. As a suggestion for further investigation, a bleeding tendency could be produced by administering heparin, or inducing obstructive jaundice, and the effect of injections of oxalic acid studied. Additional work may reveal a relationship between the prothrombin level of the blood and the oxalic acid content.

From Table III it is seen that the oxalic acid content of the urine is increased, but not nearly enough to account for all of the intake of this substance. The first few days the rabbit is fed with oxalic acid in the chow, there is a greatly enlarged volume of urine, gradually diminishing until smaller than normal. This might be accounted for by a break down of the kidneys due to the oxalate poisoning. Apparently the body is able to metabolize considerable amounts of oxalic acid. It would be interesting to carry this experiment further, and analyze the feed and feces as well as the urine, to determine just how much of the acid is actually absorbed from the intestine.

CONCLUSIONS

1. Small quantities of oxalic acid injected intravenously into rabbits and chickens do not alter the total phosphorous or the inorganic phosphorous of the blood serum.

2. The calcium in the serum of chickens is not affected significantly, but in rabbits there appears to be a reduction of calcium.

3. Rats are able to survive on diets containing as high as 5% oxalic acid or sodium oxalate, the latter being slightly less toxic in action.

4. A sharp reduction in clotting time occurs within 15 minutes after the intravenous injection of 10 mg. of oxalic acid. The maximum reduction usually occurs about 60 minutes after injection.

BIBLIOGRAPHY

1. Steinberg, Arthur, and Brown, William R. "A New Concept Regarding the Mechanism of Clotting and the Control of Hemorrhage." Am. J. Physiol., 126, 638 (1939).

2. Jackson, A. S. "Editorial." Jackson Clinic Bulletin, January, 1940, Madison, Wisconsin.

3. Page, R. C., Russell, H. K., and Rosenthal, R. L. "The Effect of Oxalic Acid Injected Intravenously on Blood Coagulation Time in Hemophiliacs." Ann. Internal Med., 14, 78-86 (1940).

4. Steinberg, Arthur, Segal, H. I., and Parris, H. K. "Role of Oxalic Acid and Certain Related Dicarboxylic Acids in Treatment and Control of Hemorrhage." Ann. Otol., Rhin., & Laryng., 49, 1008 (1940).

5. Schumann, E. A. "Newer Concepts of Blood Coagulation and the Control of Hemorrhage." Am. J. Obst. & Gyn., 38, 1002-1007 (1939).

6. Miller, R. T., and Davies, A. P. "Oxalic Acid as a Coagulative Agent." Vet. Med., 34, 544-545 (1939).

7. Foster, R. H. K. "Role of Oxalic Acid in Blood Clotting." Proc. Soc. Exptl. Biol. Med., 44, 136-139 (1940).

8. Gross, E. G. "Inorganic Ion Ration After Administration of Oxalates and Citrates." J. Biol. Chem., 66, 729-743 (1925).

9. Jodlbauer, A. "A Calcium Loss From the Blood in Poisoning With Oxalates and Fluorides." Arch. Exptl. Path. Pharmakol., 164, 464-468 (1932).

10. Mueller, Paul B. "Biology of Oxalic Acid." Verh. Schweiz. Physiol., 11, 8-9 (1937).

11. Suzuki, Saburo "Observations on the State of Oxalic Acid in the Blood After Injection of Sodium Oxalate." Japan. J. Med. Sci. II. Biochem., 3, 19-22 (1935).

12. Herz, R. W., and Kawgori, S. "The Occurrence and Determination of Oxalic Acid in the Blood." Z. Physiol. Chem., 201, 31-37 (1931).

13. Barber, H. H., and Gallimore, Eric J. "The Metabolism of Oxalic Acid in the Human Body." Biochem. J., 34, 144-148 (1940).

14. Thomsen, Axel, "The Oxalic Acid Content of the Blood." Z. Hoppe-Seyl., 237, 199-214 (1935).

15. Doneddu, Francesco P., "Oxalic Acid in Maternal and Fetal Blood." Arch. Farmacol. Sper., 58, 201-202 (1934).

16. Marcolongo, Fernando, "Pathological Changes Produced by Oxalic Acid." Clin. Med. Ital., 65, 1068-1112 (1934).

17. Kamiya, Shigeo, "Oxalic Acid Metabolism in Bone Diseases." Japan. J. Med. Sci. II. Biochem., 3, 163-169 (1937).

18. Meuller, P. S., "The Action of Oxalic Acid in Blood." Z. Hoppe-Seyl., 256, 75-84 (1938).

19. Sarvonat, F., and Roubler, Charles, "Decalcifying Action of Oxalic Acid Intoxication." Compt. Rend. Soc. Biol., 71, 104-105 (1909).

20. Giovane, Nicola di, "Lesions in the Blood Vessels or in the Viscera Produced by Treatment with Oxalic Acid, and Their Significance." Rif. Med., No. 47-48 (1908).

21. Loew, Oscar, "The Poisonous Action of Oxalates, and the Physiological Function of Calcium." Biochem. Z., 38, 226-243 (1912).

22. Gaiant, W., and Swenson, A. M., "Observations on the Action of Tartrates, Citrates, and Oxalates." J. Pharmacol., 11, 133-145 (1918).

23. Root, E., "Toxicology of Oxalic Acid and Oxalates." J. Pharmacol., 28, 257-267 (1926).

24. Hopkins, C. M. S., and Gill, D. A., "The Sorrel-Poisoning Theory of Lamb Mortality." New Zealand J. Agri., 36, 153-155 (1928).

25. Lecoq, R., "Oxalic Acid in the Diet of Pigeons." J. Pharm. Chim., 25, 53-62 (1937).

26. Okawa, Syosuke, "Excretion of Oxalic Acid in Urine and Feces Under Different Dietary Conditions." Japan. J. Med. Sci. II. Biochem., 4, 17-19 (1938).

27. Sarvonat, F., and Roubler, Charles, "Contents of Different Organs in Oxalic Acid After Intoxication by that Substance." Compt. Rend. Soc. Biol., 70, 450-451 (1909).

28. Dakin, H. D., "Experiments Bearing Upon the Mode of Oxidation of Simple Aliphatic Substances in the Animal organism." J. Biol. Chem., 3, 57-73 (1907).

29. Roe, Joseph H., and Kahn, Bernard S., "The Colorimetric Determination of Blood Calcium." J. Biol. Chem., 91, 1-14 (1929).

30. Youngburg, Guy E., and Youngburg, Annie V., "A System of Blood Phosphorous Analysis." J. Lab. Clin. Med., 15, 158-179 (1930).

AUTOBIOGRAPHY

I, J. C. Horton, was born in Waco, Texas, July 23, 1914. My elementary and high school education were received in the public schools of Texas and Oklahoma. In June, 1930, I enrolled at East Central State College, Ada, Oklahoma. Upon graduation from this institution in 1933, I was employed as high school science and music instructor in Texas and Oklahoma for the next six years.

In the fall of 1939, I enrolled in the Graduate School of the Oklahoma Agricultural and Mechanical College in Stillwater, Oklahoma. I have been employed for the past two years as counselor in Cordell Hall, and expect to receive the degree of Master of Science in Chemistry in June, 1941.

Typed By: J. A. Horton