AN EXPERIMENTAL STUDY OF OXALURIA, WITH SPECIAL REFERENCE TO ITS FERMENTATIVE ORIGIN.

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After the discovery of calcium oxalate crystals in the urine by Donné[1] in 1838, there followed a careful study of the symptoms associated with their deposit. This resulted in the description of a so-called oxalic-acid diathesis, which was thought by certain clinicians, notably Prout,[2] Golding Bird,[3] and Begbie,[4] to be of considerable importance and interest. Smoler,[5] however, found that the crystals were present in fifty-two per cent of four hundred specimens of urine, and Bacon, in forty-one per cent of nine hundred and nine cases, from patients suffering from almost every form of disease, and Neubauer[6] showed that calcium oxalate was almost constantly present in solution in the urine, even when not precipitated. These discoveries led to the belief that oxaluria is a symptom of many diseases, and not in itself of grave import. In 1896, Dunlop[7] published an article which supported the view that all the oxalic acid excreted in the urine had been ingested with the food, and that it was never formed in the animal organism by metabolism.

I have made the following series of observations and experiments: (1) to test the accuracy of Dunlop's conclusion; that is, to determine whether oxalic acid is ever formed in the animal body; (2) to study the influence of the ingestion of oxalic acid in foods upon the amount

[1] Donné, *Compt. rend. Acad. d. Sc.*, Paris, 1839.

[2] Prout, On the Nature and Treatment of Stomach and Urinary Diseases. London, 1840.

[3] Golding Bird, Observations on Urinary Concretions and Deposits. London, 1842.

[4] Begbie, *Monthly Journ. Med. Sc.*, Edinburgh and London, 1848–9, ix, p. 943.

[5] Smoler, *Prager Vrtjschr. f. d. prakt. Heilk.*, 1861, lxix, p. 157; lxx, p. 35.

[6] Neubauer, *Arch. d. Vereins f. gemeinsch. Arb. z. Förd. d. wiss. Heilk.*, 1860, iv, p. 1. Neubauer und Vogel, Analyse d. Harns. Wiesbaden, 1863.

[7] Dunlop, *Journ. Pathol. and Bacteriol.*, 1896, iii, p. 389.

excreted in the urine; (3) to study the physiological action of soluble oxalates with a view to deciding in what measure the presence of oxalic acid in the system is responsible for the symptoms attributed to the " oxalic-acid diathesis."

Occurrence of Oxalic Acid in Foods.—Oxalic acid and its salts are found widely distributed in nature. They are present in many of the common forms of vegetables, grains and fruits. Esbach [8] and Auerbach [9] have made estimations of the oxalic acid in the common foods. Some of those which they found to be rich in oxalic acid are spinach, rhubarb, dried figs, cocoa, tea, coffee, pepper, potatoes, beetroot, green beans, plums, tomatoes and strawberries. Foods which they found to contain little or no oxalic acid are peas, asparagus, cucumbers, mushrooms, onions, lettuce, rice, cauliflower, pears, peaches, grapes, melons, and wheat, rye, and oat flour.

In connection with the following experiments, a few foods were examined in order to select a diet free from oxalates, which should be more liberal than Dunlop's exclusively milk diet. The list of foods free from oxalic acid includes proteids (meat, milk and eggs) with sugar, butter, corn meal, rice, and the Huntley and Palmer breakfast biscuits.[10]

Characteristics of Calcium Oxalate Crystals.—When calcium oxalate is rapidly precipitated from a solution it falls in a very finely divided crystalline deposit, appearing like an amorphous sediment. These crystals contain only one molecule of water of crystallization. If allowed to stand a number of days, these crystals may group themselves in dendritic or star forms, similar to certain of the phosphatic crystals. After standing from ten days to three weeks, the characteristic octahedral crystals frequently form. These contain three molecules of water of crystallization.

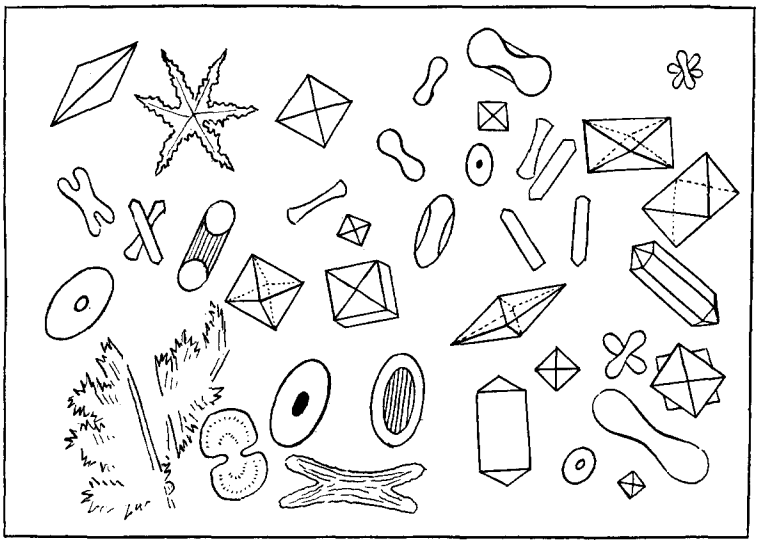
In the urine, calcium oxalate usually forms in octahedra, but it may

[8] Esbach, *Bull. gén. de thérap.*, Paris, 1883, civ, p. 385.

[9] Auerbach, Virchow's *Archiv*, 1879, lxxvii, p. 226.

[10] The food tested was cut or ground in small pieces, boiled in dilute hydrochloric acid, allowed to stand forty-eight hours, filtered and washed free from acid. The filtrate was then neutralized with ammonia, rendered very slightly acid with acetic acid, and then treated like the urine by a method described below.

be found in a variety of crystalline forms, described by Fürbringer[11] as following two types, the prismatic and spheroidal (see the accompanying figure). These crystals which are clear and colorless are insoluble in ammonia and alcohol, almost insoluble in hot and cold water (1: 500,000 (Storer) = 2 mg. per litre), slightly soluble in acetic acid (3-9 mg. in 20-60 cc. dilute acetic acid (Nickel[12]), but are readily dissolved by the strong mineral acids.[13]



Varieties of Calcium Oxalate Crystals.

Quantitative Estimation of the Calcium Oxalate in the Urine.— The quantitative estimation of the calcium oxalate found in the urine is a long and tedious process, and, unless great care is used, it is liable to large percentages of error. The method employed in these experi-

[11] Fürbringer, *Deutsch. Arch. f. klin. Med.*, 1875, xvi, p. 519.

[12] Nickel, *Ztschr. f. physiol. Chemie*, 1887, xi, p. 186.

[13] The solubility of calcium oxalate as tested in this series of experiments was found to be as follows:

1. In cold distilled water 2.2 mg. per litre.
2. In boiling water 1 mg. per litre.
3. In 2.5% acetic acid, after standing 48 hrs., 28 mg. per litre.
4. By washing with 2.5% acetic acid, 8.2 mg. per litre.

ments is founded on that of Dunlop.[14] It differs essentially from the older methods of Neubauer[15] and of Shultzen[16] in that the calcium oxalate is precipitated from an acid solution by means of alcohol, instead of from an alkaline solution by calcium chloride or calcium hydrate.

Dunlop's method (slightly modified). The urine should be thymolized as soon as passed to prevent fermentation and the precipitation of phosphates. If the specimen is alkaline, render it slightly acid with acetic acid.

To 500 cc. of a well mixed specimen of the twenty-four hours' urine, add 150 cc. of over 90% alcohol, to precipitate the calcium oxalate. Set aside for forty-eight hours. Filter, washing the beaker carefully, and removing crystals from the sides by rubbing with a rod protected by rubber tubing. Wash the sediment thoroughly with hot and cold water and with dilute acetic acid (1%). Place the filter in a small beaker and soak in a small amount of dilute hydrochloric acid. Then wash with hot water till there is no further acid reaction; filter the washings and evaporate the filtrate to about 20 cc. Add a very little calcium chloride solution to ensure an excess of calcium; neutralize the hydrochloric acid with ammonia, and then render the solution slightly acid with acetic acid. Add strong alcohol to the amount of 50% of the volume of the fluid and set aside forty-eight hours. Collect the sediment on an ash-free filter, wash with cold water and dilute acetic acid till free from chlorides. (Avoid the use of hot water, as it carries the finely divided precipitate through the pores of the filter). Incinerate first over a Bunsen burner and afterwards for five minutes in a blowpipe flame, cool over sulphuric acid, and weigh. The ash is calcium oxide, each gramme of which represents 1.6 grms. oxalic acid.

In the older methods of Neubauer and of Shultzen, the urine was rendered alkaline, and the oxalic acid precipitated by adding an excess of calcium chloride solution. In this way the phosphates, as well as calcium sulphate, are precipitated, and can with difficulty be separated from calcium oxalate; for, unless large quantities of water and acetic acid are used in washing, traces of phosphates and sulphates remain and are weighed in the ash, whereas, if enough of these is used to dissolve away the impurities, there is a weighable amount of calcium oxalate dissolved and lost. In using the method either of Neubauer, Shultzen, or Dunlop, the ash should each time be tested for phosphates and sulphates.

The following experiments (Table I) were made to test the accuracy of Dunlop's method; 500 cc. of a well mixed twenty-four hours' specimen of urine was examined for the amount of oxalic acid present and the result noted. Then to another 500 cc. of the same specimen was added a weighed amount of calcium oxalate and the mixture treated as in the first instance. When using the utmost care, the loss will be about one milligramme.

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[14] Dunlop, op. cit.

[15] Neubauer and Vogel (Huppert), Analyse d. Harns. Wiesbaden, 1898. (Fürbringer and Czapek's modification of Neubauer's method.)

[16] Shultzen, *Arch. f. Anat. u. Physiol.*, 1868, vi, p. 719.

TABLE I.

EXPERIMENTS TO TEST THE ACCURACY OF DUNLOP'S METHOD.

	Calcium oxalate added to urine.	Calcium oxalate recovered.	Calcium oxalate lost.
1.....	.0289 grm.	.0270 grm.	.0019 grm.
2.....	.0169	.0158	.0011
3.....	.0232	.0256	Trace of phosphates in ash.
4.....	.0203	.0201	.0002 grm.
5.....	.0150	.0150	.....
6.....	.0281	.0282	.....
7.....	.0202	.0208	Faint trace of phosphates.
8.....	.0281	.0280	.0001 grm.
9.....	.0453	.0455	Faint trace of phosphates.
10.....	.0289	.0270	.0019 grm.

Salkowski [17] has described a method of separating the phosphates from the oxalates by shaking out with ether. This does not seem necessary in the case of human urine when Dunlop's method is used. But in dog's urine, with high specific gravity, it is very difficult to remove the phosphates even while using Dunlop's method.

Oxalic Acid in Normal Urine.—In health the amount of oxalic acid excreted in twenty-four hours varies with the amount ingested in the food. The average is estimated by Fübringer as .02 grm. The following tables show varying amounts excreted with different diets:

TABLE II.

NORMAL URINE. DIET CHIEFLY CARBOHYDRATES. (MEAT, MILK, BREAD, POTATO, OATMEAL.)

Vol.	Sp. gr.	Oxalic acid in 24 hours.	Calcium Oxalate crystals in sediment.
1305	1017	.0058	None.
1235	1020	.0004	Few.
1615	1015	.017	Numerous.
1295	1012	.013	None.

TABLE III.

NORMAL URINE. FOOD RICH IN OXALATES. MIXED DIET WITH LARGE AMOUNTS OF TOMATO, SPINACH, TEA AND COFFEE.

Vol.	Sp. gr.	Oxalic acid in 24 hours.	Calcium Oxalate crystals in sediment.
1765	1011	.0011	Present.
1865	1017	.0012	"
1945	1015	.0006	"
1760	1016	.0028	"

[17] Salkowski, *Centralbl. f. d. med. Wiss.*, 1899, xxxvii, p. 257.

TABLE IV.

NORMAL URINE. MIXED DIET.

Diet.	Vol.	Sp. gr.	Oxalic acid in 24 hrs.	Oxalic acid per litre.	Calcium oxalate crystals in sediment.	Impurities in ash.
Ordinary mixed diet	1645	1016	.0047	.0029	None.	
"	1360	1018	.0022	.0016	"	
"	1185	1014	.0099	.0083	"	
"	1065	1019	.002	.0019	"	
"	750	1028	.0017	.0014	Numerous.	
"	865	1026	.0147	.0170	None.	Trace of phosphates.
"	730	....	.015	.021	Few.	"
" + rhubarb	480	1034	.034	.072	Large, numerous.	Trace of phosphates and sulphates.
Ordinary mixed diet	800	1022	.024	.030	Numerous.	"
"	1720	1010	.011	.006	Small.	
"	720	1022	.009	.0128	None.	
"	1115	1019	.017	.015	Numerous.	Trace of phosphates.
"	1210	1014	.0035	.0014	None.	
"	1205	1013	.0017	.0016	Few.	
"	950	1019	.0076	.008	"	
"	820	1022	.0103	.0116	Very few.	
"	950	1021	.0016	.0006	" "	
"	950	1024	.0115	.012		
"	1225	1014	.0047	.0038		
"	1250	1013	.0169	.0135		
"	1225	1013	.0031	.0026		
"	1300	1013	.0062	.0048		
"	2000	1012	.0062	.0031		
"	1700	1012	.0011	.0006		

There were also examined thirty-five specimens from patients who were receiving a mixed diet. These patients were suffering from gastric and intestinal disorders. The urinary examinations gave the same results as in the case of the healthy persons. The daily excretion of oxalic acid varied from a few milligrammes to about two centigrammes, the mean falling below ten milligrammes. It was also noted, as Fübringer had shown, that the precipitation of calcium oxalate crystals seemed to bear no relation to the amount in solution in the urine.

Characteristics of Urines Precipitating Oxalate of Lime.—The conditions causing precipitation of calcium oxalate in the urine are

not known. The characteristics of the urines of oxaluria have been studied by Begbie, Golding Bird, Maclagan and others. Fürbringer has shown that the crystals may be absent when a specimen contains a large amount of oxalic acid or present when there is only a trace. They are found with every degree of acidity, from highly acid to alkaline, with every degree of specific gravity and with every color. The following observations are collected from Dr. Herter's records.

In 370 cases in which the sediment was examined calcium oxalate crystals were found in 94, that is 25.4 per cent. The acidity varied from .091 grm. oxalic acid to alkaline, the mean acidity was .012 grm. The color varied from 1 to 7—mean 4—(Vogel's Chart). Thirty per cent of the specimens contained uric acid or urates in the sediment. The ratio of urea to uric acid varied from 23.4 to 99.2, the mean being 43.5. The lowest specific gravity was 1014, the highest 1035, the mean 1026. To determine whether the concentration of the urine affected the precipitation of calcium oxalate, five specimens of urine containing octahedral crystals were filtered and the filtrates concentrated at a low temperature until the specific gravity reached from 1032 to 1037. In no case was there a further precipitation of oxalate crystals.

ON THE FORMATION OF OXALIC ACID IN THE ANIMAL ORGANISM.

The origin and significance of the oxalic acid found in the urine, has formed the subject of much discussion. Since the observations of Prout, it has been recognized that the oxalic acid taken in the food or in drugs may in part reappear unchanged in the urine. Dunlop claims that all the oxalic acid found in the urine is taken into the body in this way. He bases his opinion on the fact that in patients placed on a milk diet, he was unable to recover any oxalic acid from the urine by quantitative analysis. In accordance with this, Gaglio,[18] Bunge,[19] and Burggraeve,[20] feeding dogs on meat alone, found no oxalic acid in the urine. But opposed to Dunlop's hypothesis are the experiments of Salkowski and of Auerbach,[21] who found oxalic acid in the

[18] Gaglio, *Arch. f. exp. Path. u. Pharm.*, 1887, xxii, p. 235.
[19] Bunge, Lehrb. d. phys. u. path. Chemie. Leipzig, 1889.
[20] Burggraeve, *Bull. Acad. roy. de med. de Belg.*, 1862, 2 s., v, p. 327.
[21] Auerbach, Virchow's *Archiv*, 1879, lxxvii, p. 226.

urine of fasting dogs, and those of Wesley Mills,[22] who found oxalic acid in the urine of dogs on meat diet. Primavera has reported an instance of a diabetic patient, with oxaluria, who, when placed on a meat diet, still had a heavy deposit of calcium oxalate in the urine. Bearing on this point, the experiments recorded in Tables V, VI and VII were made:

TABLE V.

PATIENTS ON DIET FREE FROM OXALATES.

	Date.	Diet.	Amount of oxalic acid in 24 hours.	Remarks.
Case I		Continuous milk diet, beginning Nov. 10.		
"	Nov. 16	Milk.	None.	
"	Nov. 17	"	"	
"	Nov. 18	"	"	
"	Nov. 19	"	Slight trace.	
"	Nov. 20	Milk and sugar.	None.	
"	Nov. 21	Milk and sugar, 45 grms.	None.	Sediment examined after precipitation by alcohol. No calcium oxalate crystals.
"	Nov. 22	Milk and sugar, 45 grms.	"	Calcium oxalate crystals formed.
"		Mixed diet from Nov. 23–Nov. 30.		
"		Milk diet Dec. 1–Dec. 4.		
"		Milk and sugar, 45 grms. Dec. 5–14.		
"	Dec. 8	Milk and sugar.	3.9 mg.	No calcium oxalate crystals.
"	Dec. 14	" "	1.1 mg.	Calcium oxalate crystals formed.
Case II....		Milk.	None.	
"	Nov. 3	"	"	
"	Nov. 4	"	"	
Case III ..		Milk, 1 week.	"	
Case IV ..		Meat, 1 year.	Slight trace.	
Case V ..		Milk and beeftea.	None.	
Case VI ..		Milk.	None.	
Case VII ..		Milk, 4 days.	Trace.	
Case VIII..	June 24	Carbohydrate diet free from oxalates for 1 week.	3.4 mg.	Calcium oxalate crystals rather numerous. Ash free from phosphates and sulphates.
" ..	June 25	Same.	25.5 mg.	Same as above.
Case IX ..	June 30	Same as above.	37.4 mg.	Calcium oxalate crystals large and numerous. Phosphates in ash.
" ..	July 1	" "	25. mg.	Calcium oxalate crystals large and numerous. No impurities in ash.

[22] Wesley Mills, *Journ. of Physiology*, 1885, v, p. 231.

TABLE VI.

DOG'S URINE. DIET FREE FROM OXALATES.

Diet.	Vol.	Amount of oxalic acid.	Sediment examined after precipitation with alcohol.
Meat	210 cc.	0	
"	100	0	
"	500	0	
"	180	0	
Glucose, 5 grm. Cornstarch, 5 grm. Water, 300 cc.	250	.004 grm.	Calcium oxalate crystals.
Cornstarch, glucose	285	.0012 grm.	
Meat	550	0	
"	170	.0024 grm.	
Meat, cane sugar, 100 grm. 2 days	150	.00114 "	No crystals in sediment.
Meat, cane sugar	550	.0119 "	Numerous small octahedral crystals.
Meat, 100 grm. sugar	52	Trace.	Numerous octahedral crystals.
Placed on milk Jan. 3.			
Milk, Jan. 5	650	Trace.	No calcium oxalate crystals.
Milk, Jan. 7	500	.0086 grm.	Numerous calcium oxalate crystals.
Milk, Jan. 10	650	.0053 "	
Same, Jan. 12	1750	Specimen lost.	
" " 14	1550	.0056 grm.	A few octahedral crystals.

TABLE VII.

FASTING DOG. STOPPED FEEDING JANUARY 24.

Date.	Vol.	Oxalic acid.	Date.	Vol.	Oxalic acid.
Jan. 25–28	No urine.		Feb. 1	100 cc.	.0043
Jan. 29	60 cc.	.005	Feb. 2	None.	
Jan. 30–31	No urine.		Feb. 3	101 cc.	.0014

In almost every case when a patient was placed upon a diet free from oxalates, the oxalic acid disappeared from the urine, or was present in too small quantities to be of any importance. A marked illustration of the reduction of the oxalic acid excretion by giving a diet free from oxalates is shown in the case of a patient of Dr. S. W. Lambert—a man subject to attacks of renal colic. With a mixed diet, including rhubarb, the twenty-four hours' excretion of oxalic acid was 67.5 mg. When for four days on a diet free from oxalic acid it fell to 4.1 mg.

Cases VIII and IX in Table V were in contrast to all others studied, as when placed on a diet free from oxalates (but rich in carbohydrates)

they continued to excrete an amount of oxalic acid which was above the normal. These were patients in the care of Dr. Hallock of Cromwell, Conn. Case VIII was that of an American woman, unmarried, aged 28 years. She suffered from nervous fears. She had some digestive trouble, at times with headache. Case IX was that of an American woman, aged 54 years, a widow. She had a history of nervous prostration followed by a melancholic condition. She had headache, digestive disturbance and poor sleep. These two cases in contrast with all others examined would definitely indicate that oxalic acid was formed in the body.

To determine whether the ingestion of excessive amounts of carbohydrate food would lead to the production of oxaluria, the following experiment was made:

A dog was placed under observation on Nov. 3, 1899. At that time there was a small amount of oxalic acid present in the urine.[23] The dog was placed on a meat diet, and the urine examined Nov. 18, Nov. 21 and Nov. 25 showed an absence of oxalic acid. On the last named date the dog was placed on large amounts of sugar in addition to meat. The animal took the sugar greedily, at times receiving 250 to 300 grms. in a day. For a month the dog showed no symptoms, but gained rapidly in weight. On Nov. 9 there were noted in the urine a few calcium oxalate crystals, but only a few. From that date until Dec. 27 oxalic acid was absent from the urine or, if present, was in very small amount. In the latter part of December there appeared simultaneously a group of symptoms consisting of loss of appetite, vomiting of frothy mucus, intermittent diarrhœa, the absence of free hydrochloric acid in the gastric juice, the presence of organic acids in the urine, and the precipitation of numerous large calcium oxalate crystals in the urine. On Jan. 1, the dog took almost no sugar, and there were again but few calcium oxalate crystals deposited in the urine. On Jan. 3 very large and very numerous crystals were noted, some appearing in masses of imperfectly formed crystals like microscopic calculi. Whenever the symptoms in this case

[23] To discover the presence of very small quantities of calcium oxalate in the urine, 95% alcohol was added (Salkowski, *Zeitschr. f. physiol. Chem.*, 1886) in the amount of one-third the volume of the urine to be examined. The mixture was then set aside for forty-eight hours, until the calcium oxalate, if present, had crystallized out from the solution. The sediment was then collected from the bottom of the beaker, centrifugalized and examined microscopically. This furnishes a more delicate test for oxalic acid than the complicated quantitative method of analysis.

became severe, the dog would refuse to take its food, when the symptoms (including the oxalic acid excretion) gradually became less marked. The dog was kept on rather large quantities of sugar for six months and throughout that time there was an almost continuous excretion of oxalic acid although the animal received a diet of meat and sugar only.

Two other dogs were treated in a similar way with the same result. These observations indicate that oxalic acid is formed in the animal body.

The question where in the animal organism oxalic acid is formed has been much discussed. Neubauer,[24] Bouchardat[25] and Ellis[26] have claimed that fermentative action in the stomach or intestine may lead to the production of oxalic acid. The most of the writers on this subject have believed that the oxalic acid was formed in the tissues as a result of defective oxidation and that it was probably due to a direct nervous action on the cells.

Oxalic acid, as has been noted above, is produced by many of the higher forms of vegetable life. It has also been noted as a product of lower forms, as of Aspergillus niger. Zopf[27] has described a form of saccharomyces (S. Hansenii, Zopf), found in cotton-seed meal, which in fermentable carbohydrate solutions produces oxalic acid in place of alcohol.

Experiment I.—With Dog I, noted above, the following experiment was made: On Feb. 9 the dog was fed 400 grm. of meat and 200 grm. of glucose with water. The dog vomited one hour afterwards 260 cc. The vomitus consisted of undigested meat suspended in a watery fluid which contained a considerable amount of mucus. There was no free hydrochloric acid. On adding hydrochloric acid and heating, there was a butyric acid odor given off. This vomited matter was found to contain oxalic acid.[28]

[24] Neubauer, op. cit.

[25] Bouchardat, *Annuaire de thérap.*, 1850.

[26] Ellis, *Boston Med. & Surg. Jour.*, 1888, cxviii, p. 64.

[27] Cited from Baumgarten's *Jahresbericht*, 1889, v, p. 452.

[28] The stomach contents were treated with hydrochloric acid and heated over the water-bath 24 hours. They were then filtered, neutralized with ammonia, and rendered slightly acid with acetic acid. A small amount of calcium chloride solution was then added, and 95% alcohol in the amount of one-half the volume of the fluid. The calcium oxalate was precipitated as a finely divided sediment, and only after about two weeks were the characteristic octahedral crystals formed.

Experiment II.—The same dog was given on Feb. 21, 400 grms. of chopped beef and 200 grms. of glucose and the stomach contents removed in 1½ hrs. The result was the same as before. The specimen contained undigested meat and a large amount of stringy mucus. It was acid in reaction, with no free hydrochloric acid. Oxalic acid was present.

Experiment III.—A mixture was prepared in a flask, which contained cane sugar 100 grms., beef ext., 1 grm., water 1000 cc. To this was added 1 cc. of the gastric contents from Experiment I. This was left in the incubator for two days. On examination oxalic acid was found.[29]

Experiment IV.—Dog II. Began feeding sugar, 100 grms. daily on Feb. 23. On March 9, calcium oxalate crystals were found in the urine. On that date there were given 200 grms. cane sugar, which was vomited in a half hour. This specimen was examined for oxalic acid with negative result. On Apr. 5 the dog was given 100 grms. sugar in 250 cc. of water. This was withdrawn from the stomach in 1¼ hrs. The specimen contained meat which was retained in the stomach from an earlier feeding. There was present thick frothy mucus. There was no free hydrochloric acid. Oxalic acid was present.

Experiment V.—On May 11, 1900, there was fed to Dog I 100 grms. of sugar, in 200 cc. of water. This was removed in one hour. The reaction was faintly acid; there was no free hydrochloric acid present; there was found a large amount of thick mucus. Examination for oxalic acid gave a negative result.

Experiment VI.—A portion of the stomach contents obtained in Experiment V was added to a mixture containing sugar 100 grms., beef extract 1 grm., water 500 cc. After fermenting in the incubator for forty-eight hours, oxalic acid was found.

Experiment VII.—A third dog was fed large amounts of sugar daily, beginning Feb. 22. On May 11 the urine was found to contain a

[29] In connection with the fermentation experiments described in this paper, control tests for oxalic acid were made upon uninoculated solutions of Liebig's extract of beef and sugar. These were examined by the same method as the gastric contents (see footnote 28). In fifteen specimens, no oxalic acid was found. In two cases, after diligent search, there was discovered a single small calcium oxalate crystal. The examination of mixtures of chopped beef and sugar for oxalic acid gave negative results. Salkowski (*Berl. klin. Wochenschr.*, 1900, xxxvii, p. 434) records the finding of oxalic acid in beef extract by quantitative analysis. The result of our examinations, however, seemed to prove that, if present, it was in too minute a quantity to affect the validity of the conclusions drawn from the fermentation experiments.

heavy deposit of calcium oxalate crystals. This dog was then fed 100 grms. of sugar in 200 grms. of water and the stomach contents removed in an hour. The reaction was acid. There was no free hydrochloric acid present. There was a moderate amount of mucus. No oxalic acid was found.

Experiment VIII.—A portion of the stomach contents obtained in Experiment VII was added to a mixture containing 100 grms. of sugar, 1 grm. of beef extract and 500 cc. of water. After fermenting in the incubator for two days, oxalic acid was found to be present in the mixture.

Experiment IX.—A mixture was prepared in a flask, consisting of sugar, boiled starch, Huntley and Palmer breakfast biscuits, and water. To this was added 1 cc. of the stomach contents of a patient having persistent oxaluria. There had been no free hydrochloric acid in the stomach contents after a test breakfast. After fermenting in the incubator, oxalic acid was found in this mixture.

Experiment X.—The patient mentioned in Experiment IX was placed on hydrochloric acid given after meals. The calcium oxalate crystals disappeared from the urine almost entirely under this treatment. When the hydrochloric acid had been discontinued for three days, an experiment similar to Experiment IX was made, using a portion of the gastric contents as a ferment. No oxalic acid was formed.

Experiment XI.—To a mixture of 50 grms. of sugar, 0.5 grm. of beef extract and 500 cc. of water there was added 1 cc. of the stomach contents of a patient with marked and persistent oxaluria. After fermenting for two days, oxalic acid was found in the mixture.

The absence of hydrochloric acid in the gastric contents after a test meal, is a noticeable feature in the cases in which the gastric contents were examined. In the case of Dog I, a test was made for free hydrochloric acid, an hour after feeding, on Feb. 9, Feb. 21, Apr. 20 and May 11. Each time the result was negative. In the case of Dog II, the test was made on Mar. 9, Apr. 5 and Apr. 6. Each time there was no free hydrochloric acid found. In the case of Dog III, the stomach contents were examined on March 15 and May 11 and showed an absence of free hydrochloric acid.[30]

[30] In making preliminary experiments to discover an organism producing oxalic acid from solutions of sugar and beef extract (sugar 100 g., beef extract, 1 g., water 500 cc.), it was found that in four specimens, inoculated with baker's yeast, oxalic acid was formed, but in other similar experiments it was not produced. These observations are too few to be of any importance other than to suggest that certain unknown conditions may lead to the formation of oxalic acid through the activity of baker's yeast.

In only three cases was there an examination of the stomach contents of patients having persistent oxaluria. The patient mentioned in Experiment IX had repeated examinations made after test meals, with a constant absence of free hydrochloric acid. The patient mentioned in Experiment XI had long standing oxuluria with local irritative symptoms in the urinary tract. In this case there was no free hydrochloric acid in the gastric juice, and the same result was obtained in a third case with persistent calcium oxalate deposit in the urine.

PHYSIOLOGICAL AND TOXIC ACTION OF OXALIC ACID.

As the presence of oxalate of lime in the urine is found associated with many symptoms, the question naturally arises as to what is the physiological action of oxalic acid and soluble oxalates. Do they produce any of the symptoms of the so-called oxalic-acid diathesis?

Death from oxalic-acid poisoning is ordinarily due to the local corrosive action on the alimentary canal, and large doses may be taken with impunity if they are in well diluted solution. Christison and Coindet[31] say that early in the century oxalic acid was used extensively in making lemonade, and was generally believed to be innocuous. Piotrowski,[32] in experimenting upon himself, took at several different times from 4 to 7 grammes in twenty-four hours with no noteworthy symptoms, and once he took 8 grammes within one hour.

With a view to studying the influence of rather large doses of soluble oxalates, extending over a number of days, the following experiments were made. A healthy man received a diet free from oxalates and was given daily for two weeks from .20 to .50 grm. of ammonium oxalate. This was taken well diluted immediately after meals. The oxalate was given at first alone, then with enough sodium bicarbonate to render the urine but faintly acid, then with dilute hydrochloric acid. The alkali and acid were given to determine the influence of the acidity of the gastric juice upon the absorption of the drug. Table VIII shows the result of the experiment.

[31] Christison and Coindet, *Edinb. Med. & Surg. Jour.*, 1823, xix, pp. 163; 323.
[32] Bucheim, *Arch. d. Heilk.*, 1857, n. F., i, p. 124.

The same experiment was carried out upon another subject with the result shown in Table IX (p. 42).

In the first of these two cases there was no symptom noted excepting polyuria, as indicated in the table. In the second case no symptom whatever followed the ingestion of the drug. In neither case was the excretion of oxalic acid increased above the normal.

TABLE VIII.

HEALTHY MAN. DIET FREE FROM OXALATES. GIVEN AMMONIUM OXALATE.

Date.	Diet.	Sp. gr.	Reaction.	Volume of urine.	Urea.	Uric acid.	Ratio.	Ammon. oxalate taken.	Other medicine.	Oxalic acid in urine.	Sediment after precipitation with alcohol.
Jan. 20	Beef, rice.....	1016	Acid.	1260	15.4	.359	42.9	None.	None.	.0055	Octahedral crystals abundant.
21	Milk 1200 cc., butter	1016	"	1545	23.	.487	47.2	"	"	.002	" " small.
22	Huntley & Palmer's biscuits	1012	"	2035	23.5	.436	53.7	.225 grm.	"	.0059	" " "
23	" " ..	1014	"	2045	30.	.684	43.8	.35	"	.0065	" " "
24	2 lamb chops, 2 eggs	1010	"	2410	25.9	.103	25.1	.425	"	.001	" " numerous.
25	As at first.....	1017	Faintly acid.	1565	25.4	335	75.8	.15	"	.014	Calcium sulphate crystals; small octahedral crystals.
26	" "	1020	"	1280	24.8	.324	76.6	.45	"	.0016	Octahedral crystals numerous.
27	" "	1016	"	2105	28.1	.566	49.7	.45	"	.0051	" " small.
28	" "	1013	"	2680	25.5	.407	62.8	.475	"	.024	" " few.
29	" "	1014	"	2160	31.1	.564	55.	.375	Sod. bicarb. 4.2 grm	.002	" "
30	" "	1013	"	2405	30.8	.515	59.9	.375	4.8	.019	" " very few.
31	" "	1015	"	1975	23.3	.444	52.5	.375	3.6	.036	" " numerous.
Feb 1	" "	1010	"	2400	37.4	.513	72.8	.375	3.6	.0038	" " few.
2	" "	1013	"	2530	34.43	.502	61.3	.350	HCl 3.6 cc.	.0057	No octahedral crystals.
3	" "	1010	"	2295	33.9	.437	77.6	.44	5.4 cc.	.015	Few " "
4	" "	1012	"	2370	29.8	.157	190.	.30	5.4	.013	Very few " "

Two dogs were given ammonium oxalate, in larger doses, in proportion to body weight, than the men received. The results of these experiments are recorded in Table X (p. 43).

This dog developed no symptoms excepting albuminuria which disappeared whenever the ammonium oxalate was withheld and reappeared whenever it was given. After receiving the dose intraperitoneally, it developed peritonitis and died within a few hours.

Another dog was given ammonium oxalate subcutaneously. On Feb. 28, .5 grm. of ammonium oxalate was given hypodermatically in 100 cc. of water. The dog showed no symptoms for twenty-four hours when there was noted a weakness in the hind extremities. At that time .5 grm. was again injected. There were no symptoms noted excepting increasing loss of power in the extremities until Mar. 4, when the respiration became rapid and panting and the animal died. After the first

TABLE IX.

HEALTHY MAN. GIVEN AMMONIUM OXALATE.

Diet—Bread, butter and meat.

Date.	Amount in 24 hours	Reaction.	Sp. Gr.	Urea.	Uric acid.	Ratio.	Ammon. oxalate taken.	Other medicine.	Oxalic acid secreted.	Micros. Exam.
Feb. 23	1120 cc.	Acid.	1017	13.48	.6237	21.61	0	0	.00502	No oxalate crystals.
24	945	Faintly acid.	1021	17.32	.6294	27.51	0	0	.00846	Small oxalate crystals.
25	1585	Faintly acid	1017	25.11	.5016	50.06	.24	0	.0071	Numerous oxalate crystals
26	715	Acid.	1021	14.76	.4706	31.36	.28	0	.0064	" " "
27	815	Acid.	1025	19.18	.3523	54.44	.30	0	.0112	" " "
28	760	Markedly acid.	1022	19.24	.3517	54.91	.30	0	.0007	" " "
Mar. 1	1400	Strongly acid.	1018	14.33	.4199	34.13	.30	Sod. bicarb.	.0067	" " "
2	995	Very faintly acid.	1019	18.58	.5543	33.52	.30	Sod. bicarb.	.0229	Octahedral crystals fewer.
3	945	Very faintly acid.	1019	20.03	5556	33.81	.38	Sod. bicarb.	.0030	Octahedral crystals fairly numerous.
4	1740	Very faintly acid.	1016	25.23	.7387	34.22	.42	HCl 5.4 cc.	.0179	Trace of sulphates left. Numerous cal. oxal. crystals and cal. sulphate.
5	1165	Faintly acid.	1018	18.62	.3132	59.48	.42	HCl 5.4 cc.	.0074	Calcium oxalate crystals not very numerous.
6	2100	Faintly acid.	1009	15.45	.9076	17.02	.48	HCl 5.4 cc.	.0087	Very few calcium oxalate crystals.

injection of ammonium oxalate there was no urine voided for forty-eight hours. Then 220 cc. were passed. This contained no albumin, no sugar nor other reducing agent. .1466 grm. of oxalic acid was recovered. The next day 250 cc. of urine were passed, containing a trace of albumin, no sugar or other reducing agent. There was found .078 grm. of oxalic acid. On the next day 690 cc. were voided containing .057 grm. of oxalic acid. There was thus given in all, subcutaneously, 1 grm. of ammonium oxalate, which represents .726 grm. oxalic acid. Of this there was recovered in the urine .281 grm. or 37%.

The autopsy on this dog was made one-half hour after death. The blood was still fluid throughout the body, but it slowly coagulated upon exposure to the air. The heart was in diastole, filled with liquid blood,

with a single small clot in the left ventricle. The lungs showed areas of congestion. The most interesting lesion was in the kidney. Here the section showed numerous crystals blocking up the uriniferous tubules. Most of them were of irregularly shaped masses. Some showed characteristic dumbbell and ovoid shapes. These crystals were of a very light yellow color, were unstained by eosin or hæmatoxylin, were insoluble in acetic acid and ammonia, but dissolved in dilute hydrochloric acid. There were no changes in the glomeruli. The cells lining the tubules were in places swollen and granular, and in places were torn away by the passing of a calculus.

TABLE X.

ADMINISTRATION OF AMMONIUM OXALATE TO DOG.

Date.	Amount of urine.	Albumin.	Ammon. oxalate taken.	Oxalic acid taken in form of ammon. oxalate.	Amount of oxalic acid excreted.
Feb. 4...		None.	.50 grm.	.36	
Feb. 5....		"	.50 grm.	.36	
Feb. 6....	144 cc.	"	0	0	.0285
Feb. 7....		"	.75	.54	
" 8....	315 cc.	"	1.00	.72	Specimen lost.
" 9....		"	1.00	.72	
" 10....	175 cc.	"	1.00	.72	.0242
" 11....		"	1.00	.72	
" 12....		Trace.	1.00	.72	
" 13....	565 cc.		1.00	.72	.0707
" 14....		None.	0	0	
" 15....	450 cc.		0	0	.0464
" 16....			.4	.29	
" 17....	90 cc.	Trace.	.4	.29	.0035
" 18....			0	0	
" 19....			0	0	
" 20....	980 cc.	None.	0	0	.0034
" 21....			0	0	
" 22....			0	0	
" 23....			0	0	
" 24....	110 cc.	Large amount.	1 grm. intraperitoneally.	.72	
" 25....					

The study of acute oxalic acid poisoning does not come within the scope of this paper. It may, however, be noted, in connection with the above experiments, that the symptoms of the acute poisoning have been carefully studied by Christison and Coindet,[33] R. Koch,[34]

[33] Op. cit.

[34] R. Koch, *Arch. f. exp. Path. u. Pharm.*, 1881, xiv, p. 153.

Kobert and Küssner,[35] and Krohl,[36] and the pathology has been studied by A. Fraenkel[37] and by Ebstein and Nicolaier.[38] The most common symptoms are those of the nervous system: either irritative phenomena, as muscular twitchings and tonic or clonic convulsions; or paralytic phenomena—paresis or paralysis, both motor or sensory, with abolition of reflexes, and excessive lowering of temperature before death. The pulse, respiration and blood-pressure are not influenced except in fatal cases, and there are no digestive symptoms unless the drug is given by mouth. Several observers have found in the urine a strong reducing agent, acting upon copper sulphate and bismuth subnitrate, which does not give the test for sugar by the spectroscope or polariscope. The urine may contain albumin or casts or red corpuscles or any of the forms of calcium oxalate crystals.

On the Oxidation of Oxalic Acid in the Organism.—The slight danger in taking large doses of soluble oxalates seems to be due in part to the precipitation of the oxalic acid as calcium oxalate in the alimentary canal, and its remaining unabsorbed in the fæces; and in part to the oxidation in the organism of that which is absorbed. Gaglio,[39] experimenting with a cock, recovered from the fæces all the oxalic acid that was taken; but Guinti,[40] while verifying Gaglio's experiment in the case of the cock, found that in dogs and man, a portion of that absorbed was oxidized.[41]

In the experiments described above it will be noted that in the cases of the men receiving ammonium oxalate, in amounts varying from .2 grm. to .48, and extending over a period of eleven days in

[35] Kobert and Küssner, Virchow's *Archiv*, 1879, lxxviii, p. 209, and 1880, lxxxi, p. 383.

[36] Krohl, *Arb. a. d. pharmakol. Inst. z. Dorpat*, 1891, vii, p. 130.

[37] A. Fraenkel, *Zeitschr. f. klin. Med.* 1881, ii, p. 664.

[38] Ebstein and Nicolaier, Virchow's *Archiv*, 1897, cxlviii, p. 366.

[39] Gaglio, *Arch. f. exp. Path. u. Pharm.*, 1887, xxii, p. 235.

[40] Guinti, *Ann. di chim. e di farm.*, Milan, 1897.

[41] Thus in giving a man .31 grm. he recovered in the fæces .038 grm., leaving .272 absorbed. Of this there was excreted in the urine during the first two days .062 grm. or 11%. From this he concludes that the rest was oxidized in the system. In a similar experiment on a dog he recovered 11% of that absorbed. But in giving the drug hypodermatically to a dog, he recovered at one time 43.6%, and again 51% of that injected.

one case, and two weeks in the other, only traces of oxalic acid appeared in the urine, while in the case of the dog receiving the drug subcutaneously 37 per cent was recovered. As no study was made of the fæces in these experiments it is impossible to form any opinion as to whether any oxalic acid was oxidized in the body.

CONCLUSIONS.

1. As varying amounts of calcium oxalate may be held in solution in the urine, conclusions based upon the presence or number of calcium oxalate crystals found therein are of no real value as an indication of the quantity of oxalic acid present.

2. Unless the utmost care is exercised, the results obtained by quantitative estimation of oxalic acid are subject to large percentages of error. This is especially true in the use of Neubauer's or Shultzen's methods, in which the calcium oxalate is precipitated in an alkaline solution.

3. An ordinary mixed diet regularly contains traces of oxalic acid or its salts.

4. A portion of the oxalic acid ingested with the food may be absorbed and reappear unchanged in the urine.

5. The normal daily excretion of oxalic acid in the urine fluctuates with the amount taken in the food, and varies from a few milligrammes to two or three centigrammes, being usually below ten milligrammes.

6. In health, no oxalic acid, or only a trace, is formed in the body, but that present in the urine has been ingested with the food.

7. In certain clinical disturbances which in some of the cases studied above were associated with absence of free hydrochloric acid from the gastric juice, oxalic acid is formed in the organism.

8. This formation in the organism is connected with fermentative activity in the alimentary canal.

(*a*) The prolonged feeding of dogs with excessive quantities of glucose, together with meat, leads eventually to a state of oxaluria.

(*b*) This experimental oxaluria is associated with a mucous gastritis, and with absence of free hydrochloric acid in the gastric contents.

(*c*) The oxaluria and the accompanying gastritis are referable to fermentation induced by the excessive feeding with sugar.

(*d*) The experimental gastritis from fermentation is associated with the formation of oxalic acid in the gastric contents.

9. The symptoms attributed to an oxalic acid diathesis, with the exception of those due to local irritation in the genitourinary tract, do not appear to be due to the presence in the system of soluble oxalates, but are more likely to depend on other products of fermentation and putrefaction.

I wish to express my gratitude to Dr. Herter, at whose suggestion and under whose guidance this study has been made, and to Dr. A. J. Wakeman for advice and aid in carrying on the work.