23. THE METABOLISM OF OXALIC ACID IN THE ANIMAL BODY

By HAROLD HAYDEN BARBER AND
ERIC JAMES GALLIMORE

From the Sub-Department of Physiology, University College, Nottingham

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THE origin and fate of oxalic acid in the animal body have been studied almost exclusively by means of experiments involving the oral administration of the substance and the investigation of its metabolism in the body as a whole. Thus Mills [1885] showed that on a vegetable-free diet of flesh foods oxalate occurred in the urine. On a pure milk and sugar diet Lommel [1899] observed the excretion of oxalic acid and found that even during fasting oxalate had not disappeared from the urine on the 12th day. It was thus recognized that this substance could, in part at least, be of endogenous origin.

Proteins and sugars were early regarded as precursors of oxalate. Lommel [1899] found an increase in the urinary oxalate after the administration of gelatin. Klemperer & Tritschler [1902] stated that glycine and also creatine brought about an increased excretion of oxalate. More recently Pincussohn [1919] found an increased oxalate excretion after purine compounds had been administered either *per os* or intravenously to dogs. Yudeles *et al.* [1936] found that the oxalate of the blood was increased after the intravenous injection into dogs of small amounts of glycine. Bergrom [1935] found that the complete elimination of oxalate given by mouth required about 14 days and, since the excretion was quantitative, he suggested that this substance is not oxidized in the tissues.

Many more studies have been reported on similar lines with the object of demonstrating that the excreted oxalate is not exclusively of alimentary origin but is at least in part produced in the body, and also with the object of discovering the substances responsible for its production. On the other hand, little attention has been paid to the possibility of the study of oxalate formation *in vitro* either by surviving tissues or by intestinal flora. The present paper is an account of exploratory experiments in these latter directions.

EXPERIMENTAL

The determination of oxalate in blood and tissues

The method previously published for the estimation of oxalic acid in urine [Dodds & Gallimore, 1932] has been satisfactorily adapted for blood and tissues.

In the case of blood, 5 ml., treated with heparin, are added to 25 ml. water and 5 ml. 5*N* HCl are then added with shaking. After the addition of 2 g. phosphotungstic acid in 10 ml. water, followed by a further 5 ml. of 5*N* HCl, the whole is heated at 100° for 20 min. on the water bath. After cooling and allowing to stand for at least an hour, the precipitate is filtered off with suction on a Hirsch funnel and washed 3 times with 7 ml. of 5*N* HCl for each washing. The filtrate is collected in a Claisen flask and the contents then treated in the manner outlined for urine [Dodds & Gallimore, 1932]. Typical results showing the recovery of oxalate added to blood are given in Table 1.

Table 1

Sample	Oxalic acid added mg.	Oxalic acid found mg.	Added oxalic acid recovered mg.	Added oxalic acid recovered %
5 ml. distilled water	None	0.010	—	—
5 ml. distilled water	Heparin added	0.014	—	—
5 ml. defibrinated horse blood A	None	0.035	—	—
"	None	0.027	—	—
"	None	0.025	—	—
"	1.0	0.88	0.85	85
"	1.0	0.83	0.80	80
"	1.0	0.88	0.85	85
"	1.0	0.87	0.84	84
"	0.1	0.113	0.084	84
"	0.05	0.066	0.037	74
5 ml. defibrinated horse blood B	None	0.036	—	—
"	Heparin added	0.031	—	—
5 ml. rabbit blood C	None	0.035	—	—
"	0.1	0.117	0.082	82
5 ml. rabbit blood D	None	0.048	—	—
"	0.03	0.076	0.028	93

All oxalic acid values are for anhydrous oxalic acid.

A number of such estimations of oxalate in the blood of normal adults showed the oxalic acid content to be between 0.4 and 0.6 mg. per 100 ml. of whole blood. In a patient with pronounced oxaluria the oxalic acid content of the blood was found to lie within this normal range. In the rabbit the content is slightly higher, varying from 0.4 to 0.8 mg. per 100 ml.

A similar procedure is applicable to tissues. The tissue, after being minced, is digested with a suitable amount of *N* HCl and phosphotungstic acid, the subsequent operations resembling those for blood.

Since the above assays are based on determinations involving an oxidation titration, it was thought desirable to check these values by a control experiment in which the oxidation titration was preceded by actual separation, weighing and microscopical identification of the calcium oxalate [Merz & Maugeri, 1931]. From 2.5 l. of defibrinated horse blood the proteins were removed with phosphotungstic acid and the oxalic acid was separated by distillation as ethyl oxalate; 16.2 mg. of an insoluble calcium salt were then obtained which under the microscope was identified as crystalline calcium oxalate. A potassium permanganate titration carried out on this precipitate was found to correspond with the calculated oxalic acid content.

Our findings for the oxalate content of blood support the statement of Thomsen [1935] that the methods of Merz & Maugeri [1931] and of Izumi [1933] give high and fictitious values for blood oxalate.

The effect of surviving tissues in vitro

Preliminary work showed that minced or sliced tissues did not destroy oxalic acid to any appreciable extent. The tissues studied were liver, kidney and spleen; the animals used included rats, guinea-pigs and rabbits. Sections as thin as practicable were cut from the fresh tissue with a razor and placed in saline or serum to which had been added the oxidizable substance. In some experiments the tissue was minced in a small machine such as is used for tumour implantations. In the majority of the experiments mentioned below controls

were performed alongside the actual digestion. In a typical experiment the tissue, weighed moist, was placed in a medium consisting of 25 ml. horse serum, 10 ml. normal saline and 0.2 g. creatine. The whole was maintained at 37° for 6 hr. whilst N₂ containing 5% CO₂ was bubbled continuously through the fluid. The medium was then examined for oxalic acid.

The substrates included uric acid, glycine, ethylene glycol, glycerol, creatine and glucose. In some cases Ringer solution with a phosphate buffer to give a pH of either 6.0 or 8.0 was substituted for serum.

In none of these experiments was any formation of oxalic acid observed.

The action of intestinal organisms in vitro

At various times it has been suggested that the formation of oxalate is the result of simple fermentative changes occurring within the digestive tract [Mayer, 1902; Borjström, 1936].

It occurred to us that it might be possible to effect the formation of oxalic acid in a suitable medium *in vitro* by inoculation with faeces and incubation at body temperature. Contrary to expectations it was found that oxalate was not formed under conditions simulating those within the intestine, but that on the contrary any added oxalate was rapidly destroyed.

The medium used (Medium A) had the following composition (g./l.): NaCl, 9.00; KCl, 0.42; CaCl₂.6H₂O, 0.48; NaHCO₃, 0.50; K₂HPO₄, 0.25; MgSO₄, trace; glucose, 0.50; peptone, 2.00.

The solution, with or without the addition of known amounts of oxalate, was adjusted to the required pH by the addition of dilute NaOH and then sterilized in steam at atmospheric pressure for 3 days in succession. After inoculation with a suspension of human faeces equivalent to about 200 mg. in 100 ml. of medium A, incubation was carried out in 250 ml. conical flasks at 37°. Sterile control flasks of medium were incubated at the same time. The oxalate content of each flask was then determined.

Table 2. *Incubation of oxalate solutions at various pH values*

Vol. of medium A, 100 ml. Period of incubation, 7 days

Condition of medium	Initial pH	Oxalic acid added mg.	Oxalic acid found mg.
Sterile	7.5	102	102
+ faeces	7.5	102	20
Sterile	7.0	102	108
+ faeces	7.0	102	36
Sterile	6.5	102	108
+ faeces	6.5	102	109

Table 3. *Incubation of oxalate solutions for various periods*

Vol. of medium A, 100 ml. Initial pH, 7.5

Condition of medium	Period of incubation days	Oxalic acid added mg.	Oxalic acid found mg.
Sterile	1	102	105
+ faeces	1	102	102
"	2	102	107
"	3	102	18
"	7	102	14
Sterile	7	102	102

Tables 2 and 3 illustrate the destruction of oxalate by faecal organisms at reactions of pH 7.0 and 7.5. Under these conditions the greater part of the oxalate is destroyed in 3 days.

Table 4. *Effect of oxygen*

Vol. of medium A, 100 ml. Initial pH, 7.5. Period of incubation, 7 days

Condition of medium	Oxalic acid added mg.	Oxalic acid found mg.
Aerobic, sterile	102	107
Aerobic, faecal inoculation	102	16
" "	102	7
Anaerobic, sterile	102	108
Anaerobic, faecal inoculation	102	26
" "	102	38

Table 4 indicates that the destruction of oxalate is independent of the presence of atmospheric oxygen and thus may occur under conditions resembling those which obtain within the intestine.

Table 5. *Effect of variations in the glucose and peptone contents*

Medium A without glucose and peptone; vol. 100 ml.; initial pH, 7.5;
period of incubation, 7 days

Glucose and peptone added	Condition of medium	Oxalic acid added mg.	Oxalic acid found mg.
No peptone; 0.1 % glucose	Sterile	102	102
" "	+ faeces	102	102
0.2 % peptone; no glucose	Sterile	102	100
" "	+ faeces	102	80
0.2 % peptone; 0.05 % glucose	Sterile	102	102
" "	+ faeces	102	14
0.2 % peptone; 0.1 % glucose	Sterile	102	104
" "	+ faeces	102	12
0.2 % peptone; 0.3 % glucose	Sterile	102	105
" "	+ faeces	102	102

In Table 5 the negative result obtained in the absence of peptone is possibly accounted for by the inability of the organisms to function in a peptone-free medium. The results without and with various amounts of glucose suggest that this substance is concerned in the oxalate oxidation. The absence of oxalate destruction in the higher concentrations of glucose is possibly explained by the observed rapid change of the reaction of the medium during incubation to an acidity which would inhibit this action of the organism.

Table 6. *Effect of partial sterilization of the faecal inoculum;
effect of toluene*

Vol. of medium A, 100 ml. Initial pH, 7.5. Period of incubation, 7 days

Condition of medium	Oxalic acid added mg.	Oxalic acid found mg.
+ faeces	102	32
+ faeces. Suspension previously heated at 80° for 20 min.	102	105
" "	102	102
+ faeces	25	2.5
+ faeces and 0.5 % toluene	25	23

The absence of oxalate destruction by a faecal inoculum previously heated at 80° indicates that the effect is dependent on the activity of non-sporing organisms.

The negative result in the presence of toluene eliminates the possibility of oxalate destruction by enzymes introduced in the faecal inoculum.

According to the foregoing results destruction of oxalic acid occurs only at a pH above 7.0. A few experiments were made to determine the effect of a faecal inoculation at an acidity nearer to that of the stomach. Under these conditions oxalic acid formation was found to take place.

The medium employed was the same as medium A but with the glucose content raised to 2%.

A volume of 500 ml. of this medium was adjusted to pH 2.4. After sterilization separate portions, each of 100 ml., were inoculated, (a) with a suspension of faeces, (b) with a scraping from a sound tooth, whilst a third portion without treatment acted as a control. After incubation for 10 days the control medium gave no oxalic acid, whilst the calcium oxalate isolated from the faecal inoculation corresponded to 4.2 mg. and that from the tooth deposit inoculation to 3.1 mg. of oxalic acid.

SUMMARY

1. A method for the estimation of oxalic acid in blood is described. The oxalic acid content of normal blood was found to lie between 0.4 and 0.6 mg. per 100 ml. whole blood.

2. Tissues do not form or destroy oxalic acid under the conditions described.

3. At a pH value of 7.0 or higher a suspension of faeces inoculated into a nutrient solution containing oxalate causes the destruction of oxalate on incubation at 37°. This was observed under both aerobic and anaerobic conditions. The action is probably due to a non-sporing organism.

4. At a pH value of 2.4 a similar inoculation with somewhat longer incubation at 37° was found to give rise to oxalic acid.

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