CXLVII. THE DETERMINATION OF SMALL QUANTITIES OF OXALIC ACID.

By EDWARD CHARLES DODDS and ERIC JAMES GALLIMORE¹.

*From The Courtauld Institute of Biochemistry,
Middlesex Hospital, London.*

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THE determination of small amounts of pure oxalic acid in aqueous solution by precipitation of the calcium salt and treatment of the precipitate with permanganate offers little difficulty. In the procedure elaborated by Leulier [1929] quantities of oxalic acid as low as 0.05 mg. in aqueous solution may be estimated with accuracy.

The lack of success which has attended the efforts to estimate oxalic acid in biological materials is due to the difficulty of obtaining the oxalic acid in a solution free from those substances which either inhibit the precipitation of calcium oxalate or which are carried down with the precipitate and react with the permanganate.

Investigations on the rôle of oxalic acid in metabolism have for the most part been based on the determination of oxalic acid in urine. It is not within the scope of this paper to discuss the literature in detail but a brief account of the more important investigations is given below. Salkowski [1900] estimated oxalic acid in urine by extracting the acidified urine with a mixture of alcohol and ether, afterwards precipitating the extracted oxalic acid as the calcium salt and weighing the calcium oxide formed by ignition. Autenrieth and Barth [1902] precipitated the oxalic acid directly from the urine as calcium oxalate and then transferred the oxalic acid from an acid solution into a mixture of alcohol and ether; the oxalic acid was again precipitated as calcium oxalate and the precipitate after ignition was weighed. Dakin [1907] modified the method of Autenrieth and Barth by using ether, without alcohol, in a continuous extractor. The final precipitate was either titrated with permanganate or weighed after ignition. Merz and Maugeri [1931] give references to adverse criticisms of the above methods and state that they were unable to extract oxalic acid quantitatively from its aqueous solution with ether. Van Slyke and Sendroy [1929] have described the advantages of determining small quantities of oxalic acid by a gasometric method in lieu of the many titration methods which have been used.

¹ Whole-time worker for the Medical Research Council.

EXPERIMENTAL.

The method of estimating oxalic acid by direct precipitation of the calcium salt from urine was found to give results both low and variable.

With regard to the extraction of oxalic acid from aqueous solution with ether, it was found that the extraction was incomplete with pure oxalic acid but with a high concentration of free mineral acid all the oxalic acid could be removed by this means. When applied to urine the ether also extracts from the strongly acidified concentrated urine many other substances, the presence of which, even though the calcium oxalate is precipitated from an alkaline, and reprecipitated from an acid, solution, may cause anomalous results.

The method about to be described depends on the fact that it was found possible to remove oxalic acid completely by esterification and distillation *in vacuo* from an acid alcoholic solution whilst apparently leaving behind all interfering substances.

Reagents. Alcohol, containing dry HCl, *N* solution. Lime-water, saturated solution, free from carbonate. Ammonium phosphate, 0.5 %.

Distillation of oxalic acid. 25 cc. of urine + 1 cc. *N* HCl are evaporated almost to dryness in a Claisen flask *in vacuo* below 50°. The flask is then connected up as shown in Fig. 1.

Fig. 1.

The receiver contains 13 cc. 4*N* NaOH and is kept cool by means of a rapid stream of cold water. To the residue in the flask a mixture of 5 cc. alcohol and 2 cc. benzene is added, the temperature of the water-bath surrounding the distillation flask raised to 65° and the contents of the flask dried by distilling off *in vacuo* the low boiling ternary mixture of alcohol, benzene and water. To remove the last traces of water, it is again distilled with 5 cc. acid alcohol and 2 cc. benzene.

The contents of the flask are now boiled with 20 cc. of the alcohol containing HCl on a boiling water-bath for half an hour; the flask is then reconnected to the receiver and the contents distilled *in vacuo* at 65°. The vacuum reached

about the middle of the distillation should not be less than 30 mm. The boiling and distillation are repeated in all three times.

The contents of the Büchner flask are washed into the smaller Claisen flask and the slightly alkaline solution evaporated down to 10–20 cc. *in vacuo*.

Treatment of the distillate. To free the oxalic acid from the high concentration of sodium chloride, an excess of 2 cc. 5*N* HCl is added and the solution extracted with ether in a continuous extractor for six hours. To the ether solution 5 cc. of water are added and the ether removed by distillation. The solution is filtered if necessary, made alkaline to methyl red with *N*/10 NaOH and concentrated to about 3 cc. on a water-bath.

Precipitation of the oxalic acid. The solution of oxalic acid in 3–5 cc. water in a 15 cc. centrifuge-tube is faintly acidified with *N*/10 acetic acid and mixed with 2 cc. of saturated lime-water. After standing 12 hours at 0° 0.5 cc. of ammonium phosphate is added. The precipitate is centrifuged, decanted and washed once with 2 cc. of water.

The oxalic acid in the precipitate is estimated by the gasometric method of Van Slyke and Sendroy [1929].

RESULTS.

The following are some results on urine and on urine with added oxalic acid.

Table I.

	mg. (COOH) ₂ found	mg. (COOH) ₂ recovered	% error
25 cc. Urine I	0.47	} 0.45	
" "	0.43		
25 cc. Urine I + 1 mg. (COOH) ₂	1.36	0.91	– 9
25 cc. Urine I + 0.5 mg. (COOH) ₂	0.96	0.51	+ 2
25 cc. Urine II	0.39	} 0.30	0
25 cc. Urine II + 0.3 mg. (COOH) ₂	0.69		
25 cc. Urine III	0.55	} 0.56	
" "	0.57		
25 cc. Urine III + 0.3 mg. (COOH) ₂	0.87	0.31	+ 3
25 cc. Urine III + 0.5 mg. (COOH) ₂	1.08	0.52	+ 4
25 cc. Urine III + 1.0 mg. (COOH) ₂	1.54	0.98	– 2

Comparison with results obtained by ether-extraction of the urine.

250 cc. urine III were heated for twenty minutes on a boiling water-bath with 10 cc. of 10*N* HCl and concentrated *in vacuo* to 100 cc. A further 10 cc. of 10*N* HCl were added and the concentrated urine was extracted with ether for 30 hours in a continuous extractor fitted with a stirrer. After adding 5 cc. water the ether was distilled off. Strong ammonia was added to the residue to give an approximately 2*N* solution of ammonia and the contents of the flask were washed into a 15 cc. centrifuge-tube. 2 cc. *M* CaCl₂ were added and the precipitate left overnight, centrifuged, decanted, the precipitate washed once

with 3 cc. 8*N* ammonia and once with 3 cc. water, stirring the precipitate up with a thin rod at each washing. The precipitate was then heated with 3 cc. *N* HCl, filtered and washed with a further 2 cc. of *N* HCl, and the filtrate was diluted with a few cc. of water. The clear solution was made alkaline to methyl red with ammonia, 0.5 cc. *M* CaCl₂ added and *N* acetic acid till faintly acid. After standing overnight it was centrifuged, decanted, and the precipitate washed twice, each time with 2 cc. of distilled water. The precipitate was dissolved in sulphuric acid as in the Van Slyke method, and the solution transferred to a 10 cc. flask. Aliquot portions of the solution were taken for the gasometric determination.

Duplicate results gave an oxalic acid content in the 250 cc. taken of 4.9 and 5.2 mg. respectively, appreciably lower than the content as determined by distillation (Table I).

The principle of the method has also been applied to the determination of oxalic acid in blood, from which small quantities of added oxalic acid have been satisfactorily recovered. The proteins are removed by phosphotungstic acid. Further details of this work, together with a series of determinations, will be reported in a future communication.

SUMMARY.

A method is described whereby oxalic acid can be quantitatively estimated. By a preliminary esterification and distillation the oxalic acid is freed from interfering substances and can then be precipitated as calcium oxalate and the oxalic acid in the precipitate estimated gasometrically.

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