CLXII. ESTIMATION OF LACTIC ACID IN BIOLOGICAL MATERIAL BY OXIDATION WITH CERIC SULPHATE

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FROMAGEOT & DESNUELLE [1935] proposed a method of estimation of pyruvic acid based on the oxidation of α-ketonic acids by ceric sulphate. The value of the method is diminished by the fact that numerous substances commonly encountered in biological material, e.g. lactic and citric acids, also react with ceric sulphate. The interference by lactic acid is not serious so long as the amount of the latter is small compared with that of the pyruvic acid present and so long as the temperature at which the reaction is carried out is kept low (e.g. approx. 0°). Citric acid is attacked by ceric sulphate even at low temperatures and its possible presence must be taken into account in any estimation of pyruvic acid by ceric sulphate oxidation.

Whilst investigating the magnitude of the error in pyruvic acid estimations caused by the presence of lactic acid in tissue extracts, we found that at relatively high temperatures, e.g. 50°, lactic acid is oxidized quantitatively by ceric sulphate to acetaldehyde. Analysis showed that the reaction obeyed the equation

$$CH_3.CHOH.COOH + 2Ce^{++++} = CH_3.CHO + CO_2 + 2H^+ + 2Ce^{+++}.$$

Lactic acid could be estimated with accuracy by allowing it to react with an excess of standard ceric sulphate solution at 50° for a suitable time and titrating the excess Ce^{++++} with standard ferrous ammonium sulphate solution. This procedure is of little value when applied to biological material owing to the large errors introduced by the presence of other substances attacked by ceric sulphate under the conditions employed. Estimation, however, of the acetaldehyde produced by the oxidation of lactic acid showed that its amount was not materially affected by the presence, in the lactic acid solution, of substances commonly encountered in tissue extracts, so long as excess ceric sulphate was used.

The method we have adopted for the estimation of lactic acid consists of the oxidation of the substance by ceric sulphate to acetaldehyde, followed by estimation of the latter by absorption in sodium bisulphite solution as in the well-known method of Friedemann *et al.* [1927]. We find that the presence of glucose, fructose, starch and a variety of other substances does not interfere with the lactic acid estimation so long as these are not present in abnormally large concentrations. Hence it is unnecessary to remove these substances from tissue extracts before making lactic acid estimations. An estimation of lactic acid in blood requires only treatment with trichloroacetic acid, an aliquot of the filtrate being treated at once with ceric sulphate solution. The same procedure is carried out with tissue brei, e.g. liver or brain.

Details of the estimation of lactic acid by ceric sulphate oxidation

We have found it convenient to use for this estimation the well-known Schrödter flask (Fig. 1), which, besides being relatively inexpensive, takes up little space and can be easily cleaned. Moreover, a battery of such flasks can be

used at once so that a large number of estimations can be carried out quickly. The end of the dropping funnel (*A*, Fig. 1) is bent so that it nearly touches the bottom of the flask.

5 ml. of the fluid, of which the content of lactic acid is to be estimated, are placed in the main vessel (*C*) of the apparatus. In the dropping funnel (*A*) are placed 5 ml. 10% ceric sulphate[1] in *N* H_2SO_4 solution. 5 ml. of 1% sodium bisulphite solution are placed in the absorption tube (*B*) of the apparatus. The apparatus is placed in a water-bath, or thermostat, kept at 50°. It is so arranged that the absorption tube (*B*) is well above the water level in the thermostat, the temperature of the contents of the tube being kept as near that of the room as possible. The dropping funnel (*A*) is now connected to a nitrogen cylinder, the tap of the funnel opened and a slow stream of nitrogen (3 or 4 bubbles a second) is passed through the apparatus. We have found that 60–90 min. are ample for the completion of the reaction and for the complete sweeping out of the acetaldehyde from the reaction vessel into the bisulphite solution, using the quantities of lactic acid mentioned later in this paper. If nitrogen is passed through the flask too quickly, there is incomplete absorption of acetaldehyde by the bisulphite.

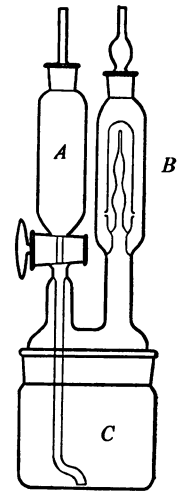

Fig. 1.

If it is found that the ceric sulphate in the reaction flask becomes nearly, or completely, reduced during the course of the experiment, it will be necessary to commence the experiment again using either less of the fluid containing the lactic acid, or a more concentrated solution of ceric sulphate. It is essential that at the termination of the experiment, there should still be excess of ceric sulphate present in the reaction flask.

At the end of the allotted time, the contents of the absorption tube are washed into a beaker. This is easily performed when making use of the type of Schrödter flask in which the reaction vessel is connected with the rest of the apparatus with a ground glass joint (as in Fig. 1). The free bisulphite is titrated with *N*/10 iodine solution and the bound bisulphite, after treatment with $NaHCO_3$, with *N*/50 iodine solution, the procedure being precisely the same as that in the method of Friedemann *et al.* The apparatus used in the latter method can obviously be used in place of the one described above.

Accuracy of the method. The error of the method appears not to be greater than ±5% when using quantities of lactic acid varying from 0·15 to 1·50 ml. of 0·032 *M* solution (i.e. 0·4–4 mg. lactic acid). Typical results are shown in Table I.

Table I

Vol. of 0·032 *M* sodium lactate solution taken ml.	*N*/50 iodine required observed ml.	*N*/50 iodine required calculated ml.	% error
1·5	4·72	4·8	−1·7
1·0	3·14	3·2	−1·9
1·0	3·06	3·2	−4·4
1·0	3·15	3·2	−1·6
0·5	1·64	1·6	+2·5
0·25	0·83	0·8	+3·8
0·15	0·50	0·48	+4·0

[1] We have found the technical grade of ceric sulphate (B.D.H.) to be quite satisfactory. The solution should be filtered before use.

Effects of impurities. The effects of the addition of a variety of substances to a zinc lactate solution on the estimation of the lactic acid by ceric sulphate oxidation were investigated and the results are shown in Table II. The substances included glucose, fructose, starch, pyruvic acid, malic acid, citric acid, ethyl alcohol, oxalic acid, β-hydroxybutyric acid, alanine, malonic acid and tartronic acid, and were added in quantities which might be expected in investigations on biological material. The error of the method did not exceed ±5%. The estimation can be carried out with accuracy in presence of trichloroacetic acid. The presence of urea or of nutrient peptone broth as used in bacteriological work introduced no error.

Table II

Solutions consisted of mixtures of 1 ml. 0·39% anhydrous zinc lactate solution and the volumes of solutions mentioned below, the total volume being made up to 5 ml. with water. The theoretical iodine titration, corresponding to the lactate taken was 3·20 ml. *N*/50.

Solution added to the lactate	*N*/50 iodine titration observed ml.	% error
Nil	3·20	0·0
1 ml. 0·2% *dl*-alanine	3·20	0·0
1 ml. 20% trichloroacetic acid	3·06	−4·3
1 ml. 0·2% glucose	3·06	−4·3
1 ml. 0·2% fructose	3·04	−4·9
0·5 ml. 1% starch	3·25	+1·6
2 ml. 0·3% ethyl alcohol	3·14	−1·9
2 ml. 0·2% pyruvic acid	3·35	+4·7
1 ml. 0·2% malic acid	3·10	−3·3
1 ml. 0·1% citric acid	3·22	+0·5
2 ml. 0·2% tartronic acid	3·12	−2·5
1 ml. 0·2% malonic acid	3·17	−1·0
1 ml. 0·2% oxalic acid	3·04	−4·9
2 ml. 0·2% β-hydroxybutyric acid	3·08	−3·7
0·1 g. urea	3·10	−3·3
1 ml. nutrient peptone broth	3·26	+1·9

The presence of large concentrations of some of these substances, e.g. citric acid, does introduce errors, these being largely due to the reduction of the concentration of the ceric sulphate. If such large concentrations of impurities are expected to be present, the initial concentration of ceric sulphate may be increased to 20%, or the solutions under investigation may be diluted.

Estimations of lactic acid in blood

Human blood, treated with oxalate (20–30 mg. potassium oxalate per 10 ml. blood), is treated with an equal, or approximately equal, volume of 20% trichloroacetic acid. The mixture is centrifuged, and lactic acid estimated directly on an aliquot of the centrifugate. Convenient quantities are 5 ml. blood, and 5 ml. 20% trichloroacetic acid, 5 ml. of the centrifugate being taken for the estimation. Some typical results are shown in Table III. The amount of oxalate used to prevent clotting does not interfere with the estimation. Citrate should not be used to prevent clotting as the presence of this in relatively large quantity causes too great a reduction of the ceric sulphate.

Lactic acid added to blood can be estimated with fair accuracy as shown in Table III and it is evident that the presence of glucose etc. in the blood has not caused any serious error.

Table III. *Lactate estimations on whole blood*

Exp.

1 *a* 5 ml. freshly drawn oxalated human blood, 1 ml. water and 5 ml. 20% trichloroacetic acid; mixture centrifuged, and lactate estimated on 5 ml. centrifugate.

$N/50$ iodine required = 0·34 ml. This is equivalent to $0{\cdot}34 \times \frac{11}{5} \times \frac{100}{5} \times 0{\cdot}9 = 13{\cdot}4$ mg. lactic acid in 100 ml. whole blood.

1 *b* 5 ml. of the blood used in exp. 1 *a*, 1 ml. (anhydrous) 0·39% zinc lactate (= 2·88 mg. lactic acid) and 5 ml. 20% trichloroacetic acid; lactate estimated on 5 ml. centrifugate.

$N/50$ iodine required = 1·70 ml.

Therefore $\frac{11}{5}(1{\cdot}70 - 0{\cdot}34) = 2{\cdot}99$ ml. $N/50$ iodine = titre of iodine equivalent to lactate added to the blood. This is equivalent to 2·69 mg. lactic acid representing 94% of the added lactic acid.

2 *a* 5·0 ml. oxalated human blood, added 1 ml. water and 5·0 ml. 20% trichloroacetic acid; mixture centrifuged and lactate estimated on 5 ml. centrifugate.

$N/50$ iodine required = 0·35 ml. This is equivalent to $0{\cdot}35 \times \frac{11}{5} \times \frac{100}{5} \times 0{\cdot}9 = 13{\cdot}8$ mg. lactic acid in 100 ml. whole blood.

2 *b* 5·0 ml. oxalated human blood used in exp. 2 *a*, 1 ml. 0·39% zinc lactate solution and 5·0 ml. 20% trichloroacetic acid; mixture centrifuged and lactate estimated on 5 ml. centrifugate.

$N/50$ iodine required = 1·72 ml.

Therefore $\frac{11}{5}(1{\cdot}72 - 0{\cdot}35) = 3{\cdot}01$ ml. $N/50$ iodine; theoretical titre = 3·20 ml.

3 *a* 2·4 ml. whipped, freshly obtained, rat blood and 5 ml. 20% trichloroacetic acid; mixture filtered, and precipitate washed thoroughly with water; lactate estimated on the combined filtrate and washings.

$N/50$ iodine required = 0·77 ml.

Therefore lactic acid present in whole rat blood $= 0{\cdot}77 \times \frac{100}{2{\cdot}4} \times 0{\cdot}9 = 28{\cdot}8$ mg./100 ml.

3 *b* 2 ml. whipped, fresh, rat blood, 1 ml. 0·39% zinc lactate solution and 5 ml. 20% trichloroacetic acid; mixture centrifuged and lactate estimated on 3 ml. centrifugate.

$N/50$ iodine required = 1·45 ml.

Therefore lactic acid present in the mixture before centrifuging $= 1{\cdot}45 \times \frac{8}{3} \times 0{\cdot}9$ mg. $= 3{\cdot}48$ mg.

From exp. 3 *a* lactic acid present in the blood $= \frac{28{\cdot}8}{50} = 0{\cdot}58$ mg.

Therefore added lactic acid estimated = 3·48 − 0·58 = 2·90 mg. The amount actually added was 2·88 mg.

Estimation of lactic acid in cerebrospinal fluid

Estimations of lactic acid in cerebrospinal fluid were made without any preliminary treatment with trichloroacetic acid. 5 ml. of the fluid were placed in the reaction vessel and treated at once with the ceric sulphate reagent in the manner already described. Results are shown in Table IV. Lactic acid added to the cerebrospinal fluid was estimated with an error of ± 5%.

Table IV

Exp.	Solution in reaction flask	$N/50$ iodine titration observed ml.	Lactic acid content mg./100 ml.
1	5 ml. cerebrospinal fluid of case A	1·03	18·5
2	5 ml. cerebrospinal fluid of case A + 0·5 ml. zinc lactate (0·39%) solution	2·72 (theoretical = 2·63 ml.)	
3	2 ml. cerebrospinal fluid of case B	0·37	16·6
4	2 ml. cerebrospinal fluid of case B + 0·5 ml. zinc lactate (0·39%) solution	1·90 (theoretical = 1·97 ml.)	

Estimations of lactic acid in tissues

Minced liver or brain was suspended in phosphate buffer solution or saline and treated at once with trichloroacetic acid. The mixture was centrifuged and lactic acid estimated on an aliquot of the filtrate. No special precautions were taken in these experiments to prevent the breakdown of tissue glucose or glycogen into lactic acid. Lactic acid added to the tissue brei was estimated with reasonable accuracy. Details and typical results of experiments are shown in Table V.

Table V. *Lactate estimations on tissues*

Exp.	
1 *a*	4 g. minced rat liver suspended in *M*/5 phosphate buffer *p*H 7·4 to make a total vol. of 12 ml. 5·5 ml. treated with 1 ml. water and 4·5 ml. 20% trichloroacetic acid; mixture centrifuged and lactate estimated on 5 ml. centrifugate. *N*/50 iodine required = 0·32 ml. This is equivalent to $0{\cdot}32 \times \frac{11}{5} \times \frac{12}{5{\cdot}5} \times \frac{100}{4} \times 0{\cdot}9 = 34{\cdot}5$ mg. lactic acid/100 g. rat liver tissue.
1 *b*	5·5 ml. liver suspension prepared in exp. 1 *a*, 1 ml. 0·39% zinc lactate and 4·5 ml. 20% trichloroacetic acid; mixture centrifuged and lactate estimated on 5 ml. centrifugate. *N*/50 iodine required = 1·71 ml. Therefore lactic acid present in mixture before centrifuging $= 1{\cdot}71 \times \frac{11}{5} \times 0{\cdot}9 = 3{\cdot}38$ mg. Lactic acid present in the liver suspension $= 0{\cdot}32 \times \frac{11}{5} \times 0{\cdot}9$ (from exp. 1 *a*) = 0·63 mg. Therefore added lactic acid estimated = 3·38 − 0·63 = 2·75 mg. The amount of lactic acid actually added was 2·88 mg.
2 *a*	5 g. minced sheep brain cortex suspended in *M*/5 phosphate buffer *p*H 7·4 to make a total vol. of 15 ml.; 5 ml. of the suspension, 1 ml. water and 5 ml. 20% trichloroacetic acid; mixture centrifuged and lactate estimated on 5 ml. centrifugate. *N*/50 iodine required = 0·76 ml. This is equivalent to $0{\cdot}76 \times \frac{11}{5} \times \frac{15}{5} \times \frac{100}{5} \times 0{\cdot}9 = 90{\cdot}3$ mg. lactic acid/100 g. sheep cortex tissue.
2 *b*	5 ml. suspension prepared in exp. 2 *a*, 1 ml. 0·39% zinc lactate and 5 ml. 20% trichloroacetic acid; mixture centrifuged and lactate estimated on 5 ml. centrifugate. *N*/50 iodine required = 2·22 ml. Therefore lactic acid present in mixture before centrifuging $= 2{\cdot}22 \times \frac{11}{5} \times 0{\cdot}9 = 4{\cdot}40$ mg. Lactic acid present in brain suspension $= 0{\cdot}76 \times \frac{11}{5} \times 0{\cdot}9$ (from exp. 2 *a*) = 1·50 mg. Therefore added lactic acid estimated = 4·40 − 1·50 = 2·90 mg. The amount of lactic acid actually added was 2·88 mg.

Estimation of lactic acid in urine

It is advisable when making estimations of lactic acid in urine to use as oxidant 20% ceric sulphate in *N* H_2SO_4 solution. Insufficient work has been carried out to determine the order of accuracy of lactic acid estimations in urine by ceric sulphate oxidation, but a few experiments have shown that relatively small quantities of added lactic acid can be estimated with fair accuracy. Typical results are shown in Table VI. In these experiments the urine is treated at once with the ceric sulphate reagent without any preliminary treatment other than filtration.

Table VI. *Lactate estimation in urine*

Exp.	
1	Lactic acid estimated directly on 5 ml. filtered urine. $N/50$ iodine required = 0·35 ml. This is equivalent to $0{\cdot}35 \times \frac{100}{5} \times 0{\cdot}9 = 6{\cdot}3$ mg./100 ml.
2	5 ml. filtered urine, added 0·5 ml. 0·39 % zinc lactate; lactic acid estimated directly. $N/50$ iodine required = 1·85 ml. This is equivalent to $1{\cdot}85 \times 0{\cdot}9 = 1{\cdot}67$ mg. lactic acid. Lactic acid present in 5 ml. urine (from exp. 1) = 0·31 mg. Therefore added lactic acid estimated = 1·67 − 0·31 = 1·36 mg. The amount of lactic acid added was 1·44 mg.

Lactic acid estimation in presence of ferricyanide

The presence of ferricyanide introduces a large error into a lactic acid estimation when using acid ceric sulphate as oxidant; ferricyanide may be easily removed by adding to the solution, made acid with trichloroacetic acid, ferrous ammonium sulphate solution, and a drop of ferric chloride solution if the presence of ferrocyanide is suspected. The amount of ferrous ammonium sulphate added should be just sufficient to react with the ferricyanide present.

The mixture is well shaken, centrifuged and lactic acid estimated on an aliquot of the centrifugate.

Lactic acid added to a 2 % solution of potassium ferricyanide was estimated in this way with 94 % accuracy.

Summary

1. Lactic acid is oxidized by ceric sulphate in acid solution according to the equation

$$CH_3.CHOH.COOH + 2Ce^{++++} = CH_3.CHO + CO_2 + 2H^+ + 2Ce^{+++}.$$

2. The reaction proceeds rapidly to completion at 50° when using the quantities and concentrations of lactic acid described in this paper.

3. The reaction is made the basis of a method for lactic acid estimation in biological material, the acetaldehyde produced being absorbed by sodium bisulphite and estimated iodimetrically.

4. The formation of acetaldehyde is independent of the presence of a variety of substances commonly encountered in biological material, e.g. glucose, malic acid etc., and no preliminary treatment of tissue fluids other than precipitation of protein by trichloroacetic acid is required for the estimation of lactic acid.

5. Details of the method as applied to the estimation of lactic acid in blood, cerebrospinal fluid, urine and tissue brei are described. The error in the estimation of lactic acid does not exceed ± 5 %.

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