LXXV. A METHOD FOR THE DETERMINATION OF LACTIC ACID PRODUCTION WITHOUT DESTRUCTION OF THE MUSCLE.

BY COLIN ASHLEY MAWSON AND ARTHUR DAVID RITCHIE.

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HILL and KUPALOV [1930] have shown that if a thin muscle such as a sartorius from *Rana temporaria* or a small *R. esculenta* is suspended in oxygen-free Ringer solution, and stimulated with not more than six single shocks per minute, the lactic acid diffuses out as fast as it is formed. In this way they were able to estimate the lactic acid formed in a series of about a thousand twitches by titrating the Ringer solution with alkali.

This method is evidently limited to experiments in which a large amount of lactic acid is formed: because (1) to prevent outward diffusion of phosphate from the muscle the Ringer solution must contain enough phosphate to cause considerable buffering; (2) there may always be some interchange of phosphate between muscle and Ringer solution which will alter the acid-base equilibrium; (3) there is some difficulty in eliminating errors due to loss of carbon dioxide from carbonates.

The ordinary method of estimating lactic acid by oxidation avoids all these difficulties. By adapting the method to deal with quantities of the order of 0.1 mg. it is possible to estimate the lactic acid produced in one hundred twitches of a pair of sartorii. The method has the advantage over all others, that the sample for estimation is removed without injury to the muscle. As the sample contains hardly anything but salt solution it can be oxidised directly without further treatment.

The principle errors in estimating small quantities of lactic acid by oxidation to aldehyde and iodimetric titration of the bound bisulphite are manifest in the form of large and variable values obtained in blank estimations. These can to some extent be avoided if, after steaming out the apparatus, an experiment is carried through using lactate solution before a blank estimation is done.

The method selected was to oxidise with freshly precipitated manganese dioxide in the presence of manganous sulphate and phosphoric acid [Friedemann and Kendall, 1929]. The flask is heated in a boiling water-bath and the acetaldehyde distilled over in a current of CO₂ [Tanaka and Endo, 1929]. The distillation apparatus is all glass, with one ground glass joint (see Fig. 1). The

method gives sufficiently low blank titrations for the purpose if the apparatus is kept thoroughly clean. The advantages of the method are considered to be:

- (1) The rubber tubing and stopper for admitting CO_2 are not heated and do not come into contact with the reagents.
- (2) There is no contamination with laboratory air.
- (3) The process is carried out in the practical absence of oxygen.
- (4) The apparatus is nowhere heated above 100° .
- (5) The considerable reflux condensation retains substances of high boiling-point.

Fig. 1. *A*, 50 cc. flask; *B*, ground glass joint; *C*, asbestos shield; *D*, asbestos lagging; *E*, CO_2 inlet.

(Made by the Scientific Glass-blowing Co., Manchester.)

(6) On the other hand, as water is condensed before entering the receiver the aldehyde is already in solution before reaching the bisulphite solution.

(7) The volume of distillate can be controlled by variation of the rate of flow of CO_2 .

EXPERIMENTAL.

A pair of sartorii was dissected out, leaving the bone attachment at both ends. The bones were trimmed down to the smallest convenient size and all other tissue cut away. A piece of silver wire was threaded through the two pieces of bone at the tibial end and another silver wire, passing through a small rubber stopper, hooked over the bone at the pelvic end. After soaking for half an hour in oxygenated Ringer solution the muscle was introduced into a narrow tube closed by the stopper at the lower end. The tube was clamped in a holder the height of which was adjustable by a screw, and the wire at the tibial end attached to an isometric tension lever. A measured quantity of the

Ringer solution to be used was introduced, the volume being adjusted so that the muscle would be just covered (4.0 cc. with the tube in use) when the sample extracted before twitches were commenced (1.0 cc.) had been removed.

The muscle was stimulated, by means of current supplied *via* the silver wires from a flashing neon lamp circuit. After the muscle had been subjected to any particular treatment a sample of the Ringer solution was removed, and the lactic acid estimated. For this estimation the apparatus was swept out with a current of CO_2 , and a slow gas current allowed to continue. The flask was immersed in the water-bath and the delivery tube dipped into 2 cc. of dilute sodium bisulphite solution (1 cc. of saturated solution in 100 cc. of water) contained in a 25 cc. conical flask. The lactate solution was run into the flask and washed through with 2.5 cc. of manganous sulphate (2.0 %) and phosphoric acid (1.0 %) mixture. 5 cc. of manganese dioxide suspension (1 cc. of $N/10 \text{ KMnO}_4 + 9 \text{ cc. of MnSO}_4 - \text{H}_3\text{PO}_4$ mixture) were run slowly in, and the flask left for one minute. A fairly rapid current of CO_2 was then passed for 12 minutes, by which time a distillate of 4 to 5 cc. had collected. A drop of starch solution was added, and the excess bisulphite removed with $N/20$ iodine in 2 % KI solution, the final adjustment being made with $N/500$ iodine. 0.5 cc. of saturated sodium bicarbonate was then added and the liberated bisulphite titrated with $N/500$ iodine.

An end-point lasting at least 60 seconds after vigorous shaking must be obtained. A micro-burette is used with a fine tip dipping under the surface of the liquid. The iodine solution is standardised against a pure specimen of lithium lactate, and blanks are done before and after a series of experiments and the mean value used as the true blank.

The total lactic acid is calculated from the lactic acid found in the sample, on the assumption that the lactate is evenly distributed throughout the water present and that muscle contains 80 % water and bone 50 %. As the volume of Ringer solution added is large compared with that of muscle and bone, and as the sample removed for estimation is usually 90 % of the whole, the errors in the calculation are small, provided uniform distribution of lactate is actually obtained. Where, however, at the end of an experiment the whole of the fluid is to be used and made up to a known volume, the muscle is extracted with successive small portions of Ringer solution.

In the following series of experiments a known amount of lactate was added to a muscle and, after soaking for 10 minutes to allow diffusion to take

Exp. No.	Muscle No.	Mg lactic acid		
		Added	Observed	Error
98 b	1	0.037	0.036	- 0.001
99 c	2	0.186	0.181	- 0.005
101 a	4	0.075	0.081	+ 0.006
101 d	5	0.075	0.080	+ 0.005
103 a	6	0.071	0.069	- 0.002
103 b	6	0.071	0.070	- 0.001
104 a	7	0.071	0.064	- 0.007
104 b	7	0.071	0.076	+ 0.005

place, the amount of lactate in the Ringer solution was estimated as described above. The muscle was kept on ice during the diffusion period to prevent formation of lactic acid due to resting metabolism.

SUMMARY.

A method is described for the estimation of amounts of lactic acid of the order of 0.1 mg. without destruction of the muscle.

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