THE LACTIC ACID MAXIMUM OF CARDIAC MUSCLE.

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THE following experiments were carried out as a first step in the investigation of the chemical changes taking place during the contraction of cardiac muscle and consist in comparative estimations of the lactic acid maximum of cardiac and skeletal muscle. The investigation has since been carried out in further detail by Hines, Katz and Long(1), using mammalian muscle, and their results are in good agreement with mine.

Estimation of Lactic Acid.

Before starting the experiments, the method by which the lactic acid was to be estimated had to be chosen and investigated. It was obvious that the amount of material available would necessitate the use of a micro-method, and the ones considered were those of Meyerhof(2,3) and of Clausen(4).

In the first place, the technique of the permanganate oxidation process of Clausen was compared with that of Meyerhof, using a solution of zinc lactate. Clausen's method was found to give excellent results both as regards the actual percentage recovery of lactic acid and its variation. In Meyerhof's method the percentage recovery was smaller and showed a much greater variation from one experiment to another. Moreover, Clausen's method presented fewer difficulties of manipulation than that of Meyerhof and afforded a considerable saving in time. The percentage recovery was of the same order of magnitude as that found by Clausen himself, that is, from 92–95 p.c. when using from 2·5–4·0 mg. of lactic acid. It was therefore decided to carry out the process of extraction from muscle and purification according to Meyerhof's technique, with the introduction of certain modifications, and to carry out the oxidation, distillation and titration according to Clausen.

The main steps of the process were as follows: the muscle was ground in a mortar with alcohol and powdered glass; the mixture was transferred to a beaker and allowed to stand over night. It was filtered first

through muslin and then through paper and the muscle residue extracted with fresh alcohol, twice for amounts of muscle under 1·5 grm. and three times for amounts over 1·5 grm. These washings were evaporated on a water bath and the original filtrate was added last and the whole taken down to 1–2 c.c.; this procedure reduced to a minimum the decomposition of lactic acid by heat. The residue, which had usually evaporated to dryness on standing, was extracted four times with 2–3 c.c. of saturated ammonium sulphate solution and filtered at the pump through a small asbestos filter, which was connected by an adapter to a separating funnel. The adapter was rinsed with 2 c.c. of 4 p.c. caustic soda and the volume made up to 15 c.c. with distilled water. The solution was extracted three times with 2 c.c. of benzene, to remove traces of alcohol, the upper benzene layer being removed by a pipette attached to the pump. The last traces of benzene were removed by evaporation under reduced pressure, by connecting the separating funnel to the pump. This was considered an improvement on the method used by Meyerhof of again evaporating on a water bath, during which there is always a risk of decomposing some lactic acid.

The solution was then roughly neutralised by the addition of 2 c.c. of 0·1 N sulphuric acid and then, according to the amount of lactic acid present, either transferred direct to the oxidation apparatus or else made up to 50 c.c., of which two or three aliquot parts of 10 c.c. were used for oxidation. The latter procedure was adopted for two reasons; in the first place it was thought that there would be a smaller relative loss in the intermediate processes of the estimation if a larger quantity of lactic acid were used; whilst in the oxidation process, perfectly consistent results had been obtained with less than 2 mg. of lactic acid, which is the amount that can be most rapidly and most efficiently dealt with. In nearly every case very close agreement (*i.e.* to within 0·1 mg. lactic acid) was obtained between the two estimations and when this did not occur a third aliquot part was used.

The oxidation was carried out in all details according to the method described by Clausen. The end-point of the titration was always found to be perfectly clear-cut and the difficulties of lack of permanence, mentioned by Clausen and also experienced by Long(5), did not occur.

Controls on the complete process were carried out on zinc lactate solution and on muscle in rigor mortis with and without added zinc lactate. Whilst estimations on two or more portions of the same muscle gave perfectly consistent results, those on zinc lactate and on muscle with added zinc lactate showed wide variation. Zinc lactate is recom-

mended by several workers as being the most suitable salt of lactic acid for control estimations and, as already stated, gave excellent results on the oxidation process alone. It was ultimately realised that these inconsistencies must be due to the small solubility of zinc lactate, which, in certain cases, prevented its being completely re-dissolved when the residue from the evaporation of the original alcoholic solution was treated with saturated ammonium sulphate solution. There is, at this stage of the process, a considerable deposit of ammonium sulphate on the dish, which would readily obscure particles of zinc lactate.

Control estimations were repeated, using lithium lactate, and quite consistent results were obtained (Table I).

TABLE I. Estimation of Lithium Lactate Solution. Complete Process.
5 c.c. solution = 8·95 mg. lactic acid.

No.	Amount taken mg. HL	Amount estimated mg. HL	Percentage loss
1	8·95	7·87	12·0
2	8·95	7·59	15·2
3	8·95	7·27	18·8
4	8·95	7·65	14·5

Mean percentage loss = 15·1.

The percentage recovery of the complete process was found to be 85 p.c.

The animals used for the comparative estimations on cardiac and skeletal muscle were the frog and the tortoise. The weight of the heart of an ordinary frog is less than 0·1 grm., but it was hoped that by using the *Rana esculenta* (variety *ridibunda*) obtained from Hungary, which is many times larger than the ordinary frog, estimations could be carried out on two or three hearts. Unfortunately, the heart was found to be small in comparison with the rest of the body, so that to obtain results of any accuracy a large number had to be used.

As the supply of these frogs was spasmodic, experiments were also carried out on the tortoise, which has a heart sufficiently large for estimations to be made on one only.

Method.

Frog. The ventricle only was used. It was cut longitudinally with scissors and the two pieces pressed between filter paper to free them from blood. The muscle from several hearts was weighed in a weighing bottle. Cotton-wool soaked in chloroform was inserted between the stopper and the bottle and the whole was placed in an oven at 40° C. for an hour.

The hearts, in any one experiment, were all taken from the same batch of frogs.

The skeletal muscle was taken from one of these frogs and consisted of either one gastrocnemius or several leg muscles.

Tortoise. The ventricle was cut into several pieces, pressed between filter paper and put into rigor as above.

The skeletal muscle was taken from the legs and from inside the shell. It was found difficult to get a representative sample, since it showed noticeable variation in composition, according to the site from which it was taken.

Results.

In calculating the true lactic acid value from that estimated, the average loss is taken to be that of the lithium lactate controls, that is, 15 p.c.

The preliminary experiments on cardiac muscle of both frog and tortoise (Tables II A and III A) were made before one or two of the

TABLE II. Lactic Acid Maximum. Cardiac and Skeletal Muscle.

FROG.

	Cardiac					Skeletal				
A. (Frog arrived 29. viii. 22):										
No.	No. of hearts	Muscle grm.	HL estimated mg.	True HL value mg.	Percentage HL in muscle	No.	Muscle grm.	HL estimated mg.	True HL value mg.	Percentage HL in muscle
1	3	0·629	0·78	0·92	0·146	—	—	—	—	—
2	4	1·006	1·22	1·44	0·143	—	—	—	—	—
		Mean percentage HL = 0·145								
B. (Frogs arrived 10. v. 23):										
3	8	1·281	1·85	2·18	0·169	8	2·572	6·24	7·34	0·285
4	6	1·160	1·80	2·12	0·182	9	2·603	8·23	9·68	0·372
		Mean percentage HL = 0·175					Mean percentage HL = 0·328			
Ratio—cardiac : skeletal = 0·53.										
C. (Frogs arrived 30. viii. 23):										
5	20	3·526	7·13	8·40	0·238	10	1·404	6·46	7·60	0·541
6	14	1·993	5·51	6·48	0·325	11	1·638	7·54	8·87	0·541
7	15	1·818	4·38	5·16	0·284	12	1·387	4·67	5·49	0·396
	—	—	—	—	—	13	2·066	9·56	11·26	0·545
		Mean percentage HL = 0·282					Mean percentage HL = 0·506			
Ratio—cardiac : skeletal = 0·56.										

later improvements in technique had been introduced. All the other estimations were carried out according to the final routine; aliquot parts were taken for oxidation except in the case of the determination on

TABLE III. Lactic Acid Maximum. Cardic and Skeletal Muscle.

Tortoise.

A.		Cardiac					Skeletal				
No.	Date	No. of hearts	Muscle grm.	HL estimated mg.	True HL value mg.	Percentage HL in muscle	No.	Date	Muscle grm.	HL estimated mg.	True HL value mg.	Percentage HL in muscle
1	Jan. 1923	1	1·157	2·40	2·82	0·240						
2	„	1	0·58	1·49	1·75	0·303						
3	„	1	0·915	1·90	2·24	0·244						
4	Feb. 1923	1	0·644	0·645	0·76	0·118						
5	„	1	0·75	2·42	2·85	0·379						
		Mean percentage HL = 0·257										
B.												
6	Feb. 1923	4	3·715	8·55	10·06	0·270	10	Feb. 1923	2·550	6·12	7·19	0·282
							11	„	2·060	9·22	10·84	0·514
7	Apr. 1923	4	3·641	9·05	10·65	0·293	12	Apr. 1923	2·946	8·07	9·50	0·322
							13	„	3·056	9·65	11·35	0·372
8	Sept. 1923	1	1·409	3·54	4·17	0·295		Sept.				
9	„	1	1·537	3·44	4·05	0·263	14	1923	1·902	7·39	8·70	0·464
		Mean percentage HL = 0·280							Mean percentage HL = 0·391			

Ratio—cardiac : skeletal = 0·72.

cardiac muscle of Tables II A and B and III A where the amount of material used was not large enough for division.

Determinations on cardiac and skeletal muscle of the same animal are tabulated on the same line. In Table III B, estimations 10 and 11 are on skeletal muscle of the same tortoise, as also estimation 12 and 13.

The lactic acid maximum of frogs' cardiac muscle is in every case lower than that of the corresponding skeletal muscle. Estimations on different batches of frogs are entered in separate tables; the values for the two maxima show a fair agreement with one another within each batch, but those of the two series differ from each other by a marked seasonal variation.

The results may be best expressed as the ratio of the cardiac mean percentage to the skeletal mean percentage, which is 0·53 and 0·56 respectively.

The lactic acid maximum of tortoise's skeletal muscle shows considerable variation from one estimation to another, even when two determinations are made on the muscle of one animal (cf. Nos. 10 and 11). These variations can only be explained as being due to differences in the composition of the muscle; that from the legs often contained much connective tissue so that it was not always possible to obtain a sample

consisting only of muscle fibres. The values for cardiac muscle are more consistent and, taking the mean of both, the cardiac maximum is seen to be definitely lower than the skeletal maximum, though the difference is less marked than it is in the case of the frog, the ratio of the cardiac mean percentage to the skeletal mean percentage being as much as 0·72.

The theoretical deductions from these results are the same as those put forward by Hines, Katz and Long.

SUMMARY.

Comparative estimations of the lactic acid maximum of cardiac and skeletal muscle show that, in every case, the cardiac maximum is lower than the skeletal maximum.

For the frog, the average ratio of the lactic acid maximum of cardiac muscle to that of skeletal was found to be 0·5, whilst for the tortoise it was found to be 0·7.

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