CLX. STUDIES ON THE PHYSIOLOGY OF PLAIN MUSCLE.

IV. THE LACTIC ACID CONTENT OF PLAIN MUSCLE UNDER VARIOUS CONDITIONS.

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APART from the very general belief that most tissues may readily be made to yield lactic acid, there is very little to indicate the presence of that substance in plain muscle. It is true that Vincent and Lewis [1901] found, as Lehmann had done before, that the normally alkaline reaction of the tissue tended towards neutrality or acidity when the tissue was kept, and further that some of the older observers, employing the rough tests which were then available, claimed to have demonstrated the presence of lactic acid in plain muscle. Yet there is a remarkable contrast between the intimate knowledge which has been yielded by the application of modern methods to the study of the metabolism of striated muscle, and the almost complete ignorance which prevails with regard to the chemical properties of plain muscle. The pivotal importance of lactic acid in the physiology of striated muscle induced me to study the relation, if any, which this substance might bear to the physiology of plain muscle.

PRESENCE OF LACTIC ACID IN PLAIN MUSCLE.

Various methods have been employed for the detection and estimation of lactic acid. When this work was commenced six years ago, large amounts (from 20 to 700 g.) of tissue were worked up by Fletcher and Hopkins' [1907] method as used for frog's striated muscle. In using the larger amounts there was no doubt some loss, but it was certain that the amount of lactic acid present was small compared with that in striated muscle. Thus, from 700 g. of muscle from the horse stomach, which had been minced and slowly heated to boiling, I obtained an amount of crystalline zinc salt corresponding to only 0.043 g. lactic acid. In other experiments with the zinc method, using muscle from the small intestine of the dog, and the ox retractor penis, the lactic acid content of the fresh tissue was found to vary from 0.012–0.076 %. It would serve no useful purpose to give details of these earlier and more

imperfect experiments; but it was possible, from them, to state that lactic acid did occur, though only in small amount.

Analysis of the crystalline zinc salt obtained in some of these experiments and dried over calcium chloride gave the following results:

Exp.	% water of crystallisation (theory requires 12.9 %)	% ZnO in dry salt on incineration (theory requires 33.4 %)
1	13.3	35.1
2	11.8	—
3	12.7	37.8
	Mean = 12.6	Mean = 36.5

The salt was obviously not quite pure.

Specimens of the salt gave Hopkins' thiophene test: when distilled with 0.25 % sulphuric acid and a little manganese dioxide, acetaldehyde was formed, which was recognised by its smell, as well as by Schiff's test, the mirror test, and the iodoform reaction. An attempt to determine the optical rotation of the free acid was unsuccessful because too little of it was obtained. I have nevertheless no hesitation in saying that sarcolactic acid is recoverable in small amounts from plain muscle from various sources.

Methods.

In addition to the method used by Fletcher and Hopkins, I have also tried the extraction method described by Wolf [1914] and the various procedures recommended by Meyerhof [1920, 1; 1921], but finally have found that, with slight alterations, the extraction method of Hirsch-Kauffmann [1924] together with the lactic acid estimation as described by Clausen [1922] gives the most regular and accurate results. The outlines of the treatment of material for resting controls are as follows: from 3–6 g. of the tissue is cooled in a beaker surrounded by a mixture of ice and salt, and weighed. The tissue is placed in a nickel crucible, which is wrapped in a duster; liquid carbon dioxide is then blown into the crucible, which is quickly filled with solid CO₂, in which the tissue is frozen. While thawing it is cut up with scissors and dropped into 10 cc. of ice-cold 4 % HCl saturated with NaCl; about 1 g. of white sand is added and the whole ground up in a mortar; 10 cc. of 5 % mercuric chloride is then added, and the mixture left overnight. It is then filtered through a dry paper into a graduated cylinder, the residue being wrung out in muslin. The volume of the filtrate is noted, and it is then carried through the process exactly as described by Hirsch-Kauffmann, removal of carbohydrate being effected by 2 cc. of 10 % CuSO₄ and 5 cc. of 25 % milk of lime. With the quantities stated, the amount of lactic acid in the final filtrate will be about half that present in the first mixture of tissue and reagents: the loss from adherence to precipitates and filter papers can, of course, be calculated from the ratios of the volumes of the materials at each stage of the process.

To the final filtrate used for the lactic acid estimation enough dilute sulphuric acid is added to make 0.5 % concentration. Oxidation by permanganate and the subsequent titration of the aldehyde bisulphite compound are carried out exactly as described by Clausen, and need not be further described. For tissues subjected to various preliminary treatments, slight and obvious modifications of the initial stages are introduced. Thus when treated with fluids, the fluid was added to the initial mixture and its volume allowed for.

The tissues employed were the following: of mammals, the intestine, uterus, oesophagus and bladder of the cat, the intestine of the dog, and the retractor penis muscle of the bullock; of cold-blooded animals the stomachs of the frog and tortoise (*Testudo graeca*). The tortoise stomach proved best of all, because of the regularity of the resting controls, due to the fact that lactic acid did not accumulate very rapidly after removal of the tissue from the body. The tissue also shows no spontaneous contractions. When an experiment on one portion of tissue was contemplated, the stomach was removed, opened, and the mucosa stripped off from the intestinal towards the oesophageal end: the tissue which remains weighs about 4–6 g. and has been shown by histological examination to consist almost entirely of plain muscle, the fibres of which run in a roughly circular direction, as in the frog. The band of tissue is divided longitudinally into two portions, one of which serves as the control, and the other for the experiment. When it was desired to carry out a number of experiments against a single control, the tissue from several stomachs was prepared, cut into pieces about 1 cm. square which were thoroughly shuffled, and samples of 3–6 g. used for control and experiments. The variation of the resting lactic acid value in different stomachs is as a rule so small that no appreciable error is introduced by this method of cutting and shuffling.

Mammalian tissues are much more difficult to employ; there is a rapid post-mortem accumulation of lactic acid and there is the further difficulty that in most cases the tissue exhibits spontaneous rhythmic contractions, whilst for various reasons the difficulty of obtaining representative samples for controls is much greater. In the small intestine, for instance, the resting lactic acid values depend on the degree of spontaneous activity, which as pointed out by Alvarez [1922] shows a diminishing gradient from above downwards. I have already shown [1923] that the oxygen usage of intestinal muscle shows a similar gradient, so that I was not surprised to discover the following resting lactic acid values, estimated by Fletcher and Hopkins' method, for the plain muscle from different levels of the dog's intestine:

Level	Exp. 4 %	Exp. 5 %	Exp. 6 %
1 (highest)	0.062	0.06	0.076
2	0.046	—	—
3	0.036	0.047	0.039
4 (lowest)	0.024	—	—

In order, therefore, to carry out a satisfactory experiment on intestinal

muscle, it is necessary to select a portion of the gut and to divide the muscle lengthwise, using one part as a control and the other part for the experiment; a cutting and shuffling method would, I think, be unsafe, so that a large series of experiments against one control could not easily be carried out.

"Resting" values of lactic acid.

From none of the tissues examined was lactic acid entirely absent, even when the tissues were in the freshest condition. The value is always low however, particularly in the plain muscle of cold-blooded animals, but not so low as in long-rested skeletal muscle. The following table gives some typical results.

Table I. *Resting values of lactic acid.*

Tissue	Lactic acid %		
Small intestine of dog	0.029	0.046	} mean = 0.042
	0.051	0.036	
	0.012	0.024	
	0.062	0.060	
	0.047	0.076	
	0.039	0.031	
	0.038	—	
Intestine of cat	0.178		
Retractor penis of bullock	0.076	0.072	} mean = 0.139
	—	0.27	
Cat uterus	0.141	0.024	} mean = .083
Cat oesophagus	0.05		
Cat bladder	0.214		
Frog stomach	0.03		
Tortoise stomach	0.056	0.029	} mean = 0.027
	0.013	0.013	
	0.032	0.029	
	0.014	0.016	
		0.020	

Methods of fixation of the tissue. It is very important to be able to kill the tissue and to fix the lactic acid precursor, whatever it may be, in such a manner as will lead to the minimal production of lactic acid during the killing. In the case of frog's striated muscle, cooling in liquid air or even in ice and salt is no doubt adequate, but it does not necessarily follow that this method will yield the best results with plain muscle, which often shows strong contractions on cooling. I have therefore compared the cooling method, using liquid carbon dioxide, with killing by crushing in ice-cold 4 % HCl, and with killing by dropping into boiling 4 % HCl, with the following results:

	Lactic acid %	
	(1)	(2)
Fixed in boiling 4 % HCl	0.014	0.026
Control frozen and fixed in cold 4 % HCl ...	0.016	0.023
Fixed in ice-cold 4 % HCl, and at once ground up	0.02	—

This experiment shows that the three methods give practically identical results; the boiling method is less satisfactory in one respect, namely, that some hydrolysis of protein occurs, so that, after treatment with mercuric chloride, there is a tendency for the liquid to froth during the subsequent passage of H₂S and air, and the cold methods seem to be preferable on that account.

The effect of stimulation on the lactic acid content.

For this purpose the tissue was suspended in a moist chamber containing air or oxygen and stimulated for 1–2 seconds at intervals of 20 or 30 seconds with a galvanic current of sufficient strength to cause the maximum contractions, these being recorded by a lever. The current required varied from 5–20 milliamperes. This stimulation was repeated, by means of a rotating key, until the muscle showed definite signs of fatigue. Meanwhile, the control sample of tissue was suspended in a similar moist chamber and both samples were fixed simultaneously.

Table II. *Effect of repeated stimulation.*

Tissue	Stimulation	Lactic acid %
12 frog stomachs (20°)	0 (control)	0.03
	5 ma. at 45'' intervals for 2 hrs.	0.04
Cat bladder (38°)	0 (control)	0.214
	15 ma. at 20'' intervals for 1 hr.	0.241
Tortoise stomach (20°)	0 (control)	0.029
	5 ma. at 60'' intervals for 1 hr.	0.085
Tortoise stomach (20°)	(Control)	0.013
	25 ma. at 20'' intervals for 2½ hrs.	0.068
	25 ma. at 20'' intervals for 3½ hrs.	0.086
Frog striated muscle (20°)	(Control. Not quite resting)	0.10
	45 mins. tetanus	0.34

The experiments show that there is an undoubted production of lactic acid during contraction, but the amounts so formed are small when compared with those set free by striated muscle. This must be either because the recovery process is abnormally rapid, or because the liberation of lactic acid comes early to a standstill, or else because the content of lactic acid precursor is lower in plain than in striated muscle. As we shall see, the last reason is an important one, but the second one probably also holds good.

The effect on plain muscle of conditions which in striated muscle cause rigor mortis.

Table III.

Tissue	Treatment	Lactic acid %
(1) Dog intestine	Gradually heated to 70°	0.083
	Control	0.029
(2) Dog intestine	30 mins. in CHCl ₃ -Ringer	0.032 0.069
	Control	0.012 0.047
(3) Bullock retractor	Gradual heat to 80°	0.144
	Control	0.076
	Sudden heat to 80°	0.079
(4) Bullock retractor	4 hrs. CHCl ₃ -Ringer	0.32
	Control	0.27
(5) Cat uterus	CHCl ₃ vapour 2 hrs.	0.14
	Control	0.14
(6) Tortoise stomach	Gradual heat to 60°	0.078
	Control	0.056
(7) Tortoise stomach	30 mins. in <i>M</i> /15 Na ₂ HPO ₄	0.043
	plus 0.4 % caffeine	
	30 mins. in <i>M</i> /15 Na ₂ HPO ₄	0.059
	Resting control	0.026
(8) Frog skeletal muscle (May)	CHCl ₃ vapour for 2 hrs.	0.182

This table shows that, with the exception of series 5 and 7, treatment, which applied to skeletal muscle would lead to the onset of *rigor*, causes also in plain muscle a rise in the lactic acid content. The maximum for plain muscle is, however, usually lower than that which would be expected for striated muscle. Perhaps this is partly due to the less efficient buffering power of plain muscle, but the store of lactic acid precursor being smaller also limits the amount of acid formed.

In series 5, exposure to chloroform vapour produces no alteration in the lactic acid content; this is perhaps due to the fact that the control already shows the lactic acid maximum.

In series 7 it is seen that, although there is an increase in the lactic acid after 30 minutes' immersion of the minced tissue in a phosphate solution containing 0.4 % of caffeine, this increase is much smaller than that found on immersion in the phosphate alone; the caffeine appears to have inhibited the production of lactic acid. Although it would be unsafe to draw a conclusion from the result of a single experiment, this fact, if confirmed by subsequent study, might perhaps be related to the further fact that whereas caffeine exerts a stimulating action on striated muscle, its effect on plain muscle seems to be more usually to cause relaxation. This question, together with the further question of the relation of lactic acid production to contraction must be deferred for the present.

The effect of mincing in buffered solutions.

The rate of formation of lactic acid in tissue which has been minced in buffer solutions of different hydrogen ion concentrations was studied in a manner similar to that used for striated muscle by Laquer [1914] and Meyerhof [1921]. The tissue, 3–6 g., was weighed, placed in 5 cc. of the buffer solution, minced therein with scissors, left for the desired time, and then killed by the addition of 10 cc. of ice-cold 4 % HCl followed by immediate disintegration with sand in a mortar.

Table IV. *Immersion of minced tissue in buffer solutions.*

Duration of exp.	Tortoise stomach muscle (18.5°). % lactic acid formed in				
	$M/15 \text{ NaH}_2\text{PO}_4$ (p_H 4.5)	$M/15 \text{ Na}_2\text{HPO}_4$ (p_H 9.0)	$M/15 \text{ Na}_2\text{HPO}_4$ plus 0.30 % Na_3AsO_4	0.8 % NaHCO_3 (p_H 9.5)	Control
15 mins.	—	0.043	0.047	—	0.026
30 "	—	0.059	0.061	0.051	0.026
1.25 hrs.	—	0.081	0.086	—	0.023
2.5 "	—	0.078	—	0.087	0.023
3.25 "	0.054	—	—	—	0.024
3.25 "	—	0.089	—	—	0.032
5.75 "	0.058	—	—	—	0.024
5.75 "	—	0.087	—	—	0.032
19.5 "	0.162	0.165	—	—	None
24 "	—	—	0.13	—	0.026

In some of the experiments 0.3 % of sodium arsenate was added to the disodium phosphate solution to compare the results with those obtained under similar conditions by Meyerhof with striated muscle. The results are shown in Table IV and are plotted in Fig. 1.

Fig. 1.

The experiment shows that, as in striated muscle, there is a rapid accumulation of lactic acid in the disodium phosphate solution, and a still more rapid one in the alkaline arsenate solution; the amount finally formed (in 20 hours) in tortoise stomach muscle would seem to be about 0.13–0.165 %, and it would appear that this represents complete conversion of all the precursor into lactic acid; at all events, I have not been able to exceed that amount by any treatment of the tortoise stomach muscle.

In the acid phosphate solution, on the other hand, owing to the acid reaction, the rate of lactic acid formation is much slower, in which again there is close resemblance to what happens in skeletal muscle; but bicarbonate solutions do not seem to differ sensibly from the alkaline phosphate solution. This may be either because the amount of tissue was great when compared with the volume of the bicarbonate solution in which it was suspended, so that the phosphate concentration was still adequate, or because the phosphates do not play such an important part in the conversion of lactacidogen into lactic acid in plain as in skeletal muscle. The point must be decided by further experiments.

Comparative experiments made with the striated muscle of the tortoise showed that it behaves in a similar manner to that of the frog under comparable conditions. Although the lactic acid maximum attained with plain muscle is much below that for striated muscle, it would appear that the relative rate of development is at least as great, if not greater, in plain muscle; thus in disodium hydrogen phosphate with or without arsenate, more than half the

maximal yield is obtained within an hour, the increase over the resting value being about 200 % in the arsenate solution, though only about 50 % in monosodium dihydrogen phosphate solution.

Lactic acid formation in anaerobiosis and in survival in oxygen.

So much light has been thrown on the chemical processes in striated muscle by the study of the effects of anaerobiosis, that the results of similar experiments on plain muscle cannot fail to be instructive. Experiments were therefore carried out to compare the lactic acid content of the tissue after various periods of aerobic and anaerobic survival; anaerobiosis was effected by immersing the tissue in frog Ringer solution containing $N/1000$ NaCN, or by exposing the tissue without immersion in fluid to a continuous current of moist commercial nitrogen; in the one case comparison was made with tissue suspended in oxygenated Ringer, and in the other case with tissue placed under similar conditions in a stream of commercial oxygen. In all cases the tissue was used in pieces about 1 cm. square; undue injury due to mincing was thus obviated, but the procedure had the drawback that in spite of the thinness of the pieces of tissue, the oxygen supply during the aerobic periods could not have been completely efficient; the tissue suspended in oxygenated Ringer was agitated by shaking at frequent intervals in an atmosphere of oxygen, but in the experiments in which the tissue was exposed directly to the moist gas, the latter could usually only have access to one surface of each piece of tissue. Therefore the aerobic periods were in reality periods in which a supply of oxygen was offered. The results indicate that the access of oxygen was slightly behind the requirement, so that there was some accumulation of lactic acid; this was also the case in most of the experiments of Meyerhof on striated muscle.

The results of these survival experiments are given in Figs. 2 and 3. These graphic results speak for themselves; during anaerobiosis there is a rapid accumulation of lactic acid in the resting tissue, whilst during aerobic survival the lactic acid mounts up very slowly indeed. It would seem that the lactic acid maximum is almost reached at the end of about 8 hours' survival at room temperature; when the asphyxiated muscle is returned to oxygen, the lactic acid begins to disappear again; this disappearance occurs even after the tissue has been in nitrogen for 20 hours, as the ensuing 7 hours in oxygen show. It would seem that with protracted survival, the efficiency of recovery gets less and less, so that after 20 hours in nitrogen followed by 24 hours in oxygen (not shown in the figure), there is more lactic acid present than there was after the tissue had been in nitrogen for 20 hours and in oxygen for 7. Nevertheless, in spite of this, there was less lactic acid after the survival of 44 hours, the first 20 of which were in nitrogen, than there was after a survival of only $6\frac{1}{2}$ hours all of which was spent in nitrogen.

The maintained low resting level of lactic acid when the tissue is kept in oxygen, together with the demonstrated removal of lactic acid when the

anaerobically surviving tissue is restored to oxygen, provide incontrovertible evidence that, as in striated muscle, there is a continuous removal, by oxidative means, of the lactic acid which is being formed continuously as a result of metabolic processes in the tissue. There is, then, in oxygen, some sort of a recovery process, and the next step is to enquire whether or not this is of the same nature as that which occurs in striated muscle. There are three ways by which this problem may conveniently be approached, namely, by the study of the thermal aspects, from the oxygen consumption, and from the carbohydrate content. I have carried out attempts along the last two directions.

Fig. 2.

Fig. 3.

Fig. 4.

The oxygen requirement of resting plain muscle.

The oxygen requirement of resting plain muscle was determined by the use of the microrespirometer, a sensitive instrument of the Krogh pattern, bottles of about 10 cc. capacity being used. Thin strips of tissue from the

experiment of Fig. 2 were cut with a sharp razor, and floated in 1 cc. of Ringer's solution: the apparatus was shaken horizontally, with the bottles immersed in a water-bath at room temperature (17°). The rate of oxygen usage was determined each hour for 6 hours, and the results per g. per hour are given in Fig. 4. The results were high during the first half-hour and were rejected; this high value is probably due to oxidative removal of lactic acid formed in the process of preparation involving handling and oxygen lack. In the succeeding hours the oxygen usage also showed a slow decline towards a steady value of about 15 mm.³ per g. per hour. I have shown in a previous paper [1923] that after a period of oxygen lack there is an accelerated rate of oxygen usage, and the evidence which has now been put forward indicates that this is associated with oxidative removal of lactic acid, much as happens in striated muscle.

It is easy to calculate, as Meyerhof has done for striated muscle, to what extent the oxygen utilisation corresponds to the lactic acid reduction. Each milligram of lactic acid which is oxidised necessitates the utilisation of 744 mm.³ of oxygen. In the experiment shown in Fig. 2 there has been in nitrogen an accumulation of lactic acid which has raised the percentage in 6 hours from 0.049–0.116 %, or an increase of 0.67 mg. per g. of tissue, whilst in oxygen the increase in the same time is only 0.12 mg. per g.; thus, the presentation of oxygen has in 6 hours prevented the formation of 0.55 mg. lactic acid. (If there had been complete lack of oxygen from the very beginning (*i.e.* no free oxygen present in the tissue), even more lactic acid would have accumulated.)

This amount of lactic acid would require 410 mm.³ of oxygen to oxidise it, and, if the lactic acid were removed entirely by oxidation, the tissue should use 410 mm.³ oxygen in 6 hours; but adding up the oxygen usage from the experiment shown in Fig. 4, we find that actually only 123 mm.³ of oxygen were used in that time. That is to say that only about one-third of the lactic acid is oxidised (actually 1/3.35), the remainder being removed in some other form. This so closely resembles what happens in skeletal muscle, that, although I have no final proof for it, it seems almost certain that what has happened to the remaining two-thirds of the lactic acid is a restoration to the lactic acid precursor, presumably glycogen.

Relation of carbohydrate to the origin of lactic acid.

For the investigation of this question I have made some determinations of the glycogen content, and of the total hydrolysable carbohydrate content of plain muscle under various conditions, but with results which are at present puzzling, and which require further examination. Glycogen has been estimated by a micro-adaptation of Pflüger's method, the glycogen thrown down by alcohol being collected by centrifugation, and being finally hydrolysed by 2 % HCl at 100° for 3 hours: determination of the glucose so produced was carried out by the Shaffer-Hartmann method. Hydrolysable carbohydrate was determined by initial hydrolysis of the fixed tissue by 2 % HCl, depro-

teinisation by HgCl_2 followed by removal of mercury by H_2S , neutralisation, and determination by Shaffer-Hartmann. In other cases extraction has been carried out by cold alcohol, glycogen estimated in the residue, and soluble carbohydrate in the filtrate after deproteinisation by colloidal ferric hydroxide [cf. Meyerhof, 1920, 2]. Some results are given in Table V.

Table V. *Carbohydrate content of plain muscle.*

Tissue	State	Glycogen %	Soluble carbo- hydrate %	Total carbo- hydrate %	Lactic acid %
Retractor	Fresh	0.012	—	—	0.072
	Rigor	0.022	—	—	0.079
Cat uterus	CHCl_3	None	—	—	0.141
Intestine (dog)	Fresh	0.005	—	—	0.178
Retractor	Fresh	0.018	0.061	—	0.27
	Fresh	0.032	0.055	—	0.22
	CHCl_3	0.014	0.062	—	0.32
	Fresh	0.05	—	0.51	0.02
Tortoise stomach	Cyanide (21 hrs.)	0.014	—	0.495	0.13

There are three striking features about these estimations; (1) the low value of the glycogen, (2) the relatively high value of the remaining carbohydrates, especially in the tortoise tissue, (3) the fact that where there is a comparison between the lactic acid and glycogen of resting and treated tissues, the increase in the lactic acid is many times greater than the decrease in the glycogen content. Three explanations suggest themselves: (1) that the lactic acid does not arise from carbohydrate at all, (2) that it arises from some carbohydrate other than glycogen, but that the results of the total carbohydrate determinations are spoilt by the production of carbohydrate from some other source in the course of hydrolysis, (3) that the glycogen estimations are unreliable. I incline to the last-mentioned opinion, for reasons now to be stated.

Similar experiments carried out on frog's striated muscle gave satisfactory results: for these, I used 1 or 2 g. of muscle. Expecting, from the lactic acid maximum, that the glycogen content of plain muscle would be of the order of 0.1–0.2 %, I therefore used 5 or 6 g., or in some cases 20 g. of tissue, but the results were always low. Now the technique used was in both cases the same, namely, for each g. of tissue there was used 1 cc. of 60 % KOH for hydrolysis, then 2 cc. water were added, and 4 cc. alcohol: in the reprecipitations 2 cc. water and 4 cc. alcohol were used; the volume of fluid from which precipitation took place would thus be disproportionately great when the glycogen content of the tissue was small, and the relative loss would tell very seriously on the result.

In order to test this explanation, I have made the following simple experiment: frog's striated muscle was minced at -5° , and was divided into samples of various weights; in two of these the glycogen was estimated in the usual

way, but in others the alcohol precipitation was carried out with much larger volumes of fluid. The results are shown below:

Exp.	Weight of tissue g.	Volume of fluid from which glycogen was precipitated (cc. per g. tissue)	Minimal weight of glycogen present (calculated from Exp. 1)	Volume of fluid per mg. minimal glycogen content cc.	Yield of glycogen %	Apparent glycogen content of tissue %
1	1.8	8.3	14.7	1.06	100	0.82
2	0.46	13.0	3.75	1.6	62	0.50
3	0.44	34.0	3.6	4.3	23	0.19
4	0.26	58.0	2.13	7.3	14	0.12

It is evident from these results that the greater the volume of dilute alcohol from which the glycogen is thrown down, the lower the results obtained.

This is also shown by the following results of experiments in which equally small amounts of glycogen were estimated, but in which the volume of precipitating fluids was kept as small as possible throughout:

Exp.	Wt. of tissue g.	Vol. of fluids per mg. minimal glycogen content cc.	Yield of glycogen, calc. from Exp. 1 (%)	Apparent % glycogen in tissue
1	1.73	0.32	100	0.88
2	0.63	0.31	103	0.91
3	0.32	0.47	98	0.86

Hence, in carrying out the estimation of such small amounts of glycogen as must be present in plain muscle, the volume of alcohol should be adjusted, not to the weight of the tissue taken, but to the weight of glycogen likely to be present. Experiments of this nature are now in hand.

In this lack of correspondence between the glycogen content and lactic acid maximum, as well as in some other respects, plain muscle appears to bear a strong chemical resemblance to cardiac muscle, which has recently been investigated by Katz and Long [1925] and by Hines, Katz and Long [1925].

SUMMARY.

1. Lactic acid is present in small amount in "resting" plain muscle. (0.03–0.08 %; the larger amounts in mammalian tissue.)
2. The amount of lactic acid is increased when the muscle is made to contract. The lactic acid maximum is never so high as in skeletal muscle from the same species.
3. The amount of lactic acid is increased by treatment which in skeletal muscle produces *rigor*. The highest amount thus found was 0.32 %, and usually it was below 0.2 %.
4. When placed in solutions of phosphates, the lactic acid production is much greater when the p_H of the solution is 9, than when it is 4.5. The rate of lactic acid formation in disodium hydrogen phosphate solutions is accelerated by the presence of arsenates. The rate of acid production, under the conditions of experiment, was much the same in 0.8 % NaHCO_3 as in disodium hydrogen phosphate solution.

5. The lactacidogen content for the muscle of the tortoise stomach (Autumn) is of an order corresponding to about 0.15 % lactic acid.

6. Lactic acid rapidly accumulates in plain muscle when this is kept under anaerobic conditions, but scarcely at all when kept in oxygen.

7. The oxygen usage of resting plain muscle indicates that the recovery process in oxygen is of much the same nature as that in skeletal muscle; actually the fraction oxidised under experimental conditions was about one-third.

8. Owing, it is thought, to the errors incidental to the determination of small amounts of glycogen, it has not been possible, up to the present, to demonstrate that lactic acid arises from glycogen, or indeed, from any carbohydrate. In any case the glycogen content of the tissue is small, though, when allowance is made for the possible errors of experiment, perhaps large enough to make it possible that glycogen is the parent substance from which lactic acid is formed.

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