ON THE ALLEGED FORMATION OF LACTIC ACID IN MUSCLE DURING AUTOLYSIS AND IN POST-SURVIVAL PERIODS.

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CONTENTS.

THE evidence available at present for determining the type of chemical action involved in the formation of lactic acid in muscle, or in other cells, is not only scanty but, so far as it goes, conflicting.

The actual course of the formation of dextro-rotatory lactic acid which proceeds spontaneously in excised muscle has been described[1], and this has been shown to present two notable features. First, the yield of acid during the survival periods of declining irritability proceeds by equal increments in equal times, and second, the yield comes to a standstill when irritability is finally lost. Thenceforward, during post-survival periods after complete loss of irritability, the lactic acid content of the muscle remains constant. From these, in connection with other observations, we concluded that it is only living, or surviving muscle, which has the means of lactic acid production.

On the other hand, it has been urged by several observers in recent years that considerable and continued production of *d*-lactic acid may be found during the autolysis (aseptic or antiseptic) of minced or crushed

[1] Fletcher and Hopkins. This *Journal*, xxxv. p. 247. 1907.

muscle, long after the extinction of irritability and destruction of structure; and in addition to this evidence of an enzymic and hydrolytic production of the acid from muscle substance or muscle *detritus*, it has been claimed that enzymes can be found in muscle, or prepared from it, which are able to produce lactic acid from added carbohydrate. These muscle enzymes are said to simulate the action of the known ferments whose property is to yield lactic acid as an intermediate stage in the final production of CO_2 and alcohol from carbohydrate material.

Obviously, if these results of autolytic study were firmly established, the distinction previously drawn between living (or surviving) muscle and dead muscle would become meaningless, except in so far as it would become necessary to determine the manner in which the enzymes producing lactic acid, as it is alleged, and acting freely in dead muscle or in muscle extracts, were subject to control in the living muscle. The main object of the present paper however is to show that the evidence which has so far been produced in favour of an enzymic yield of acid from muscle in post-survival periods or in autolysis is not trustworthy, and that we have no reason to suppose that either the muscle cell or its forcibly released contents have any power of lactic acid formation under other conditions than those of life, or of "survival" periods coupled, probably, with a greater or less degree of subsisting irritability.

It will be urged that the results of autolytic experiments have been commonly misinterpreted through misapprehension of the effects upon muscle of the treatment incidental to the methods employed. It is obvious that a necessary preliminary for the proper measurement of autolytic change is the determination not only of the spontaneous changes to be expected after excision in the particular tissue under examination, but also of the changes produced in it by the agencies of heat or of the antiseptic media which a given method may involve before autolysis begins. Some account of these will be given therefore now before proceeding to the results of autolysis.

I. Lactic acid formation in injured mammalian muscle.

The experiments in autolysis to which critical reference is to be made later deal chiefly with mammalian muscle, and, from the nature of the case, with injured muscle. The present section is concerned only with estimations of *d*-lactic acid in injured mammalian muscle; I hope to publish shortly an account of the production of acid in uninjured mammalian muscle.

Methods. In all the observations to be given in this and the later sections, the *d*-lactic acid was estimated by gravimetric determination of anhydrous zinc lactate, according to the methods fully described in the paper already referred to, and it will not be necessary to repeat these here. No modification in manipulation was called for, except that it was found advisable, and in some cases necessary, to increase by 50 % the weight of the blood-charcoal used for the treatment of the alcohol residues. The filtrate from the charcoal flask was always crystal clear. Control observations showed that the use, within wide limits, of additional charcoal introduced no errors if due care was taken in washing the charcoal on the filter with hot water.

The final crystalline products obtained had in all cases the same characters as those previously described for amphibian muscle. They were quite free from pigment, they gave crystals characteristic of pure zinc lactate and yielded zinc oxide in percentages close to those expected by theory.

In all the experiments with mammalian muscle to be given below the muscle was rapidly dissected immediately after cessation of circulation and minced at once in a freshly cleaned mincing machine. When two or more similar samples were required for purposes of comparison, the fragments of minced muscle were thoroughly mixed and shuffled before the separate samples were rapidly weighed out. This is an important precaution if closely similar samples are to be obtained, and of special importance in the case of the rabbit, where the anatomical segregation of the white and red muscle-fibres is well marked. When an estimation was to be made, the minced muscle was ground in a mortar with sand under 98 % alcohol; the alcohol extraction was then carried out as usual. In autolysis experiments, where chloroform-Ringer was used, the supernatant fluid, drained from the muscle before grinding under alcohol, was taken down on the water-bath to dryness and the residue added to the ground muscle under alcohol for extraction.

It was found impossible to obtain similar muscle samples—and without these there can be no fair results—by using muscle from more than one rabbit in the same experiment without special precaution. It was found difficult to obtain them even with careful mixing of the muscle among all the different samples. Many factors may disturb the weight relations of the muscles, and chief among these seems to be the amount of fat present: the weight of zinc lactate expressed in percentage of original muscle for one sample may vary widely—by 25 %—from that of another sample similarly treated throughout but

obtained from another rabbit. The difference may be unimportant or absent if care be taken that the rabbits are of exactly similar breed and condition, but this is not always easy to secure. The point is emphasised here because it does not seem to have been attended to by previous observers: in many published experiments the weights of muscle used show that two or more rabbits were used, mixing has not been referred to, and variations in the final lactic acid estimations of the different samples, expressed in percentage of original muscle weight, are derived, or are at least as probably derivable, from original disparity of sample, as from other supposed causes to which they have been assigned.

The rate of development of lactic acid after injury.

The effects of mechanical injury and of other agencies upon the yield of lactic acid have been described already for the case of amphibian muscle[1]. It has been shown that freshly excised frog's muscle, after the severe traumatic injuries of cutting or mincing, exhibits a greatly accelerated rate of lactic acid production. It was found, however, that the infliction of cutting injury to the muscle "does not produce instantaneously an acid yield, maximal for that injury, but rather sets up a rapid acid-yielding breakdown which at first quickly, and then more slowly, approaches a maximum."

The course followed in time by the changing rate of acid production after injury was determined: at a room-temperature (15° C.) the maximum yield was not attained until 10 hours from excision and injury, though after five hours 90 % of the maximum had been reached.

For the case of mammalian muscle I find similar relations. The rate of acid yield after severe injury is accelerated, but here also, even after the most extensive mechanical injuries, such as the finest mincing of the muscle, its maximum though very rapidly approached is not reached instantaneously. Having reached its maximum development—the maximum being determined by the character and previous condition of the muscle—the lactic acid content remains thereafter constant for many hours, and even, as we shall see, for many days.

The acceleration due to injury, like the rate of the spontaneous survival production in undamaged muscle, is closely dependent on the temperature. In the case of the frog muscle it was comparatively easy by successive estimations at a temperature so low as 15° C., which is within physiological limits for the frog, to follow the curve relating the

[1] Fletcher and Hopkins. *Loc. cit.* p. 261.

acid yield to lapse of time, and it was shown that this curve, unlike the linear graph for undamaged muscle, had exponential characters. In the case of warm-blooded muscle the difficulties of obtaining a comparable curve for acid yield after injury are very great. If the temperature be maintained at or near the body-temperature (38—39° C. for the rabbit) from the moment of excision, throughout the manipulations of mincing and weighing, up to the time of extraction and estimation, the increase of acid occurring in the muscle fragments may be so rapid that its course in detail can hardly be followed: the maximum yield may be reached in less than half an hour. The following experiment illustrates this.

Exp. I. Room-temp. 24·5° C. Rabbit. All manipulations conducted as closely as possible to body-temperature. Mincing machine warmed to 39° C. Rapidly dissected muscle minced, mixed and divided to five equal sets, each 90·2 gms. Ringer solution saturated with chloroform used for antisepsis.

		Anhydrous zinc lactate
(*a*)	Extracted for estimation immediately* after mincing and weighing	·397 %
(*b*)	Incubated at 39° C. in 200 c.c. chloroform-Ringer for ½ hour ...	·489
(*c*)	,, ,, ,, ,, ,, 2 hours ...	·493
(*d*)	,, ,, ,, ,, ,, 4 ,, ...	·495
(*e*)	,, ,, ,, ,, ,, 8 ,, ...	·493

* Within three minutes from mincing the muscle was ground with sand in alcohol for extraction, the interval being occupied by shuffling and weighing from the other sets.

By lowering the temperature during the infliction of injury, the acid production is slowed, and even if the injured muscle be brought immediately after the injury to body-temperature again, the attainment of the acid maximum is delayed. Without elaborate precautions lowering of the muscle temperature must inevitably happen when the manipulations are carried out at room-temperature: during removal of the skin and excision of the muscle, during mincing or chopping, and during mixing and weighing, the temperature must fall below, and often falls far below, the normal 38—39° C. The muscle just afterwards will always show a lactic acid yield decidedly below the maximum attainable later. If now the muscle be placed for antiseptic incubation in chloroform-Ringer at body-temperature, in which on being stirred every fragment of muscle is quickly brought again to normal temperature, the acid increases very rapidly at first, and then less rapidly approaches the acid maximum, which is not attained fully for two hours or even more. A typical experiment is given in Exp. II, and the results shown graphically in Fig. 1. In this particular case the acid maximum shown

(in percentage of original muscle weight) is a high one. For rabbit muscle the maximum varies in individual cases between ·45 % and ·65 % of anhydrous zinc lactate.

Exp. II. (Fig. 1.) Room-temp. 18·7° C. Rabbit. Muscle excised and minced without special precaution. Mincing and weighing occupied 7 mins. during which cooling towards room-temp. was not prevented. Muscle mixed and divided to five weighed sets. Of these, one was ground at once in alcohol for extraction, the others placed each in 270 c.c. chloroform-Ringer which had previously been brought to 39° C.

			Zinc lactate
(*a*)	76·3 gms.	estimated at once	·302 %
(*b*)	72·5 ,,	incubated at 39° C. for ½ hour ...	·570
(*c*)	72·5 ,,	,, ,, 1½ hours ...	·645
(*d*)	74·7 ,,	,, ,, 4 ,, ...	·674
(*e*)	73·6 ,,	,, ,, 11 ,, ...	·667

(ZnO determination, (*d*) and (*e*) combined, gave 33·38 %. Theory 33·42 %.)

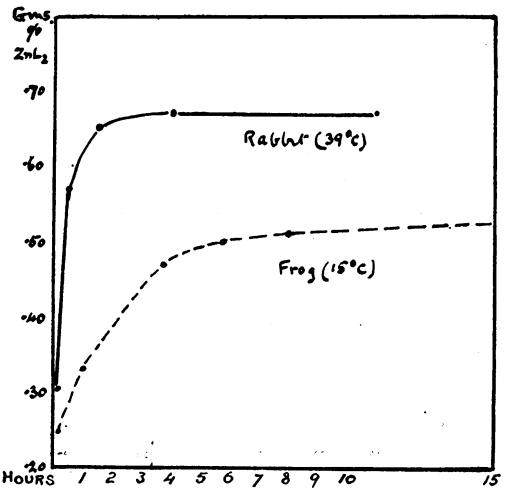

Fig. 1. The course of lactic acid production after severe mechanical injury. The rabbit muscle was minced between body (39° C.) and room (18·7° C.) temperatures. The first estimation was made immediately: thereafter the muscle was incubated at 39° C. in chloroform-Ringer solution (Exp. II). The dotted line gives for comparison the course of production by frog's muscle at 15° C. (with no antiseptic solution added) from Fletcher and Hopkins (*loc. cit.*), Fig. 1, p. 261.

The course followed by the successive rates of acid yield in a warm-blooded muscle, injured in the cold but allowed thereafter to return to body-temperature, is of course not strictly comparable with the course followed by the acid yield of the amphibian muscle kept at constant temperature throughout. It will be noticed, however, that the mammalian muscle shows after the injury an acid development more rapid

at first and slower afterwards (see Exps. II and IV), in spite of the earlier periods including, as they do, the lower temperatures from which the muscle is being raised. The present object however is not to establish the detailed time relations of the acid yield after injury, but to show, for the purposes of the next section, that the muscle just after injury gives an acid yield inevitably below that reached later at body-temperature. How far it will be below the later acid maximum in a given case will depend on the degree to which the temperature has sunk during the infliction of injury, and the rapidity with which estimation is made afterwards.

In the autolysis experiments to be referred to later, the preparation of the muscle by mincing or chopping has been carried out, in previously published observations, without special precautions at room-temperature, and for comparison with these Exp. II probably offers typical relations.

It is clear, in both the typical experiments I and II, that when the acid maximum has been attained it remains at constant level for many hours, and indeed, as will be seen later, for as long as observation is maintained, supposing no interference to come from outside, by bacterial infection or otherwise. Any pair of observations made after the establishment of the maximum (after about three hours at body-temperature) will give nearly identical estimations of lactic acid. This is seen again in later experiments, and is shown in Exp. III.

Exp. III. Room-temp. 13° C. Rabbit. Muscle minced, mixed and divided into two samples. Each incubated with 200 c.c. chloroform-Ringer, with toluol, added at room-temp.

		Zinc lactate
(*a*)	79 gms. incubated at 38° C. for 3½ hours ...	·486 %
(*b*)	85·5 gms. ,, ,, ,, 24 ,, ...	·525

If cooling of the muscle during injury be carried further, below room-temperatures, the retardation of acid yield and the delay in attainment of the acid maximum are of course more markedly shown. An example is given here.

Exp. IV. Room-temp. 13·5° C. Rabbit. Muscle rapidly excised and frozen, minced in machine cooled in freezing mixture. Mixed and divided in cooled dishes into three parts.

		Zinc lactate
(*a*)	102·9 gms. ground at once in ice-cold alcohol and estimated at once	·176 %
(*b*)	95·6 ,, incubated at 38° C. for ½ hour	·286
(*c*)	88·7 ,, ,, ,, 10 hours	·474

(Chloroform-Ringer in double volume, and toluol, added for incubation.)

The important point to be emphasised here, especially in view of the methods ordinarily used in published experments upon autolysis, is that the lactic acid development in muscle after very severe mechanical disruption *does not reach its maximum instantaneously.* At body-temperature the acid development is very rapid after injury, but if the muscle be injured (as by cutting or mincing) at ordinary room temperature, the acid maximum will not be fully attained for a time to be measured by hours, while the muscle estimated immediately, or at room-temperature after several minutes, after the injury will commonly show 50 % less than the maximum which would be reached later.

It is wholly erroneous to assume that minced muscle, taken immediately for estimation, is in a state of chemical equilibrium in regard to its properties of lactic acid production. It would seem unnecessary to point this out if the assumption had not very commonly in the past been made the basis of experimental work.

The influence of chloroform-water.

The irritant effect of chloroform upon muscle is well known, and it has probably been a common assumption that the use of this agent as an antiseptic during autolysis has secured that an almost immediate maximum of the acid yield of the tissue, apart from possible autolytic processes, has been attained. In point of fact, however, the application even of chloroform vapour to muscle does not induce a rate of acid production notably greater than that following severe mechanical injury[1]. The following experiment shows the difference in yield of acid shown by minced mammalian muscle kept at 38° C. in Ringer solution, and in Ringer solution saturated with chloroform, respectively, and it will be seen that though the addition of chloroform to the Ringer solution has hastened the approach to the maximum yield of acid, it has not hastened it so to change markedly the order of change in time. The irritant effect of the chloroform is nearly overshadowed by the results of the previous mechanical disruption.

EXP. V. Room-temp. 19·5° C. Rabbit. Muscle minced rapidly, mixed and divided to four nearly equal sets.

			Zinc lactate
(*a*)	67·9 gms. + 160 c.c. Ringer,	after 2 hours at 38° C.	·555 %
(*b*)	67·5 gms. + 160 c.c. chloroform-Ringer,	,, ,, ,,	·604
(*c*)	67·6 gms. + 160 c.c. Ringer,	after 4 hours at 38° C.	·600
(*d*)	67·7 gms. + 160 c.c. chloroform-Ringer,	,, ,, ,,	·628

(ZnO determination, (*a*) and (*b*) combined, gave 33·6 %. Theory, 33·42 %.)

[1] Fletcher and Hopkins. *Loc. cit.* p. 265.

This experiment illustrates again what was shown in the last section; the muscle was manipulated at room-temperature, and it is seen that the acid production has not reached its maximum in either pair of samples before the fourth hour of incubation at 38° C.

It will be noticed that in many published experiments on the autolysis of tissues in which a comparison is made between a control estimated "at once" and a similar sample incubated with chloroform-water, we have presented to us not only the expected difference in acid yield due to the non-attainment of the acid maximum by the former sample of the pair, but, added to this, an increment in the yield of the latter sample, due to the chloroform employed.

The "fixing" effect of rapid heating.

The effect of rapid heating in arresting chemical change in surviving muscle, which was first noticed by Du Bois Reymond[1], was described in some detail by Fletcher and Hopkins[2] for amphibian muscle. We showed that while resting muscle may yield ·04 % of zinc lactate, which would be increased to ·50 % or more if the muscle were brought to full heat-rigor at 40—45° C., similar samples killed rapidly at 100° C. may yield no more than ·08 %.

The point is easily demonstrated in the case of frog's muscle because individual and almost undamaged muscles can be brought very rapidly to a high temperature. In the case of rabbit muscle rapid heating of all parts of the muscle is best secured by mincing the muscle masses directly into boiling water. Even so, although mechanical injury and some time delay must precede the heating, the fixing effect is well marked. The boiled muscle invariably shows a lower lactic acid content than a corresponding sample of minced muscle allowed to proceed near body-temperature towards its maximal yield.

Exp. VI. Room-temp. 13° C. Rabbit. Muscle minced, rapidly mixed, divided and weighed to two samples.

		Zinc lactate
(*a*)	157 gms. thereupon boiled immediately ...	·416 %
(*b*)	196·5 gms. incubated at 38° C. for 45 minutes	·526

Exp. VII. Room-temp. 15° C. Rabbit. Muscle minced, rapidly mixed and divided to two samples.

		Zinc lactate
(*a*)	79·0 gms. thereupon thrown into boiling water	·250 %
(*b*)	81·2 gms. + 250 c.c. chloroform-Ringer, incubated at 39° C. for 7 hours	·543

(ZnO determination, (*a*) and (*b*) combined, gave 33·46 %. Theory, 33·42 %.)

[1] *Muskel- und Nervenphysik*, II. p. 18. Leipzig, 1877.

[2] *Loc. cit.* p. 267.

In both experiments the estimation of (*a*) includes of course the extract from the water used in boiling.

In Exp. VII four muscle masses also were dissected with the least possible cutting injury to the fibres from the back and the upper and lower hind leg (to give a mixture of muscle equivalent to a "shuffled" set), and these were plunged into boiling water and so kept at boiling temperature for 45 minutes. The muscles weighed 79·5 gms. On parallel estimation with set (*a*) already given, it was found that

	Zinc lactate
(*c*) muscle in bulk, nearly uninjured, boiled at once, gave	·375 %
[(*a*) similar muscle, minced and boiled at once, gave ...	·250 %.]

The result shows that whether minced to small pieces or not, the muscle rapidly heated shows less lactic acid than the survival maximum, but the reduction in yield is less if large pieces of muscle are used. This is to be expected since the minor parts of large muscle masses escape rapid heating and probably proceed far towards their acid maximum at high but not supra-physiological temperatures before the upper temperature limit for the survival yield of acid is reached.

It is worth notice in passing that during the processes of estimation it is made obvious to casual inspection that other chemical changes in the muscle besides those leading to lactic acid formation have been influenced by the rapid heating. The muscle which has been allowed after injury to undergo change at body-temperature [(*b*) in each of the two preceding experiments] when compared with the muscle rapidly heated [(*a*) in each case] gives not only more lactic acid on estimation but can be distinguished from it by the appearances presented by the successive extracts prepared, and very obviously also by an increased pigmentation in the water extract prepared for shaking with ether.

II. The alleged formation of lactic acid in the autolysis of muscle.

In his early observations upon the autolysis of muscle, Salkowski[1] found no formation of ether-soluble acids after the prolonged autolysis of minced mammalian muscle placed under ten times its bulk of chloroform-water, and he concluded that "it is much more probable that muscle produces lactic acid not because it is dying but because it is living, and it produces it only so long as it lives."

The confidence to be placed in his results, and in the suggestion he based upon them, is lessened by the circumstance that he found no lactic acid at all in the freshly minced mammalian muscle, for this is contrary to general experience. Schwiening[2], however, using similar

[1] *Ztschr. f. klin. Med.* xvii. (Supp. Bd.), p. 77. 1890.
[2] *Virchow's Arch.* 136, p. 444. 1894.

methods, did find lactic acid present in the minced muscle (though only in "very slight quantity"), and he confirmed Salkowski's observation that in the process of auto-digestion the yield of acid was not increased. His quantitative results however show that his methods of extraction or of estimation were imperfect, since only ·09 % of zinc lactate is given for the yield of the mammalian muscle after mincing (instead of the ·4—·6 % which is to be expected with confidence) and only ·08 % for the yield after 48 hours autolysis.

These results in Salkowski's laboratory have been the object in recent years, as is well known, of much hostile criticism. Magnus Levy[1] found that lactic acid increased during the autolysis of liver tissue and he made the not very reasonable objection that Salkowski had not taken long enough periods for his autolyses, though these extended over 68 hours. Certainly Magnus Levy's own results are not free from the objection that he disregarded the possibility of survival change in the disrupted liver tissue—change which may fairly be presumed to occur until the contrary has been shown—and reckoned this as autolytic change.

A group of Japanese observers have extended Levy's methods to the study of other tissues. Mochizuki and Arima[2] have observed an increase of lactic acid in the antiseptic autolysis of the testis, Kikkoji[3] finds the same, using the same methods, for the spleen, Saito and Yoshikawa[4] for the thymus and for the lungs, and Inouyi and Kondo[5] for mammalian, and other muscle. All these have used a smaller bulk of antiseptic medium than Salkowski: they have used in relation to the tissue mass examined twice instead of ten times the bulk of chloroform-water. Inouyi and Kondo found their results opposed to those of Salkowski whether they used the double bulk or his ten-fold bulk of medium: and they concluded that an increase of dextro-rotatory lactic acid is a result of muscle autolysis.

Frew[6], under Salkowski's direction, has published the results of a few experiments in opposition to Inouyi and Kondo. He employed a confessedly rough method of quantitative estimation. Using Salkowski's ten-fold volume of chloroform water, without toluol, in the hopes of avoiding any bacterial growth, he found a marked disappearance of lactic acid after autolysis in two cases, but a nearly three-fold increase

[1] *Hofmeister's Btr.* II. p. 283. 1902.
[2] *Zeitschr. f. physiol. Chem.* XLIX. p. 108. 1907.
[3] *Ibid.* LIII. p. 415. 1907.
[4] *Ibid.* LXII. p. 107. 1909.
[5] *Ibid.* LIV. p. 481. 1908.
[6] *Ibid.* LX. p. 15. 1909.

in a third case. Using, like Inouyi and Kondo, Levy's double volume of chloroform-water, with toluol, he found putrefaction to occur in all cases, with an increase of lactic acid in two cases and a decrease in one. These results are obviously inconclusive as they stand, while many of the individual estimations depart so widely from probability (*e.g.* ·04 % of zinc lactate, or only "traces" of it, from freshly minced muscle; ·03 %, ·07 %, ·06 %, found for muscle after 72 hours' autolysis), that they lose significance.

Neither Salkowski's early results then, nor those of Schwiening or of Frew in his laboratory, can fairly be set against those of Inouyi and Kondo, for the reasons which have been mentioned, and the Japanese results must so far be considered to hold the field. In spite however of the accuracy and coherence of their quantitative estimations, it can be shown that grave fallacies underlie their experimental methods. It is in the methods of control observation that these fallacies appear, and it may be worth while to set these out with some fullness because, though we are only concerned here with the case of muscle autolysis, they may be found commonly among observations made in recent years upon autolysis in a variety of other tissues.

In the experiments referred to, two chief methods are adopted for the demonstration of an autolytic increase of lactic acid in the muscle preparation. In each the autolysed muscle is compared with a similar muscle used as a control which has not been the seat of autolysis, either (*a*) because it was extracted and its acid yield estimated immediately after mincing, or (*b*) because it was boiled for the destruction of enzymes before autolytic digestion began. In either case it has been generally assumed, and is so assumed by Inouyi and Kondo, that the control muscle preparation, whether obtained by method (*a*) or (*b*), may be regarded as a controlling standard of maximum spontaneous acidity, and that any excess of lactic acid beyond this standard which may be found many hours later in similar muscle after autolytic digestion is due wholly to autolytic changes. No evidence to support it has been advanced by any of the observers who have relied upon this assumption, and the facts given in the preceding sections show that it is unfounded and misleading.

Under the method of control (*a*), the control muscle, estimated directly after excision or mincing, will invariably contain less lactic acid than similar muscle kept near body-temperature (as in autolytic digestion) for a few or for many hours. To what degree the acid yield of the control muscle estimated early will fall short of the maximum of

acid attained later by its companion preparation will depend upon two chief factors. Its yield will be diminished (1) as the temperature of the mincing and extractive processes is lowered below body-temperature, and (2) as the time occupied by manipulation between excision and estimation is shortened. Whatever the degree of difference may be in a given case, it is the difference between an incomplete and a complete attainment of survival acid maximum, and it illustrates nothing else. Yet in all their experiments of this type Inouyi and Kondo assign to the results of autolysis the whole increase of acid found in muscle digested for many hours as compared with muscle freshly excised. They give no particulars of the temperature of mincing, and it may be presumed to have been at room-temperature, or at least below body-temperature, and they make no mention of the time relations of their processes. It seems clear that the whole "autolytic" increase claimed in four out of their eleven published experiments may be assigned to survival changes after injury, and the most significant part of the increase claimed in two others.

The method of control (*b*) provides an experimental fallacy of another kind, not less serious. It has been used not only by Inouyi and Kondo but by other observers of autolytic change. In this a sample of freshly minced tissue is boiled and then digested, side by side with an equivalent but unboiled sample. If the unboiled sample yields more lactic acid than the control—and this, in the case of muscle at all events, is always to be expected—the increase is assigned wholly to the action of enzymes not destroyed. From what has been said of the "fixing" effects of rapid heating in muscle it will be seen that the arrest of lactic acid formation in the boiled control will take effect at a lower point in the scale, in proportion as it occurs rapidly after excision and as the temperature between excision and boiling has been lowered. In some of the experiments of Inouyi and Kondo (and of others) the tissue is not boiled until after rapid mincing, presumably at room-temperature and decidedly below body-temperature, and the injury survival changes must have been well advanced. But the results already given on p. 294 show that even so the discrepancy between boiled and unboiled samples is well marked. The discrepancy is due to unarrested survival change in the unboiled sample, and there is no justification for claiming it as the result of autolytic digestion.

Of the eleven experiments in which Inouyi and Kondo show an increase of lactic acid during autolysis of muscle, we have seen that four depend upon the fallacies already discussed under control method (*a*);

six others are based on the control method (*b*) in which the arrest of acid formation due to rapid heating is ignored, and the results of these again cannot be accepted as demonstrating an autolytic increase.

The manner in which "survival" changes after injury have been reckoned as autolytic changes may be seen most clearly by comparison of two actual experiments. Inouyi and Kondo give, for instance, the following figures for rabbit muscle, in their Exp. IV (*loc. cit.*), p. 486:

(*a*)	boiled at once, then incubated 2 days at 38° C. ...	·29 % lactate
(*b*)	incubated 2 days at 38° C.	·54 ,,

In Exp. VII, given above (p. 294), we have for comparison

(*a*)	boiled at once, and estimated	·250 ,,
(*b*)	incubated at 39° C. for 7 hours	·54 ,,

The difference in lactic acid content between (*a*) and (*b*) is assigned by Inouyi and Kondo to the action of autolytic enzymes. An intermediate observation would have shown that the same difference is present after two hours, or a little more, as after two days. Their experiment gives in fact a demonstration that between the earliest hours of incubation and the end of the second day there has been no further increase due to autolytic change. They have shown also that the acid yielded is the dextro-rotatory acid. This is of course to be expected, since it is the acid produced by the "survival" processes of muscle which follow injury.

The only evidence which has been advanced of autolytic increase by Inouyi and Kondo which is not explicable in terms of survival change is that derived from experiments extending beyond the first day from excision. This evidence is much less striking; it shows a relatively slight increase of lactic acid in autolysis after the first day in three cases, a marked but irregular increase in one case, and a decrease in another. Reference will be made to the results of long extended autolyses below, when the agencies used for avoiding bacterial contamination are discussed.

In my own experiments I have found no indications of any increase of lactic acid in muscle incubated under antiseptic conditions, when the early increase attributable to accelerated survival change is ended. It has already been seen that after the maximum acid yield of survival following injury has been attained the muscle shows no further increase beyond this maximum during the succeeding hours of incubation at body-temperature (38—39° C.)

Further examples showing the same relations will appear incidentally in the experiments to be given in a later section, and it will be unnecessary to multiply them here. I have never obtained any evidence pointing to an autolytic increase of acid in addition to the survival yield. The maximum yield after injury for the case of rabbit muscle I find to vary from ·45 % to ·65 % of anhydrous zinc lactate; the variations in different individuals depend probably in largest part upon variations in fat content. Increase of fat will lower the apparent yield of lactic acid found for the muscle, since the yield is expressed in percentage of the original gross muscle weight.

Volume of the antiseptic medium. The importance of the methods used for avoiding bacterial contamination increases as the periods for observation of the maintenance of the "plateau" of acid maximum at physiological temperature become prolonged.

Inouyi and Kondo, like Mochizuki and Arima and their fellow-workers, have used, in relation to the mass of tissue incubated, twice its bulk of chloroform-water. They obtained however the same results when they repeated their experiments using the ten-fold volume of chloroform-water recommended by Salkowski, and this, so far as their experiments escape the more obvious objections already raised, enables them to meet Salkowski's criticism that the two-fold volume of chloroform-water, with toluol, is inadequate for the prevention of bacterial attack.

But there is no doubt, or at least so I find, that the two-fold volume is not often adequate for antisepsis, and perhaps never adequate unless used in very narrow cylinders. The muscle mass with so shallow a margin of fluid above it cannot be often stirred without serious risk of infection, and even without stirring it very commonly becomes infected.

Frew[1], repeating Inouyi and Kondo's methods, always found a "strong putrefactive smell," and had reason to think, from change in the relations of the water of crystallisation, that part of the lactic acid yield was optically inactive and due to bacterial fermentation.

It is probable that there has been another factor in this failure of antisepsis. Kikkoji[2], at Salkowski's suggestion, has made a special investigation into the antiseptic properties of various combinations of chloroform-water with toluol, and of other bodies. He makes the valuable observation that chloroform-water with toluol may actually have less antiseptic influence than chloroform-water alone. This he

[1] *Loc. cit.* [2] *Zeitschr. f. physiol. Chem.* LXIII. p. 109. 1909.

explains by the relations of partition of chloroform between toluol and water respectively: chloroform is much more soluble in toluol than in water. There seems no doubt that the addition of a layer of toluol to a relatively small bulk of chloroform-water, such as that used by Inouyi and Kondo, may dangerously reduce the amount of effective chloroform dissolved in the water.

The following is an example of the use of a two-fold volume of chloroform-water, with toluol:

Exp. VIII. Rabbit. Muscle minced, mixed and divided to four samples; double volume of chloroform-Ringer, with toluol, added to each.

								Zinc lactate
(*a*)	97·5 gms.	incubated	at	37° C.	for	30 hours,	yielded ...	·435 %
(*b*)	116 ,,	,,	,,		45	,, ,,	...	·287
(*c*)	108 ,,	,,	,,		45	,, ,,	...	·375
(*d*)	110 ,,	,,	,,		72	,, ,,	...	·267

Bacteria found in (*b*), (*c*) and (*d*). Faint putrefactive smell in (*b*) and in (*d*). None in (*c*).

This experiment shows what I have most commonly found to accompany bacterial invasion. Using Inouyi and Kondo's methods I have in almost all cases obtained not an increase but a well-marked decrease of lactic acid on autolysis during two or more days. In some cases a relatively slight decline in lactic acid was found where no positive evidence of bacterial infection appeared, but since diminution of lactic acid always occurred, with exceptions to be noticed below, when infection was evident, it is reasonable to suppose that in these cases of slight diminution the fall in lactic acid yield was due to infection which escaped demonstration. An example of this kind is given in Exp. IX: another instance of the same effect may be noticed in estimation (*d*) of Exp. XIV (p. 307).

Exp. IX. Rabbit. 175 gms. of minced and mixed muscle weighed to two equal samples, and each incubated under double volume of chloroform-Ringer, with toluol, at 38° C.

(*a*)	after 10 hours	yielded	...	·47 %
(*b*)	,, 54 ,,	,,	...	·39

No bacteria found. No suspicion of putrefactive smell.

While the commonest type of bacterial infection found in unsuccessful incubations leads to a loss of lactic acid, it may happen (as would be expected) that specific infection by lactic acid producing organisms shows itself upon occasion. An example of this will be given in Exp. XI (p. 305) in the next section.

It has been seen that the results of prolonged autolyses of muscle given by previous observers have been irregular and conflicting. Since we have the three possibilities of (1) no bacterial infection, (2) infection leading to lactic acid loss, and (3) specific infection leading to lactic acid gain, both quantitative irregularity and qualitative contradiction in results are to be expected. Where infection occurs at all, the habits and accidents of the laboratory will determine its nature. Under conditions of partially successful antisepsis, the results of infection will not often be gross, and a demonstration that the lactic acid finally weighed in a given estimation is dextro-rotatory does not exclude the presence in it of some added fermentative acid. The amount of such an addition, reckoned as *d*-lactic acid, may then appear in a table of results as an important increase due to a supposed autolysis.

While the small volume of chloroform-water used by Inouyi and Kondo and their predecessors is inadequate for antisepsis, and probably still more inadequate when toluol is added, it has been found to be inconvenient and unnecessary to use Salkowski's ten-fold volume: no advantage appeared to be gained from the use of so large a volume, and the preparation of the alcoholic extract of the residue from the fluid was lengthened. I have used in ordinary routine a volume of chloroform-water (or chloroform-Ringer) 3–4 times the bulk of the tissue, without the addition of toluol, and I have not found evidence of infection under these conditions. The following experiment may be regarded as typical, and it shows the long maintained constancy of lactic acid yield when the antiseptic conditions are preserved.

Exp. X. Room-temperature, 24° C. Rabbit. Muscle minced, mixed and divided to five nearly equal samples. All incubated under chloroform-Ringer (350 c.c. to each) at 39° C.

			Zinc lactate
(*a*)	99·7 gms. incubated 1 day, yielded	...	·418 %
(*b*)	100·4 ,, ,, 2 days ,,	...	·402
(*c*)	100·4 ,, ,, 3 ,, ,,	...	·443
(*d*)	100·4 ,, ,, 4 ,, ,,	...	·412
(*e*)	100·4 ,, ,, 5 ,, ,,	...	·434

It will be seen that the estimations given in such an experiment do not give identical results, though they are arranged closely about a maintained "plateau" of acid yield. The quantitative variations, so far as they are not due to possible errors in the lengthy processes of estimation, might well be due to the difficulties of obtaining as many as five exactly equivalent samples of muscle. As the number of samples to be obtained for observation is increased the difficulties of rapid and

completely successful shuffling increase, and delay in manipulation will introduce further errors from irregular drying of the muscle samples and consequent alteration in weight-values. It has been noticed, however, in practice that the minor irregularities of the order seen here are commonly found when long periods of incubation are taken, and they are absent or less noticeable over shorter periods—as in Exps. I, II, XIV, for instance. It seems possible that they may be due to minimal and undetected bacterial action. It is clear, however, that the results give no evidence in favour of autolytic production of lactic acid during prolonged incubation.

III. The alleged relation of lactic acid formation to a glycolytic enzyme in muscle.

Much prominence has recently been given to the experimental results of Stoklasa and his colleagues[1] which have suggested that the lactic acid formation in muscle is due to a fermentative glycolysis, during which lactic acid appears as an intermediate product in the changes which finally yield CO_2 and alcohol. Upon this view, the absence of autolytic production of lactic acid after the attainment of the acid maximum following injury which it has been attempted to establish in the preceding section might be accepted as showing not the absence of a glycolytic and acid-forming enzyme in the muscle preparation, but rather the exhaustion or absence of available substrate material. If this were so, the addition of dextrose to the muscle during survival or during autolysis should lead to an increase in the lactic acid yield.

Stoklasa's experiments in which he obtained glycolysis with muscle preparations were not directed specially to the question of lactic acid formation. He showed that lactic acid appeared during the yield of CO_2 and alcohol from the fermented sugar: the acid was not determined by direct quantitative methods: it was not dextro-rotatory, and the direct application of his results, even as they stand, to the discussion of muscle physiology is hardly justifiable. It is doubtful moreover whether Stoklasa's fermentation was directly connected with the muscle extract he used. Harden and McClean[2] have recently shown the extreme difficulty of carrying out experiments by Stoklasa's methods without bacterial contamination. Their results make it appear probable that no such process as alcoholic fermentation occurs in excised animal

[1] *Ctrlb. f. physiol.* XVI. p. 712. 1902. *Ztsch. f. physiol. Chem.* L. p. 303. 1907. *Etc.*

[2] This *Journal*, XLII. p. 64. 1911.

tissues in the absence of bacteria, and, at the least, they have shown that the only evidence which has been advanced up to the present time in favour of such fermentation is inadequate.

The recent observations of Ransom[1] appear to supply, in support of Stoklasa's views, direct evidence of an increase of lactic acid in muscle-plasma due to the addition to it of dextrose, and the acid is spoken of as the same as that yielded during the onset of *rigor mortis*. He finds that in the presence of added dextrose (or glycogen) the amount of lactic acid formed in muscle-plasma prepared from frozen muscle is greater, and the resulting coagulation firmer, than is the case when no carbohydrate is added. The addition of dextrose causes a "super-rigor due to the extra production of lactic acid," and it is concluded that, as Stoklasa suggested, muscle-plasma contains a ferment or ferments capable of converting dextrose or glycogen into lactic acid, CO_2 and alcohol.

It must be considered doubtful however whether Ransom's conclusions are justified by the experimental results actually obtained. The fermentation of the added dextrose, with lactic acid formation, is said to begin almost at once in the case of frog's muscle-plasma after exposure of the preparation to temperatures of 15°—32° C. But only two experiments are given. In one, varying amounts of dextrose were added to similar samples of plasma, and the number of gas-bubbles present *after* 16 *hours at* 32° *C.* was found roughly to be in proportion to the amount of added sugar: no antiseptic is mentioned and no estimations were made. In the other, frozen frog's plasma to which dextrose had been added was found to become rapidly acid on thawing at 15° C. But there was no control observation, and frozen muscle-plasma, as is well known, becomes rapidly acid on thawing, without the addition of sugar.

In the case of the plasma from fowl's muscle, the fermentation is said by Ransom to be slower, but to be observable within an hour: no experimental evidence of it, however, is given after less than 16 hours' incubation at 32° C., and this apparently without antiseptics. The results of these lengthy incubations show that the addition of sugar increases the gas-bubbles formed, and increases the acidity found on titration. The increase of acid is assumed to be increase of lactic acid without any reason except that lactic acid was shown to be present by qualitative test; the gas was assumed to be CO_2 without analysis. The results irresistibly suggest bacterial activity, and Harden and McClean

[1] This *Journal*, XL. p. 1. 1910.

indeed (*op. cit.*), having repeated the experiments and obtained the same phenomena, find the gas-bubbles to be mostly hydrogen.

My own results with added sugar are not in harmony with those of Ransom, except when bacterial contamination was allowed to become evident. I have not succeeded in observing an increase of lactic acid in surviving or in autolysed muscle, mammalian or amphibian, to follow the addition of dextrose, as will be seen in the experiments to be given below.

Mammalian muscle. The addition of dextrose to the minced muscle is not found to affect the yield of acid during antiseptic incubation under chloroform-Ringer. Yet a constant feature of the experiments has been that whenever bacterial growth has occurred by partial failure of the antiseptic conditions it has been favoured by the presence of the sugar and its results have been magnified accordingly. This is well shown in Exp. XI, in which, after a first stage of complete antisepsis, bacterial invasion was intentionally secured.

Exp. XI. Room-temp. 26° C. Rabbit. Muscle minced, mixed and divided to four similar samples. 250 c.c. chloroform-Ringer, without toluol, added to each. To samples (*b*) and (*d*) 1·5 gms. pure dextrose were added. All were incubated at 39° C. for 24 hours, when (*a*) and (*b*) were extracted for estimation.

		Zinc lactate
(*a*)	69·6 gms. incubated at 39° C. for 24 hours, yielded	·550 %
(*b*)	67·7 ,, ,, ,, ,, (+1·5 gms. dextrose), yielded	·553

The stoppered cylinders of (*c*) and (*d*) were now opened and a large part of the chloroform allowed to evaporate. Each was stirred again, closed, and incubated for a further period of 24 hours. At the end of this time bacteria were found readily in (*c*) and in great profusion in (*d*). Both smelt very strongly of chloroform, neither had any putrefactive smell, (*d*) smelt strongly of *sour milk*. On estimation it was found that

		Zinc lactate
(*c*)	56·3 gms. incubated at 39° C. for 2 days, yielded	·585 %
(*d*)	54·3 ,, ,, ,, ,, (+1·5 gms. dextrose), yielded	·895

It will be noticed that after 24 hours' autolysis the sample of minced muscle incubated with 1·5 gms. of dextrose—immersed, that is, in a ·6 % dextrose solution—gave the same yield of acid as the equivalent control sample without sugar.

In the cases of the other pair of similar samples, infection was encouraged in each, and occurred in each, though the bacterial growth was restrained by the presence of much remaining chloroform. Without sugar a very small increase of acid was found: with sugar, the yield was increased by 65 % in 24 hours. This large yield of fermentative lactic acid from the sugar must be compared with the increased acid yield

obtained in many cases by Ransom on the incubation of muscle-plasma.

It has been found in practice that where a yield of the fermentative acid is being estimated its behaviour in the preparation of its anhydrous zinc salt is different from that of the dextro-rotatory sarcolactic acid. The latter loses its water of crystallisation completely on the water-bath: though in routine it has always been kept in the oven at 105° C. before cooling and weighing, it may be cooled at once, straight from the bath, and then be found already to have reached constant weight, unlowered by subsequent prolonged heating in the oven at 105° C. This is not so in the case of the fermentative acid: it reaches constant weight much more slowly, sometimes not before an hour or more in the oven, and it is not safe to accept any weighing as final until after reheating has been found to give no loss of weight. This difference in behaviour is useful in practice in giving indication of the addition of a fermentative yield of acid to a survival sarcolactic yield when the yields are too slight in absolute amount for proper polariscopic determinations.

The substitution of glycogen for sugar in experiments of the same kind has given similar negative results: the following example is typical:

Exp. XII. Room-temp. 15° C. Rabbit. Muscle minced, mixed and divided to two similar sets. 300 c.c. chloroform-Ringer added to each. 1 gm. pure glycogen (prepared from rabbit's liver) added to one; both thoroughly stirred before, and at intervals during, incubation at 39° C.

		Zinc lactate
(*a*)	82·5 gms. incubated 24 hours alone	·610 %
(*b*)	84·5 ,, ,, ,, with 1 gm. glycogen ...	·615

It has been seen already (p. 301) that the use of smaller volumes of chloroform-water, with toluol, is attended by increased risk of bacterial invasion. I have found that under these conditions the result of adding dextrose to the incubated muscle has generally been to disturb slightly the amount of acid yield, either by increase or decrease, and more commonly by slight decrease. It has been pointed out already that the bacterial growth, often so small or so local as to escape detection, will be associated either with destruction of lactic acid, or with formation of new fermentative acid, according to its specific nature.

The following experiments show this:

Exp. XIII. Room-temp. 18° C. Rabbit. Muscle minced and mixed for two sets. 100 c.c. chloroform-Ringer, with toluol, added to each. 2 gms. pure dextrose added to (*b*). Incubated at 38° C.

		Zinc lactate
(*a*)	84·7 gms. at 38° C. for 44 hours, yielded	·534 %
(*b*)	83·5 ,, ,, ,, (+2 gms. dextrose), yielded	·56

(ZnO determination, (*a*) and (*b*) together, gave 33·25 %. Theory 33·42 %.)

Exp. XIV. Room-temp. 20·5° C. Rabbit. Muscle minced and mixed for four equal sets of 66·7 gms. each. 150 c.c. chloroform-Ringer, with toluol, added to all, 1 gm. pure dextrose to one.

		Zinc lactate
(*a*)	incubated at 38° C. for 6 hours, yielded	·607 %
(*b*)	,, ,, 23 ,, ,, ...	·626
(*c*)	,, ,, 23 ,, ,, ...	·631
(*d*)	,, ,, 23 ,, (+1 gm. dextrose), yielded	·596

[ZnO determination, (*c*), =33·5 %. Theory 33·42 %.)]

(*b*) and (*c*) were similarly treated throughout, for control of methods of shuffling and estimation.

In each it will be seen that the addition of sugar has affected, though very slightly, the acid yield. It is possible that the disturbance in quantitative results produced by the sugar, if not due to accidental error, may be due to some effect upon the methods of extraction and estimation caused by the presence of the sugar in solution. There seems no obvious reason for supposing this, and I am inclined to attribute it to minimal and unnoticed bacterial action, present perhaps in all the muscle samples of the experiments just quoted, but so encouraged in the presence of the sugar as to affect in a small degree the yield of acid. Here again we are to suppose that the result, whether in slight increase or decrease of yield, will depend on the specific nature of the otherwise unnoticed infection.

But however this may be, it is quite obvious that the results of addition of sugar to the incubated muscle provide no evidence at all of any glycolytic formation of lactic acid due to enzymes in the muscle itself. No production of lactic acid which passes the limits of possible experimental error has been found in any case except that of obvious and gross bacterial contamination.

Amphibian muscle. Further experiments have been made with frog muscle because with this the effects of adding sugar during survival at room-temperature and without antiseptics can be followed, as well as the effects of adding it during incubation at 38° C. for autolysis. In all cases the results have been negative: no evidence has been obtained of a glycolytic yield of lactic acid.

At physiological temperatures the addition of dextrose to finely divided muscle does not affect the acid yield, as may be seen in the following experiment.

Exp. XV. Room-temp. 18·3° C. Hind limbs of 16 frogs in two sets of 8 each. Muscles removed and weighed, and each set ground with sharp sand in a mortar until a perfectly smooth "*magma*" was obtained. During grinding, 15 c.c. Ringer solution was added to (*b*), 5 c.c. Ringer + 10 c.c. 10 % dextrose to (*a*). The two muscle masses (*a*) and

(*b*) were then put in covered dishes, kept in air at 19° C. for 24 hours, and finally extracted as usual with alcohol for estimation. At the end both were exactly similar in appearance and no signs of bacterial change were found. On estimation,

		Zinc lactate
(*a*)	45·9 gms. + 1 gm. dextrose in Ringer, after 24 hours at 19° C. gave	·493 %
(*b*)	45·3 gms. + Ringer only ,, ,, ,, ,,	·510

In other experiments the frog's muscle was incubated for short periods at supra-physiological temperatures, but again no evidence was gained of glycolytic lactic acid formation. In cases of this kind the addition of dextrose secured most commonly a small, but sometimes a large, decrease of lactic acid, and this I attribute to the early onset of bacterial change accelerated in the presence of the sugar.

Exp. XVI. Room-temp. 16° C. Limbs of 24 frogs mixed and divided into three sets. The muscles of sets (*a*) and (*b*) were ground with sand as in the last; during grinding, 20 c.c. Ringer solution was added to (*a*), the same containing 1·5 gms. pure dextrose was added to (*b*). Both muscle masses were then incubated in closed flasks at 39° C. for 4½ hours. The limbs (*c*) were left uninjured, and incubated with 20 c.c. Ringer in a flask with the others, so reaching the full development of heat-rigor. On estimation,

		Zinc lactate
(*a*)	49·3 gms. + Ringer only after 4½ hours at 39° C. gave	·478 %
(*b*)	45·1 gms. + 1½ gms. dextrose in Ringer ,, ,, ,, ,,	·295
(*c*)	41·7 gms. uninjured for heat-rigor ,, ,, ,, ,,	·471

After incubation no difference in appearance between (*a*) and (*b*), and no signs of bacterial change were evident. No examination for bacteria was made.

It will be noticed in this experiment that a practical identity of lactic acid yield was reached by samples (*a*) and (*c*). The extreme of injury done to (*a*) in the grinding with sand brought it to the same acid-maximum as that for the full heat-rigor of the undamaged muscles of (*c*). In this, as in the next experiment, the diminution in final acid yield shown by the samples containing added sugar, must be assigned to the beginnings of bacterial infection. In these short experiments, as in Ransom's longer experiments, no antiseptics were used.

Exp. XVII. Room-temp. 18·5° C. Limbs of 24 frogs shuffled to three sets. The muscles of each set ground to a smooth "*magma*" with sand. 20 c.c. of Ringer were added during grinding to each; 2 gms. dextrose were dissolved in the Ringer in the case of (*b*), 1 gm. dextrose in the case of (*c*). All were incubated in closed flasks at 34° C. for six hours. No bacterial changes were evident at the end of incubation. On estimation,

		Zinc lactate
(*a*)	41·9 gms. + 20 c.c. Ringer only after 6 hours at 34° C. gave	·452 %
(*b*)	41·3 gms. + 2 gms. dextrose in Ringer ,, ,, ,, ,,	·376
(*c*)	43·3 gms. + 1 gm. ,, ,, ,, ,, ,, ,,	·364

In other experiments the muscle, after being soaked in a dextrose solution, was cooled to freezing point and subsequently allowed to thaw.

It was thought possible that the dextrose, thus presented to the muscle, might be available if any glycolytic action had its seat in the muscle as the fibres, when allowed to thaw, showed the accelerated survival processes characteristic of thawing muscle. It should be noted that Ransom obtained the effects which he claimed as due to tissue-glycolysis, by the use of frozen muscle-plasma subsequently allowed to thaw in the presence of dextrose. In my observations the presence of added dextrose has never affected the acid yield of uninjured muscle in the hastened survival changes of thawing after freezing: the results have been as negative as those found for the hastened muscle processes after mechanical destruction.

Exp. XVIII. Room-temp. 18° C. Frog. 16 pairs of limbs to two sets. Each set placed uninjured with 10 c.c. Ringer in closed flasks maintained at 0° C.—1° C. for 24 hours. In the Ringer of (*b*) ·5 gm. dextrose had been dissolved. After 24 hours in the cold the flasks were kept at 18° C. for 24 hours, when stiffness was beginning. No difference of any kind was observable between the two muscle samples at the end. The estimations were

		Zinc lactate
(*a*)	32·4 gms.* without dextrose	·32 %
(*b*)	33·3 gms.* with ,,	·29

* The nett muscle weights were calculated from experience of the average relation of the muscle weight to the gross weight of the whole limbs.

Exp. XIX. Room-temp. 18° C. Frogs. 20 pairs of limbs to two sets. ·5 gm. dextrose in 35 c.c. Ringer added to (*a*), 35 c.c. Ringer alone added to (*b*). After two hours at room-temperature, both sets were frozen for 48 hours in a mixture of ice and salt, renewed from time to time. The muscles were now frozen in a solid mass with their plasma and Ringer. This was left to thaw at 20° C. The onset of stiffness and the decrease of irritability were observed from time to time and after 10 hours both were stiff, but not clotted to opacity, and both were unirritable by the strongest induced shocks. No difference between the two muscle sets could be detected at any stage. After a further period of 10 hours at 20° C., the muscles were removed for estimation.

		Zinc lactate
(*a*)	52 gms. with ·5 gm. dextrose, after 20 hours thawing, gave ...	·43 %
(*b*)	51·5 gms. without dextrose, ,, ,, ,, ,, ...	·42

The results obtained with amphibian muscle agree then with those found for mammalian muscle. In no case has any evidence been found for a glycolytic formation of lactic acid by living muscle or by dead and disintegrated muscle. In the incubations performed without antiseptics the addition of sugar has always led to a diminution in the amount of lactic acid present by favouring the onset of bacterial invasion.

IV. Conclusions and summary.

It has been contended that we have no trustworthy evidence for thinking that any additional production of lactic acid occurs in the long continued self-digestion of muscle fragments which have already reached their normal maximum of acidity upon death, and, further, that there is no reason to believe that the dead muscle cells or their expressed contents have the power of producing by glycolytic enzyme action a further yield of lactic acid—whether dextro-rotatory or inactive—from added carbohydrate material. That is to say, we have no knowledge of any production of lactic acid by muscle cells excepting that which occurs during life or during the survival processes leading to death.

What light we have on the question of the sources and the mode of origin of the dextro-rotatory acid so produced is very slight, and comes only from a consideration of the outlines we have already of the course of the survival production of acid by undamaged and by damaged muscles respectively.

The survival production of acid "spontaneously" by undamaged muscle after the blood-flow has ceased (and in the absence of oxygen) is in equal amounts in equal times, and it has been pointed out[1] that it is not easily explained as being due to intra-cellular enzymic action. It is provisionally explicable on the hypothesis that the acid is derived from unstable material previously elaborated, and the peculiar linear relations of the spontaneous yield may be taken to indicate that the unstable precursor is maintained in constant amount by an unknown process which ends as irritability ends. Without supposing such a maintenance of the precursor at constant "head" it is difficult to account for the absence of a change in the rate of acid production as it proceeds.

The yield of acid by muscle dying after severe mechanical injury has different characters. It is rapid at first and then progressively slower as it reaches its predetermined maximum. This yield has been spoken of more than once as a "survival" yield, in order to distinguish it from such a yield of acid as that which has been alleged to result in autolysis from the action of supposed free enzymes in muscle preparations, and it seems probable, in justification of this, that the yield after injury is an accompaniment of subsisting but rapidly declining irritability. If calling it a "survival" yield for the present, however, is to use what is at least

[1] Fletcher and Hopkins. *Loc. cit.*

a convenient figure of speech, it is nevertheless most important that until experimental enquiry has gone further we should not prejudge the question whether the injury yield is identical in its sources and mode of origin with the yield from undamaged muscle, or not.

It is natural to suppose that the yield of acid by the muscle dying after injury is derived from the same sources as that produced by the undamaged, or at least un-disrupted, muscle, and that the acid after injury is delivered from the same preformed unstable material, whose amount has been determined by previously existing conditions. This view is strengthened by a consideration of the remarkable constancy of the maximum yield attained by similar samples of muscle allowed under very different circumstances of injury, or heating, or chemical irritation, to reach it. But this is at present uncertain, and it is still more doubtful whether the mode of origin of the acid after mechanical destruction of the muscle fibres has any counterpart in the mode of origin of the acid yielded by the undamaged muscle deprived of oxygen. Both questions must be left for further experimental analysis.

Meanwhile it is a great gain if they can be freed from the prejudgements which have come inevitably from the common beliefs that lactic acid producing enzymes can be demonstrated in muscle autolysis, and that glycolysis, with lactic acid formation, can actually be effected by dead muscle preparations. It seems clear that, whatever be the nature of the acid yield after injury, no further production of lactic acid takes place after the characteristic acid maximum has been reached. The maximum appears to be predetermined by previous intra-cellular events, and its amount is probably fixed because it arises from a fixed amount of specific unstable precursory material.

The results may be summarised as follows:

1. Lactic acid production in excised mammalian muscle, as in amphibian muscle, is accelerated by the mechanical injuries of cutting, mincing or grinding, and at body-temperature it leads to a final maximum yield within an hour or less. But the maximum yield of acid is not reached instantaneously even upon the severest injuries. The rate of production depends chiefly on the temperature: if the muscle has cooled, for instance, to room-temperature, during the infliction of injury, the attainment of the maximum yield of acid is markedly delayed.

Muscle, accordingly, which is examined immediately after even the severest injuries always contains less, and often much less, acid than the maximum it would have reached later.

2. Mammalian muscle, like amphibian muscle, after rapid destruction by heating, yields less lactic acid than it would have produced if allowed to proceed to the maximum of its spontaneous acid production in survival. The diminution is more marked the earlier the heating is inflicted during survival.

3. The evidence hitherto produced of an autolytic production of lactic acid by muscle cannot be accepted. The greatest weight of this evidence relates to periods of autolysis including the first day, and it has been based upon fallacious methods of control in which the factors in survival change just mentioned have been ignored; as a consequence survival changes have been unwittingly reckoned as autolytic changes. Less striking and more scanty evidence has been advanced for an increase of the acid in later periods of autolysis, but this is weakened by the probabilities of bacterial invasion under the described conditions.

4. Muscle which has reached its maximum of acid yield in survival produces no further lactic acid during subsequent periods, early or late. There is no subsequent "autolytic" production of lactic acid by muscle.

5. No glycolytic enzyme leading to lactic acid formation appears to exist in muscle. After the addition of dextrose to intact surviving muscle, or to preparations of disintegrated muscle, no increase of lactic acid is found in the absence of bacteria. In the presence of bacteria the results depend upon the specific nature of the infection: the *d*-lactic acid already present in the muscle is commonly diminished, but with specific infection a large yield of optically inactive acid may occur.