XCII. THE ORIGIN OF PYRUVIC ACID IN MUSCLE.

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INTRODUCTION.

It was shown by Case and Cook [1931] that it is possible under a variety of conditions to detect the presence of methylglyoxal and pyruvic acid among the products of the metabolism of muscle. With regard to methylglyoxal in particular, our results constituted an addition to the already considerable quantity of evidence which has accumulated in favour of the hypothesis that this substance is of importance in intermediary carbohydrate metabolism. From purely chemical considerations, the participation of methylglyoxal in the transformation of carbohydrate to lactic acid has long appeared probable; biological evidence for such a view may be said to have begun with Dakin and Dudley's [1913] demonstration of the existence in animal tissues of the enzyme glyoxalase, which converts α -ketonic aldehydes into the corresponding hydroxy-acids. At about the same time Neuberg [1913] observed the conversion of methylglyoxal to lactic acid in tissues, and Levene and Meyer [1913] showed that leucocytes could bring about the same transformation. Since then evidence has accumulated which has established conclusively the facts that diverse animal tissues which are known to produce lactic acid are also capable of forming methylglyoxal, and that methylglyoxal is transformed to lactic acid by these tissues as readily as, or more readily than, carbohydrates. These two circumstances, taken in conjunction with the widespread occurrence of glyoxalase in nearly all tissues, make the probability strong that methylglyoxal is indeed a normal precursor of lactic acid. Some stress is laid upon this because, in what follows, this hypothesis is assumed.

In addition to methylglyoxal, Case and Cook were able to identify the corresponding keto-acid, pyruvic acid, as a metabolite under similar conditions. They were led to make the tentative suggestion that this pyruvic acid which appeared was a product, not of lactic acid oxidation, but of the chain of processes leading up to the formation of lactic acid.

Such a conclusion is supported by the results of Utewski [1929], who estimated the lactic acid produced by autolysing muscle in the presence and absence of sodium pyruvate, and reported that much more lactic acid appeared when pyruvate was added. Mendel, Bauch and Strelitz [1931] found that the

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anaerobic glycolysis of animal tissues is more than doubled by the addition of pyruvic acid, and they also state that tumour cells, which are remarkable for their high glycolytic activity, produce pyruvic acid in large amounts. Hahn and Haarmann [1930] found that muscle which had been thoroughly washed, and hence presumably freed from lactic dehydrogenase (as Hahn and Fischbach [1929] had previously stated), readily produced pyruvic acid from fructosediphosphoric acid.

That in tissues other than muscle, pyruvic acid can be reduced to lactic acid, appears likely from the work of Khouvine, Aubel and Chevillard [1929, 1930] who in a series of investigations demonstrated the power of liver to bring about this transformation either aerobically or anaerobically. It has been shown [*e.g.* Mayer, 1912] that lactic acid can be found in the urine of animals which have been given pyruvic acid by mouth. It is of course not permissible to draw any conclusions with regard to the metabolism of muscle from observations on the liver, and the above examples are mentioned simply as indicating that the change pyruvic acid $\rightarrow$ lactic acid can and does occur in some animal tissues. Again, it is unsafe to draw too close an analogy between lactic fermentation by muscle and fermentations by yeast and bacteria. It is important to remember, however, that it has been thoroughly established by Neuberg and his associates that the formation of pyruvic acid is a normal stage in the fermentation of carbohydrate by yeast; but ordinarily it undergoes subsequent changes which do not occur in muscle. As an indication of the part played by pyruvic acid in lactic fermentation by bacteria, we may cite its isolation by Kostychev, Gwaladse and Eliasberg [1930], who employed semicarbazide as a fixative in the lactic fermentation by *B. caucasicum*.

It has recently been made fairly certain [*e.g.* by Neuberg and Kobel, 1928, 1929, 2; by Gottschalk, 1928; and by Bayo, 1929], by the use of co-enzyme-free yeast preparations, that the formation of methylglyoxal is the step intermediate between phosphorylation and the formation of pyruvic acid. The same has been shown to be true with respect to certain lactic acid-forming organisms, as for instance by Neuberg and Kobel [1929, 1] in the case of *B. delbrücki*.

These changes may be expressed as above (following the continuous lines). The left-hand branch represents the case of lactic acid-forming organisms, or yeast under some special conditions, while the right-hand branch represents ordinary alcoholic fermentation.

But animal tissues are strongly characterised by their possession in marked degree of the enzyme glyoxalase, which converts methylglyoxal directly into lactic acid; this transformation appears to be effected by an addition of the elements of water so that the change methylglyoxal $\rightarrow$ lactic acid takes place in one step. In other words, an internal oxidation-reduction seems to be involved, in contradistinction to a phase of oxidation (with formation of pyruvic acid) followed by one of reduction (with formation of lactic acid). The scheme given above must therefore be amplified by addition of the direct conversion of methylglyoxal into pyruvic acid, represented by the dotted line, to include the case of animal tissues [*cf.* Gottschalk, 1926].

By such considerations as these a good deal of doubt is cast upon the likelihood of pyruvic acid formation as a stage in the course of normal lactic acid formation in muscle, and shortly after the completion of the above-mentioned paper by Case and Cook the present author began to feel that the validity of such a hypothesis was questionable. The purpose of this communication is to report some of the experimental work which has been performed since that time and to discuss its bearing upon the problem of pyruvic acid formation and oxidation in general in muscle.

EXPERIMENTAL.

Methods.

Pyruvic acid was used in the form of its sodium salt, prepared in the manner described in a preceding paper [Case, 1932]. The method used for its determination is given in full in the same place. Continued use has also been made of the Simon and Piaux [1924] reaction which was employed in former work.

Solutions of methylglyoxal were obtained either by the method of Neuberg and Hofmann [1930] or by the following procedure: *isonitrosoacetone* (methylglyoxal aldoxime) was prepared from acetone and the *isonitroso*-group removed by boiling with dilute sulphuric acid [Pechmann, 1887]. On distilling, the methylglyoxal was contained in the distillate. This latter method was found on the whole the more satisfactory, as it is extremely difficult by Neuberg and Hofmann's procedure to obtain a product free from impurity, notably formaldehyde. The solutions of methylglyoxal could be further purified by redistillation with phosphorus pentoxide and concentration *in vacuo*. The methylglyoxal was standardised in the first place by weighing the yield of 2:4-dinitrophenylosazone obtained from a known volume, and subsequent estimations were carried out by a slight modification of the method of Barrenscheen and Dreguss [1931] which, although laborious, seems to be the most satisfactory yet devised.

The preparation of muscle extracts and the determinations of lactic acid were performed exactly as has been customary in former work.

The first experiments to be reported are concerned with the confirmation of the previous findings that while pyruvic acid production is increased by the addition of starch or glycogen, the addition of lactate has little effect. Flasks were set up with the following contents, and incubated at room temperature:

	A (cc.)	B (cc.)	C (cc.)
Rabbit muscle extract	50	50	50
Phosphate buffer, p_H 7.2 (10 times strength)	5	5	5
M Na_2SO_3	3	3	3
2 % soluble starch	—	10	—
$M/5$ sodium lactate	—	—	10
Water	to 100 cc.		

Nitroprusside reactions were carried out from time to time upon 3 cc. samples, and at the end of 3 hours pyruvic acid estimations were performed. The results appear in Table I.

Table I. *Nitroprusside reactions.*

Time after commencement of exp. (mins.)	A	B	C
0	—	—	—
20	—	++	—
60	+	+++	+—
120	+	+++++	+
180	+	+++++	+

Pyruvic acid after 180 mins., expressed as mg./100 cc. incubation mixture.

A	B	C
7	53	8

It is obvious that while the presence of starch has given rise to a large increase of pyruvic acid over the amount formed in the control, the addition of lactate has had no such effect. It is to be noted however that the experiment was carried out with muscle extract, in common with most of the rest of the work to be reported in this paper. Since there is no recorded instance of a lactic dehydrogenase having been obtained in an aqueous extract from muscle, it cannot be concluded from the above result that in muscle tissue itself pyruvic acid does not originate from lactate. Therefore the experiment was repeated using chopped rabbit muscle. Also, since it was thought possible that the configurative difference between the *dl*-lactate added and the lactic acid actually produced by muscle itself might account for the result, flasks were included in which a preparation was added which had been made by mincing rabbit muscle, allowing to stand for 2 hours, extracting with buffer, boiling, filtering and concentrating *in vacuo*. This preparation was found to contain about 30 mg. of lactic acid per 10 cc.

The experimental flasks were as follows:

	A	B	C	D
Chopped rabbit muscle ...	10 g.	10 g.	10 g.	10 g.
Buffer, p_H 7.2 ($\times 10$) ...	3 cc.	3 cc.	3 cc.	3 cc.
M Na_2SO_3 ...	2 cc.	2 cc.	2 cc.	2 cc.
2 % soluble starch ...	—	5 cc.	—	—
$M/5$ sodium lactate ...	—	—	5 cc.	—
Muscle-lactic acid preparation ...	—	—	—	30 cc.
Water ...	to 50 cc.			

The results of the periodical nitroprusside tests, and the final estimations of pyruvic acid, are given in Table II.

Table II. *Nitroprusside reactions.*

Time after commencement of exp. (mins.)	A	B	C	D
0	—	—	—	+
60	—	+++	—	+
120	—	++++	+-	+
Pyruvic acid after 120 mins., expressed as mg./100 cc. incubation mixture.				
	A	B	C	D
	4.5	69	9.5	18

Here again then the findings are similar. The flask containing starch shows a great increment in pyruvic acid content. There is a slight increase in those containing lactate, the possible significance of which will be discussed later on. The slight positive reaction shown throughout by the contents of flask D is clearly due to preformed pyruvic acid in the added preparation.

Similar experiments, employing chopped frog muscle instead of rabbit muscle, gave results of the same type. Weight for weight, rather more pyruvic acid was formed by the frog muscle than by the rabbit muscle.

The next subject for investigation was the effect upon pyruvic acid production of agents which inhibit the formation of lactic acid from carbohydrates. Sodium fluoride, which prevents the breakdown of phosphoric esters, and amylase, which inhibits at an earlier stage [Case and McCullagh, 1928], were of course obvious substances to try. A typical experiment is recorded below:

	A (cc.)	B (cc.)	C (cc.)
Muscle extract ...	50	50	50
Buffer, p_H 7.2 ($\times 10$) ...	5	5	5
M Na_2SO_3 ...	3	3	3
2 % soluble starch ...	10	10	10
$M/10$ sodium fluoride...	—	10	—
Saliva (1 in 5) ...	—	—	10
Water ...	to 100 cc.		

(It was known from experience that these quantities of sodium fluoride and dilute saliva were sufficient to cause complete suppression of lactic acid formation.)

Incubation was, as usual, at room temperature; nitroprusside tests were performed initially and at intervals. Table III gives the results.

Table III. *Nitroprusside reactions.*

Time after commencement of exp. (mins.)	A	B	C
0	-	-	-
20	+++	-	-
60	+++	-	-
90	+++++	-	-

Pyruvic acid after 90 mins., expressed as mg./100 cc. incubation mixture.

A	B	C
65	3	4.5

This experiment then shows that both amylase and sodium fluoride, which are powerful inhibitors of glycolysis, have completely suppressed the formation of pyruvic acid which otherwise takes place to a large extent from starch. Some experiments which were done with iodoacetic acid will be reported in detail in a later communication, as their significance is not fully clear. So far, it has been found that concentrations of this substance which totally inhibit lactic acid production may bring about only a partial inhibition of pyruvic acid formation. In this connection it has to be remembered (*a*) that iodoacetic acid may exert its inhibitory action at two stages of glycolysis at least [Dudley, 1931], (*b*) that in higher concentrations it inhibits respiration as well as glycolysis, and (*c*) that its effect is not instantaneous. Hence in drawing conclusions from experiments involving its use there are a number of factors to be taken into account.

Now, so far as all these results go, they certainly indicate that for pyruvic acid to be formed, glycolysis must occur and must not be inhibited. But they do not settle the problem as to whether pyruvic acid is actually a stage in lactic acid formation, though such a hypothesis is not contradicted by the facts so far reported. More light can be thrown upon this question by experiments to determine the fate of pyruvic acid when it is added to muscle. Some of the evidence in the literature which suggests the possibility that pyruvic acid goes to lactic acid has already received mention in the introduction.

It will be remembered that in the work of Case and Cook [1931] added pyruvic acid was found to disappear rapidly from minced muscle. Before the publication of that paper, a rough experiment had been done which indicated that a corresponding increase of lactic acid took place, as had been found by Utewski [1929], and it was intended to repeat this later on, making accurate determinations. When the present author attempted to do this, however, it was found to be a less straightforward undertaking than had been supposed. The results were extremely variable in that sometimes pyruvic acid disappeared at a rapid rate and sometimes at a slow one. In three cases only, out of sixteen tried, was there any formation of lactic acid in excess of the amount simultaneously formed in the controls without added pyruvate, and even then the increase accounted for less than 10 % of the pyruvate disappearing. This series of sixteen tests embraced experiments with minced

rabbit muscle, rabbit muscle extract, and chopped frog muscle, at room temperature and at 37°. It is to be noted here that Toenniessen and Brinkmann [1930] and Toenniessen [1930] showed that if pyruvic acid were perfused through the musculature of eviscerated rabbits it very rapidly disappeared. But it did not form lactic acid; succinic acid and formic acid made their appearance.

There cannot be said then to be sufficient evidence for the belief that the transformation of carbohydrate to lactic acid in muscle involves the intermediate formation of pyruvic acid, and some other explanation must be sought for the appearance of this latter substance. It is at least certain from the work already reported that pyruvic acid occurs in the greatest quantities under conditions which are accompanied by breakdown of carbohydrate to lactic acid, *viz.* glycolysis *in vitro*, and activity as a result of stimulation *in vivo*. It might be argued from this that the result of glycolysis is merely to give rise to the production of large amounts of lactic acid, from which pyruvic acid is formed by a simple dehydrogenation. But this is rendered unlikely by the fact, demonstrated by Case and Cook and here confirmed, that the formation of pyruvic acid brought about by the addition of lactate to the system is not comparable in amount with that brought about by the addition of carbohydrate. If also, then, pyruvic acid is to be excluded as an intermediary in glycolysis, the most feasible supposition remaining is that it is formed as a result of a side-reaction from something which is itself an intermediate. Methylglyoxal of course suggests itself. The next experiments to be described deal therefore with the influence of added methylglyoxal upon the appearance of pyruvic acid.

The following incubations were made:

	A	B
Muscle extract ...	50 cc.	50 cc.
Buffer, p_H 7.2 ($\times 10$)...	5 cc.	5 cc.
M Na_2SO_3 ...	3 cc.	3 cc.
Methylglyoxal ...	0.2 g.	—
	} to 100 cc.	
Water ...		

Table IV. *Nitroprusside reactions.*

Time after commencement of exp. (mins.)	A	B
0	—	—
20	—	—
60	+ —	—
120	+	+
240	++	+
		
18 hours	+++	+
Pyruvic acid after 4 hours, expressed as mg./100 cc. incubation mixture.		
	A	B
	15	6
Pyruvic acid after 18 hours.		
	A	B
	39	9.5

After 4 hours, it is thus seen, there was surprisingly little formation of pyruvic acid from methylglyoxal, compared with the amounts obtained from an equivalent quantity of carbohydrate. It is true that after standing overnight a considerable increase in pyruvic acid was found to have occurred, and this is recorded in Table IV; but of course a figure obtained after so long an incubation is of doubtful value owing to the possibility of bacterial contamination. It was at first thought that the experiment might be faulty, but after a large number of repetitions the above results were shown to be quite typical.

As a necessary control, an experiment without sulphite was performed, lactic acid being estimated instead of pyruvic acid, and it was found that about 40 % of the methylglyoxal was transformed rapidly into lactic acid. The details of this are as follows:

	A	B
Muscle extract ...	50 cc.	50 cc.
Buffer, p_H 7.2 ($\times 10$)...	5 cc.	5 cc.
Methylglyoxal ...	—	0.2 g.
Water	to 100 cc.	

Table V.

Methylglyoxal, expressed as mg./100 cc. incubation mixture.

Time after commencement of exp. (mins.)	A	B
0	8	203
15	6	38
60	6	8

Lactic acid, expressed as mg./100 cc. incubation mixture.

Time after commencement of exp. (mins.)	A	B
0	47	51
15	58	125
60	63	136

In this control, without sulphite, the nitroprusside tests were throughout negative, or at most faintly positive. The fate of that portion of the methylglyoxal which under these conditions disappears but does not give rise to lactic acid is not at present clear; the same phenomenon has been encountered by many other workers.

Hexosediphosphate was next tried as a substrate, and was found in presence of sulphite to yield pyruvic acid to about the same extent as did starch, and therefore much more readily than did methylglyoxal. An experiment is reported below. The sodium hexosediphosphate which was used here was prepared from the calcium salt in solution by quantitative decomposition with sodium oxalate.

	A	B	C	D
Muscle extract ...	50 cc.	50 cc.	50 cc.	50 cc.
Buffer, p_H 7.2 ($\times 10$) ...	5 cc.	5 cc.	5 cc.	5 cc.
M Na_2SO_3 ...	3 cc.	3 cc.	3 cc.	3 cc.
Na hexosediphosphate...	—	—	0.4 g.	0.4 g.
Methylglyoxal ...	—	0.2 g.	—	0.2 g.
Water	to 100 cc.			

The results are set forth in Table VI.

Table VI. *Nitroprusside reactions.*

Time after commencement of exp. (mins.)	A	B	C	D
0	—	—	—	—
20	—	—	++	++
60	+—	—	+++	+++
120	+	+	+++++	+++++
18 hours	++	+++	+++++	+++++
Pyruvic acid after 2 hours, expressed as mg./100 cc. incubation mixture.				
	A	B	C	D
	8	12	71	77
Pyruvic acid after 18 hours.				
	A	B	C	D
	14.5	49	88	113

It is clear from this that pyruvic acid is formed very much more rapidly from hexosediphosphate than from methylglyoxal. Once again, after standing overnight, there is very appreciable production of pyruvic acid in the flask with added methylglyoxal. Although this probably indicates a slow reaction of the same type as the comparatively rapid one occurring in flask C, it is subject to the criticism already made in connection with the corresponding results in Table IV. Flask D is a control intended to demonstrate that the slow formation of pyruvic acid from methylglyoxal as compared with that from hexosediphosphate is not due to poisoning of the enzyme system by impurities present in the methylglyoxal preparations used, as seemed quite possible. It is seen that addition of methylglyoxal does not retard the production of pyruvic acid from hexosediphosphate.

The last set of experiments to be described in the present communication is concerned with the effect of antiglyoxalase on the formation of pyruvic acid. The following flasks were set up:

	A	B	C	D	E	F
Muscle extract ...	50 cc.	50 cc.	50 cc.	50 cc.	50 cc.	50 cc.
Buffer, p_H 7.2 ($\times 10$) ...	5 cc.	5 cc.	5 cc.	5 cc.	5 cc.	5 cc.
M Na_2SO_3 ...	3 cc.	3 cc.	3 cc.	3 cc.	3 cc.	3 cc.
Soluble starch ...	0.2 g.	0.2 g.	—	—	—	—
Na hexosediphosphate...	—	—	0.4 g.	0.4 g.	—	—
Methylglyoxal ...	—	—	—	—	0.2 g.	0.2 g.
Antiglyoxalase ...	—	10 cc.	—	10 cc.	—	10 cc.
Water ...	...	...	...	...	...	...

to 100 cc.

The antiglyoxalase here used was made from an aqueous extract of commercial pancreatin which had been digested for 24 hours with 1 % sodium carbonate to remove trypsin and amylase. Tests showed that the preparation was active as an antiglyoxalase. The muscle extract used in the above experiments was incubated for 2 hours at 37° with the antiglyoxalase and buffer before adding the other substances, while in those flasks from which antiglyoxalase was to be absent, the muscle extract and buffer alone were

incubated at 37° for the same length of time. The subsequent "experimental" incubation was, as usual, at room temperature.

Table VII. *Nitroprusside reactions.*

Time after commencement of exp. (mins.)	A	B	C	D	E	F
0	-	-	-	-	-	-
60	++	++++	++	++++	-	+-
120	+++	+++++	++++	+++++	+-	++
18 hours					++	++++
Pyruvic acid, expressed as mg./100 cc. incubation mixture.						
	A	B	C	D	E	F
After 2 hours	47	88.5	59	104	10	15.5
After 18 hours					33	48

In all three cases it is to be observed that the presence of antiglyoxalase has brought about an increase in the quantity of pyruvic acid which appears. As in the preceding experiments, the production of pyruvic acid from methylglyoxal is very much slower and less marked than from starch or hexosediphosphate.

DISCUSSION.

The question of the origin of pyruvic acid in muscle assumes great significance if the hypothesis be regarded as legitimate that the occurrence of this substance is associated with the oxidative phase of the much-discussed "carbohydrate cycle." A clue may be afforded by the present work as to the actual stage of the cycle where oxidation comes into play. In considering the aerobic removal of lactic acid it has long been a disputed problem as to whether part of the lactic acid itself is the material which is oxidised in order to provide energy for the resynthesis of the remainder, or whether some other member of the metabolic chain is more directly concerned. On the assumption that lactic acid as such undergoes oxidation, the operation of a lactic dehydrogenase is generally postulated, which would yield pyruvic acid as the first oxidation product. And indeed the phenomena which are observed if lactic acid be added to an intact muscle seem to be entirely compatible with this conception; oxidation does take place, and pyruvic acid is formed and can be detected by suitable means. Nevertheless, it has not so far proved possible to obtain from muscle an extract possessing the property of a lactic dehydrogenase, and even if the tissue be merely chopped or minced so that its structure is disintegrated it now yields pyruvic acid very much less readily from added lactate, as is shown in the present work. It is well known that the synthetic activity of muscle is severely impaired by such treatment as chopping, and may be destroyed altogether. It appears quite likely that this is connected with the simultaneous loss of the power of producing pyruvic acid from lactic acid; in other words, the formation of pyruvic acid from lactic acid may result not from direct oxidation of the latter, but from the oxidation of some compound intermediary between lactic acid and carbohydrate. On the whole there has,

until recently, been little available evidence to assist in arriving at a decision in favour of one or the other of these alternatives. The information afforded by the present work points rather strongly to the probability that the second is the correct one.

Results obtained by methods involving the use of tissue extracts must always be interpreted with circumspection when they are applied to considerations of events taking place in intact tissues or organs; at the same time, the employment of such procedures has a definite advantage in that it facilitates a certain degree of separation and differentiation between various correlated systems and processes. In the present instance, for example, the use of muscle extracts eliminates complications ensuing from what may be called the synthetic half of the carbohydrate cycle. The fact that in such a system pyruvic acid formation can still be observed, while lactic acid when once formed plays no further part in the metabolic changes, may be of prime importance from the point of view of the problem under discussion.

Again, the fact must be borne in mind that throughout this work a fixative, namely sodium sulphite, has been employed. This of course is quite justifiable when, as was here the case, the primary object was to gain information as to the precursor of pyruvic acid. The actual magnitude of the extent of the reaction thus brought into prominence may or may not be considerably modified by the use of such a technique, and this has to be remembered when attempting to relate the findings thereby obtained with the extent to which the process occurs under normal conditions. The modification in this case can only be a quantitative, and not a qualitative one, however, for it has previously been shown that it is possible to demonstrate the presence of pyruvic acid in muscle in the absence of any fixative.

When we come to consider the identity of the precursor of pyruvic acid, some interesting points are raised. The experiments with inhibitors show that this precursor must occupy a place in the glycolytic chain subsequent to that occupied by hexosephosphoric esters. It has been shown that it is extremely unlikely to be lactic acid. Therefore, the only substance definitely known to occur which fulfils the necessary conditions is methylglyoxal (see the scheme given in the introduction). It seems very probable then that pyruvic acid arises from methylglyoxal, and by analogy with the generally accepted theory of yeast fermentation this probability is strengthened. This suggestion has been made before, *e.g.* by Toenniessen and Brinkmann [1930], and the present work certainly lends it support. It seems at first sight surprising therefore that, as has been shown here, added methylglyoxal does not readily yield pyruvic acid, while hexosediphosphate does. But consideration shows that a parallel exists in the case of yeast fermentations where, although it is practically sure that methylglyoxal is an intermediate, the fact remains that added methylglyoxal is almost unfermentable. This is explained by the supposition that the methylglyoxal participates in the fermentation in a form which is not structurally identical with that of extraneously added methylglyoxal. Precisely

in what this difference consists is still a matter for conjecture. It seems very possible that methylglyoxal as actually produced in fermentation either by muscle or by yeast is in the form of a hydrate, in which case the formation of pyruvic acid would be the result of a direct dehydrogenation, while the action of glyoxalase would be a structural rearrangement, the necessary elements of water being already present in the molecule. This hypothesis, though not a new one by any means, is very attractive and would account for the results reported in this paper. On the other hand, it has been suggested [*e.g.* Giršavičius, 1930], with some experimental support, that the $\text{CH}_3 \cdot \text{CO} \cdot \text{CHO}$ form is the fermentable one, while the hydrated form is the one existing in solutions of the substance. Again, other suggestions postulating the existence of tautomeric forms have been put forward, notably by Neuberg. At present it is not possible to decide which is the correct interpretation of the known facts, but the data presented in this paper almost certainly call for explanation on these lines. The experiments with antiglyoxalase carry a good deal of significance, since they indicate that when the methylglyoxal is prevented from being transformed to lactic acid—the reaction which normally preponderates—it is more available for the process which results in the appearance of pyruvic acid. Many interesting issues are involved which must be made the subject of future work. For example, in view of what has been said above the possibility arises that there may in reality be no true lactic dehydrogenase in muscle. This question is at present receiving attention.

SUMMARY.

The occurrence of pyruvic acid in muscle is more fully investigated with the object of ascertaining its mode of origin. The conclusion is drawn that it is not an intermediate in lactic acid formation and that it is not a product of direct oxidation of lactic acid. It is suggested that it is derived from the modification of methylglyoxal which is widely believed to participate in carbohydrate metabolism. The problem is discussed in relation to oxidative processes in muscle and the situation with regard to methylglyoxal in fermentations in general.

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