XLVII. A QUANTITATIVE STUDY OF SUCCINIC ACID IN MUSCLE. I.

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Introduction.

That muscle-tissue contains a very powerful enzyme for the oxidation of succinic acid has been known since the work of Thunberg [1909], and specially of Batelli and Stern [1911], and this fact inevitably suggests that the acid may have an important rôle in muscle respiration. In the present paper is described an attempt to discover, by following quantitatively the changing concentrations of succinic acid under various conditions, the dynamic part played by the acid in the muscle.

Einbeck [1913, 1914] showed that perfectly fresh muscle tissue contains succinic acid; he thus disproved the older view that the succinic acid found in muscle extracts was always a product of putrefaction [Wolff, 1904, and many others], or a product (in the course of analysis) of a complex substance, carno-phosphoric acid [Siegfried, 1903]. Einbeck succeeded in preparing 0·112 g. of pure succinic acid from a kilogram of beef two hours after the death of the animal, but his method was unsuitable for quantitative work; he showed an increase in the succinic acid content of the meat, but only after long periods of storage (two and six weeks at 2°), when attack by moulds and bacteria had begun.

In the present investigation, absolutely fresh muscle was always (except in the case of the frog) found to yield a small quantity of succinic acid; this yield could be increased by incubating the minced muscle in suitable buffer solutions, and the effect on this yield of various factors (such as changing hydrogen ion concentration, added sugar, amino-acids, toluene, tissue extracts, etc.) has been studied. Beef which had been exposed for sale was found to contain no succinic acid, or not more than 0·5 mg. per 100 g. This must mean that the power of the muscle to oxidise the acid persists longer than the power to produce it; so it is not surprising that the older investigators, such as Wolff, whose freshest samples were one day old, should have entirely missed the presence of succinic acid before putrefactive changes set in.

Unfortunately, the increase in the succinic acid yield found, though relatively large (in most cases 4–6 fold) is absolutely very small, and this fact has made it impossible to obtain so far any direct evidence as to the precursor of the acid.

Method of Estimation.

The essential part of the method employed was the volumetric estimation of silver in the silver succinate precipitate in the muscle extract after the removal of all other interfering muscle constituents.

The minced muscle was first ground with sand and 50 % alcohol (according to Batelli and Stern [1911] this concentration of alcohol is sufficient to inhibit the action of the succinoxydase) and left to stand overnight. The mixture was strained through muslin, and the residue extracted twice more with 50 % alcohol. After filtering through paper, the extract was evaporated on a boiling water-bath nearly to dryness in a large dish. The protein which separated out was filtered off, and washed with boiling water. The filtrate was then evaporated down to dryness in a small dish.

The brown fatty residue was rubbed up very thoroughly with successive small quantities of cold saturated ammonium sulphate solution, and filtered in a special apparatus. This consisted of a 10 cc. cylindrical funnel, fitted with a capillary tap. At the top of the capillary, just above the tap, was placed a small flat piece of porous pot, and above this a pad of asbestos, about 2 mm. thick, was made. The capillary tube of the funnel passed through a rubber stopper, into an adapter, which communicated by a side-tube with the filter-pump, and which in turn fitted into the rubber stopper of a 100 cc. measuring cylinder. Not more than 20 cc. of saturated ammonium sulphate solution were used; the residue which collected on the pad was well stirred to ensure complete washing during the latter part of the filtration. This treatment with ammonium sulphate solution, as described by Meyerhof [1921] in his modification of Parnas' method of lactic acid estimation [1915], removes all the remaining protein and fat, and a beautifully clear yellow or brown filtrate is obtained; the high salt concentration also has a favourable effect on the partition coefficient in the ether extraction stage.

One-tenth of the volume of concentrated sulphuric acid and a little solid ammonium sulphate were added, and the solution was extracted with thrice its volume of ether five times, by shaking by hand. The ether was each time siphoned off through a dry filter paper into a flask containing about 10 cc. of water. The ether was distilled off, and the aqueous residue cooled, filtered, and made up to about 100 cc.

To this were added 3 cc. of concentrated sulphuric acid, and the flask was heated on a boiling water-bath; $N/10$ potassium permanganate was run in from a burette until a permanent brown precipitate was obtained. This permanganate titration is primarily to remove lactic acid, and acts as a fairly dependable rough indication of the amount of lactic acid present; sugar has

been removed in the ether extraction, and the only other substances to be oxidised are possible traces of fumaric, malic, and hydroxyglutaric acids, etc.

The oxidised solution was evaporated down to about 10 cc.; it was saturated with ammonium sulphate, and again extracted with pure ether five times in the same manner as before. This second extraction stage is necessary to get rid of the large amounts of sulphuric acid and manganese salts present. After removal of the ether, the faintly acid aqueous solution was made faintly alkaline with very dilute potassium hydroxide solution (to decompose any ammonium salts, which interfere with the precipitation of the silver succinate) and evaporated down to 10 cc. It was then made faintly acid again with dilute nitric acid, and 1 cc. of 10 % silver nitrate was added; the solution was neutralised with dilute ammonia and the silver succinate suspension warmed on a water-bath to coagulate the precipitate and then allowed to stand for 3–12 hours. It is important to precipitate in acid solution, neutralising afterwards, as it is often difficult to *start* the precipitation in neutral solution.

The precipitate was filtered off into a Gooch crucible, and washed four times with very dilute ammonium nitrate solution. It is very important that a standard method of washing should be used for all estimations. Silver succinate is appreciably soluble in cold water (17·6 mg. per 100 cc. water at 18°) and it was found, on titrating the washings with potassium thiocyanate, that silver-free filtrates could not be obtained even after eight to ten washings; but by the fourth washing the silver content had become constant at a very small amount. The silver precipitate was dissolved in hot nitric acid (about 1 : 4), the solution cooled and titrated against $N/100$ potassium thiocyanate solution, using iron alum as an indicator.

The method was tested by adding small known amounts of succinic acid to muscle, and comparing the succinic acid content of this and of untreated muscle. The beef used in these experiments had been exposed for sale and contained only traces of succinic acid; the values given in the following table were obtained by subtracting these small amounts from the values found for the treated muscle.

Succinic acid added mg.	Found	% recovered	No. of ether extractions	
			First stage	Second stage
5·0	5·1	102	5	4
4·8	4·3	90	6	6
5·2	4·9	94	6	6
5·2	4·7	92	4	6
4·5	4·2	95	6	6
4·5	4·4	97	8	8
4·5	4·5	100	6	6
4·5	4·1	91	6	6
4·5	4·0	90	6	6

These examples of results show that on an average 95 % of the succinic acid present in the muscle extract is accounted for, with an error of ± 5 %.

In order to test the purity of the silver succinate obtained in the final stage, the silver in it was estimated; the free acid was also prepared (without recrystallisation), and its melting point taken.

850 g. of beef, fresh from the slaughter-house, were treated exactly as described, and the solution, ready for precipitation, was divided into two parts.

(*a*) The silver salt was precipitated; it was carefully washed by decantation, then filtered through a Gooch crucible, and washed further. The washings were free from sulphate. The crucible was then dried at 110°–115° to constant weight. The precipitate was dissolved in nitric acid and the silver estimated with potassium thiocyanate. Percentage found = 62·1. Calculated percentage = 65·0.

(*b*) The silver salt was precipitated and washed in a Gooch crucible. It was then suspended, together with the asbestos, in water and H_2S was passed. The liquid was filtered and the dark brown filtrate was evaporated down to small bulk to coagulate the colloidal silver sulphide. The evaporation and filtration were continued alternately until, on evaporating completely to dryness, a residue was obtained, which was completely soluble in water. This was dried at 110°. M.P. 177·5°. Mixed M.P. (with Kahlbaum's succinic acid) 177·5°.

On another occasion, beef in which the maximum amount of succinic acid had been allowed to accumulate, was worked up. The melting point of the free acid was 179°; the mixed melting point was also 179°. The percentage of silver in the silver salt was found to be 63·9.

It is thus seen that the silver salt obtained is silver succinate in a state of very fair purity, and also that the added succinic acid can be quantitatively recovered. There is, however, one reservation which should be borne in mind with regard to the trustworthiness of the results. It is possible that under certain conditions, *e.g.* incubation in phosphate solution, traces might be formed of some unstable substances (of which the most likely seems to be α-hydroxyglutaric acid) which in the course of the method of estimation might be converted into succinic acid. Thus, suppose that α-hydroxyglutaric acid was formed in the muscle, it is possible that this would yield succinic acid on oxidation with permanganate; this is a difficult point to investigate, but work is being carried out on the subject.

Succinic acid formation in minced muscle.

The method adopted in the hope of encouraging as great an accumulation of the acid as possible was the suspension of the minced muscle in an approximately isotonic buffer solution (Na_2HPO_4) at an alkaline p_H. To prevent oxidation of the acid formed, either nitrogen was bubbled through the mixture or potassium cyanide was added. In some cases the nitrogen was carefully freed from oxygen, and in other cases taken straight from the cylinder; similarly, the concentration of potassium cyanide was varied within

fairly wide limits, but these changes had no detectable effect on the yields of succinic acid.

Details of the experiments with muscle from different animals are given below.

(A) *Beef. Exp.* 1. The muscle was removed from the neck of the bullock immediately after death and brought to the laboratory in a pan surrounded by ice and salt. The muscle was quickly dissected out, and minced into a cooled dish, where it was well mixed. Two lots of 100 g. each were weighed out, and placed in two flasks containing 175 cc. of 1·4 % Na_2HPO_4 solution (p_H 8·4). To one flask 5 cc. of $N/10$ KCN solution were added (the concentration of KCN thus being about 1 in 5000), the other was connected to a nitrogen cylinder, and a slow stream was kept bubbling. The flasks were placed in a thermostat (36–38°) for 3·5 hours. At the end of the time 300 cc. of 97 % alcohol were added to each. As soon as the samples in the thermostat were ready, 50 g. of the muscle were extracted at once with 50 % alcohol.

	cc. of $N/10$ permanganate required %	Succinic acid in mg. %
Initial	118	5·6
After 3·5 hrs. KCN	260	34·0
„ „ nitrogen	270	30·0

The working up of the samples was carried out exactly as described, except that the 50 % alcoholic extract was neutralised before evaporation. Controls were performed to show that all phosphate and cyanide are removed in the course of the estimation and do not interfere with the silver precipitation. The precipitate obtained was always white on first coming down.

(B) *Dog. Exp.* 2. Muscle from the thigh was removed in one piece and cooled in a pan surrounded by the freezing mixture. When the temperature had fallen to about 20° some uninjured muscle was dissected out and further cooled to 7°. It was then minced and two lots of 50 g. each were weighed out. One lot was extracted at once, the other was suspended in 100 cc. of 1·4 % sodium phosphate solution.

	cc. of $N/10$ permanganate required %	Succinic acid in mg. %
Initial	73	2·0
After 3·5 hrs. at 33° in nitrogen	204	24·3

(C) *Sheep. Exp.* 3. The mutton was obtained about half-an-hour after the death of the animal; the lactic acid maximum, as indicated by the permanganate titration had already been reached before the experiment began, but evidence of succinic acid formation was obtained.

	cc. of $N/10$ permanganate required %	Succinic acid in mg. %
Initial	138	4·9
After 3·5 hrs. at 37° in phosphate at p_H 7·0	142	14·7
Ditto, in Tyrode's solution	118	11·0

(D) *Frog.* The results obtained here were surprising as at first no succinic acid could be detected either in the fresh or in the incubated muscle.

Exp. 4. July. The hind-limbs were cooled in ice; the muscle was dissected without injury, and extracted at once with ice-cold 50 % alcohol. Two samples were worked up and no succinic acid was found in either.

Exp. 5. November. The hind-limbs of 15 frogs were put into chloroform rigor (in an atmosphere of nitrogen) in order to see whether the increase of lactic acid obtained in this way was accompanied by an increase in succinic acid. The muscles from 15 control frogs were extracted at once with 97 % ice-cold alcohol.

	cc. of $N/10$ permanganate required %	Succinic acid in mg. %
Initial	8	0·7
$CHCl_3$ rigor	100	1·4

Exp. 6. March. Two lots of 50 g. of muscle, suspended in 100 cc. of phosphate solution at p_H 7·0, were kept at 37–40° for 3·5 hours, and KCN added to make the solution about 1 in 5000. The control was extracted at once with 97 % alcohol.

	cc. of $N/10$ permanganate required %	Succinic acid in mg. %
Initial	20	0·0
After 3½ hrs.	290	0·0
,,	280	0·0

Exp. 7. December. In view of Laquer's work [1921] showing that the formation of lactic acid by muscle from added glucose does not take place at 45° while it does at 30°, it was thought that the high temperature might have had a deleterious effect on the factors concerned in succinic acid formation. A suspension of muscle in buffer solution (1·4 % phosphate) was therefore kept for 24 hours at room temperature (KCN about 1 in 5000) with the result that about 5 mg. % of succinic acid were found. Unfortunately no frogs were available for a control.

(E) *Rabbit.* When the mixed muscle of the whole animal was used, the succinic acid formation was found to be very small, though quite definite. 1·4 % phosphate solution was used and KCN as before.

Exp. 8.

	cc. of $N/10$ permanganate required %	Succinic acid found in mg. %
Initial	183	0·0
	165	0·0
After 1·5 hrs. (33°)	180	4·0
3·25 hrs.	204	5·3
4·0	197	5·4
5·0	208	5·9

In another experiment under similar conditions 4·2 mg. % of succinic acid were found after two hours.

Succinic acid formation in red and white muscle.

In consideration of the fact that Batelli and Stern [1912] found considerable differences between the activity of the succinoxydase in the red and white muscles of the hen and the calf—the enzyme being much more active in the red muscle—it was thought worth while to test the two kinds of muscle in the rabbit separately for succinic acid formation.

Two rabbits were used in each experiment. It had been thought that the smallness of the amount of succinic acid formed might be due to an injurious effect of the very rapid lactic acid formation in the mixed muscle of the rabbit; and that if the lactic acid formation could be delayed by thorough cooling, the succinic acid formation might get a better chance. After the rabbits had been skinned and drawn, therefore, the carcases were placed in a pan surrounded by a freezing mixture for over an hour before the muscle was dissected off and minced. The reddest muscle was dissected out and minced separately. One sample of red muscle and one of white were extracted at once; other samples were incubated in phosphate solution ($p_H = 8{\cdot}4$) with KCN at a concentration of 1 in 1700.

Exp. 9.

		cc. of $N/10$ permanganate required %	Succinic acid found in mg. %
White.	Initial	165	0·0
	3·5 hrs.	250	3·7
Red.	Initial	150	2·9
	3·5 hrs.	210	9·4

Exp. 10.

White.	3·5 hrs.	180	4·2
	3·5	185	4·7
Red.	3·5	114	10·4
	3·5	140	8·8

It must be pointed out that the red muscle used was very tendinous and that much of it was of a light red colour; probably if a sufficient quantity of the really dark muscle could be obtained, a still higher figure for the succinic acid would be found. The permanganate titration figures show clearly the much slower formation of lactic acid in the red muscle, as was found by Fletcher [1912]. It is interesting to note that, in Exp. 10, the sample giving the higher lactic acid figure (and therefore probably containing more pale muscle) gives a lower figure for succinic acid. Even with the greater precautions in cooling, the lactic acid formation in the white muscle goes on much more rapidly than in the muscle from the ox and from the dog.

The effect of added sugar, amino-acids, and toluene.

(*a*) *Sugar.* The addition of glucose to incubating beef muscle increases the lactic acid formation to an extent quite outside experimental error, without affecting the production of succinic acid. The samples were suspended in 1·4 % phosphate, and incubated at 37° for 3·5 hours.

	cc. of $N/10$ permanganate required %	Succinic acid found in mg. %
Exp. 11.		
No glucose	294	29·1
	295	33·8
Glucose added	370	34·7
	371	30·8
Exp. 12.		
No glucose	257	22·9
Glucose added	326	23·9

(*b*) *Amino-acids. Exp.* 13. The amino-acids tried were glutaminic and aspartic; 0·2 g. of each acid (in neutral solution) was added to one sample of muscle, suspended in phosphate solution at p_H 8·4, in an atmosphere of nitrogen. The sample and a control were kept at 37° for 3·5 hours.

Control	15·6 mg. % succ. acid
Glutaminic and aspartic acids added	83·4 ,, ,,

Thus a markedly increased yield of succinic acid resulted from the addition of these amino-acids.

(*c*) *Toluene.* The addition of toluene interferes seriously with succinic acid formation, though only in certain cases with that of lactic acid. In the following table this is shown. Each sample of 50 g. of muscle was suspended in 100 cc. of 1·4 % Na_2HPO_4 solution, 5 cc. of toluene and 1 g. of sugar were added to certain of the samples as pointed out in the table.

Exp. 14. Conditions of experiment				cc. of *N*/10 permanganate required %	Succinic acid found in mg. %
Initial				(*a*) 115	6·9
3 hrs.	KCN 1 : 4000.	No sugar.	Toluene	(*b*) 251	14·8
3	N_2	,,	,,	(*c*) 247	21·7
3	N_2	,,	No toluene	(*d*) 257	22·9
18	KCN 1 : 1500.	,,	Toluene	(*e*) 257	16·5
18	KCN 1 : 4000.	,,	,,	(*f*) —	15·6
3	KCN 1 : 4000.	Sugar	,,	(*g*) 261	14·0
3	N_2	,,	,,	(*h*) 296	20·5
3	KCN 1 : 4000.	,,	No toluene	(*i*) 326	23·9
Exp. 15.					
3	KCN 1 : 5000.	No sugar.	No toluene	(*j*) 294	29·1
3	KCN 1 : 5000.	Sugar.	,,	(*k*) 370	34·7
3	KCN 1 : 5000.	,,	Toluene	(*l*) 340	12·0

These figures show:

1. The inhibitory effect of toluene on succinic acid formation. Compare (*b*) and (*g*), also (*c*) and (*h*), (*e*) and (*f*), with (*d*) and (*i*). The much smaller effect in the cases of (*c*) and (*h*) is probably due to the carrying away of the toluene vapour by the stream of nitrogen.
2. The inhibitory effect of toluene on the formation of lactic acid from *added* glucose. Compare (*g*) and (*h*) with (*i*), and (*l*) with (*k*). On the other hand the non-effect of toluene on the formation of lactic acid from the carbohydrate of the muscle itself. For this compare (*b*), (*c*) and (*e*) with (*d*).
3. Further examples of the non-effect of added glucose on succinic acid production. Compare (*b*) and (*c*) with (*g*) and (*h*).

It is extremely improbable that the larger increase in succinic acid found in the absence of toluene is due to bacterial action. In the first place, there is quite as much chance for bacterial action in the case of the frog and rabbit muscle, where the succinic acid production is nil or very small; and, in the second place, the time relations of the formation, as described later, are against the probability of bacterial action.

EFFECT OF THE ADDITION OF VARIOUS PANCREAS PREPARATIONS.

It was thought possible that the smallness of the succinic acid production might be due to the presence of only a very limited amount of its immediate precursor, and that some internal secretion—normally brought in the circulation, but absent from excised muscle—might be necessary for the production of new supplies of the precursor. For example, it was suggested that succinic acid production might be an alternative course to lactic acid production in carbohydrate metabolism, and that the former acid might arise from the breakdown of some special form of sugar. Now it seems that the pancreas is concerned in the production of the special form of sugar found in the blood [Winter and Smith, 1923, 1, 2]; moreover, Winfield and Hopkins [1916] had shown that the addition of ground pancreas to chopped muscle caused a marked inhibition of lactic acid formation. Experiments were therefore performed to see whether the carbohydrate which, in the presence of the pancreas, had not been converted into lactic acid, might possibly have been turned into succinic acid. Instead of the ground pancreas having a stimulating effect, however, a well-marked inhibition of the succinic acid formation (approximately proportional in most cases to the lactic acid inhibition) was observed.

Exp. 16. As before, perfectly fresh beef was obtained. A rabbit's pancreas was used, and ground with sand in Tyrode's solution, the p_H of which was adjusted to 7·0 by adding dilute hydrochloric acid. In order to get as thorough mixing as possible of the pancreas preparation with the muscle before any considerable acid production could take place, 150 g. of the muscle were weighed out and pounded in a mortar with the ground pancreas; this mixture was then divided into three equal samples. Two samples were suspended each in 100 cc. of phosphate solution at p_H 5·8, and well mixed. Two control samples were each suspended in the same amount of phosphate solution. Finally the requisite amount of soda to bring the p_H of the solution to 7·0, not allowing for the acidity of the tissues, was added to each flask. Two flasks, one control, and one with pancreas preparation, were connected to a nitrogen cylinder and kept in an atmosphere of nitrogen; to the other two flasks KCN was added (1 in 4000). The third lot of 50 g. was suspended in Tyrode's solution at p_H 7·0, and a control in the same solution was also prepared. All were kept for 3·5 hours at 37°.

			cc. of *N*/10 permanganate required %		Succinic acid found in mg. %	
Phosphate.	N_2.	No pancreas extract	302	60 % inhib.	21·5	55 % inhib.
Phosphate.	N_2.	Pancreas extract	126		9·8	
Phosphate.	KCN.	No pancreas extract	294	57 % inhib.	18·0	80 % inhib.
Phosphate.	KCN.	Pancreas extract	128		3·5	
Tyrode.	N_2.	No pancreas extract	160	19 % inhib.	17·0	20 % inhib.
Tryode.	N_2.	Pancreas extract	130		13·0	

Exp. 17. This experiment was performed in July, and the effect, both on

lactic and succinic acid production, was much less. This was probably due to the greater difficulty in cooling the tissue.

	cc. of $N/10$ permanganate required %	Succinic acid found in mg. %
No pancreas extract	310	20·8
Pancreas extract	220 } 30 % inhib.	16·3 } 22 % inhib.

Exp. 18. Mutton was obtained from the slaughter-house, but about 30 mins. had elapsed between the killing of the animal and the cooling of the meat. The experiment was carried out just as for the beef, two samples being suspended in phosphate solution, and two in neutralised Tyrode's solution. It was found that the lactic acid maximum had already been reached at the beginning of the experiment, and no inhibition was seen, *i.e.* no resynthesis of lactic acid seems to have taken place; but succinic acid formation and inhibition are clearly seen.

		cc. of $N/10$ permanganate required %	Succinic acid found in mg. %
Initial		(*a*) 138	4·9
Phosphate.	No pancreas extract	(*b*) 142	14·7
Phosphate.	Pancreas extract	(*c*) 130	10·4
Tyrode.	No pancreas extract	(*d*) 118	11·0
Tyrode.	Pancreas extract	(*e*) 122	8·2

Effect of absence of buffer. In this experiment the effect of the absence of the buffer, and the consequent rise in hydrogen ion concentration of the medium is seen in the smaller yield of succinic acid in (*d*) and (*e*).

Other pancreas preparations were tried in order to see whether the succinic acid production was always affected in a similar way to that of lactic acid.

Exp. 19. Commercial pancreatin was used, 0·4 g. being added to each of samples (*c*) and (*d*). The pancreatin used for sample (*e*) was first digested with alkali in order to destroy trypsin: 1 g. was mixed with 50 cc. of 1 % Na_2CO_3 solution and left for 20 hours at 37° with toluene. The neutralised solution was found to be trypsin-free, for there was no digestion of casein after 48 hours; the toluene was removed by warming to 40°. 20 cc. were used for sample (*e*). The experiment was carried out just as was Exp. 16, except that the p_H was made up to about 7·6, not allowing for the acidity of the tissue, and the muscle was left for about 30 minutes in the *acid* phosphate solution with the pancreatin, before the alkali was added.

	cc. of $N/10$ permanganate required	Succinic acid found in mg. %
Control	(*a*) 244	27·6
Control	(*b*) 260	31·8
With pancreatin	(*c*) 96 (62 % inhib.)	13·8 55 % inhib.
With pancreatin	(*d*) —	13·3 „ „
With digested pancreatin	(*e*) 225 No inhib.	27·1 No inhib.

Exp. 20. The effect of added insulin was tried, 5 mg. freshly dissolved in a little Ringer solution being added to each of samples (*d*) and (*e*). The experiment was carried out just as was Exp. 16 except that the p_H was made up to 8·0, not allowing for the acidity of the tissue.

	cc. of $N/10$ permanganate required %	Succinic acid found in mg. %
Initial	(*a*) 84	5·1
No insulin	(*b*) 220	16·3
No insulin	(*c*) 200	17·8
Insulin	(*d*) 210	16·0
Insulin	(*e*) 226	15·1

Exp. 21. The last experiment was repeated, with the differences that care was taken to prevent the p_H from rising above 7·0, for fear of destruction of the insulin by the alkaline reaction during the experiment, and that nitrogen was used.

	cc. of $N/10$ permanganate required %	Succinic acid found in mg. %
Initial	110	8·8
No insulin	280	27·7
Insulin	304	26·4
Insulin	300	24·3

TIME-RELATIONS OF SUCCINIC ACID PRODUCTION.

Exp. 22. The muscle was removed from the leg of a dog immediately upon the death of the animal, and dropped into a pan sunk in ice. The muscle was minced when it had cooled down to about 8°. Four lots of 50 g. each were weighed out into four flasks, each containing 100 cc. of Na_2HPO_4 solution at p_H 8·4 and KCN. The flasks were placed in an incubator at 33°. An initial example was extracted at once.

	Succinic acid in mg. %
Initial	8·0
1 hr.	17·4
2 hrs.	22·0
3·5 hrs.	26·9
3·5 hrs.	29·4

These results are plotted on the accompanying curve (Fig. 1). The contents of the flasks required some time to attain the temperature of the incubator, so that the earlier part of the curve is somewhat flattened; but, of course, all the samples were under the same conditions.

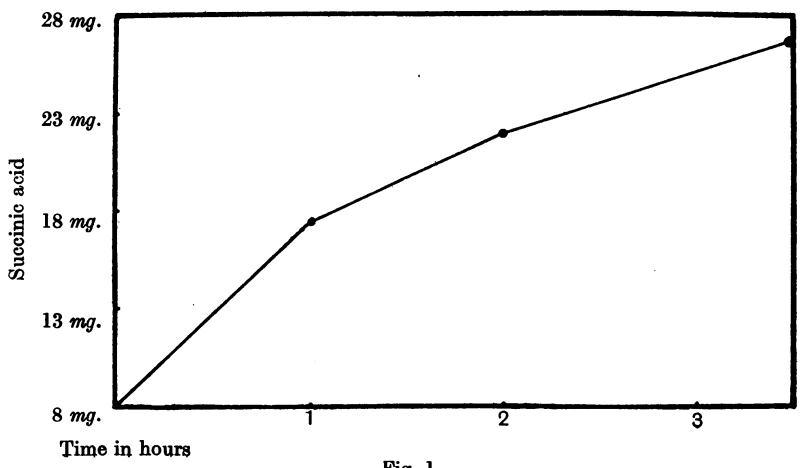

Fig. 1.

Exp. 23. With beef the experiment was carried out at 25°. The muscle samples were weighed out into cooled beakers, and then, when all were ready, transferred as quickly as possible to the flasks of phosphate solution containing 1 : 4000 KCN, already in the bath. In this way the constant temperature was very quickly attained.

	cc. of $N/10$ permanganate required %	Succinic acid found in mg. %
Initial	114	10·0
0·5 hrs.	170	20·3
3·3 hrs.	198	21·4
6·0 hrs.	210	21·3

Exp. 24. The last experiment was repeated.

Initial	120	8·6
20 mins.	160	20·6
40 mins.	192	20·6
2 hrs.	260	22·5

These results show that, in beef, the succinic acid maximum is very quickly reached. The fact that this maximum remains unchanged even after six hours makes it practically impossible that bacterial action can be responsible.

Discussion of results.

The results show formation of succinic acid in all the different kinds of muscle tested, with the possible exception of that of the frog. The very small increase in succinic acid concentration in the pale muscle of the rabbit and in the muscle of the frog is interesting; it has been shown that, after 3·5 hours' incubation the red muscles of the rabbit contain about three times as much succinic acid as do the corresponding weights of white muscle. It is possible that this fact should be correlated with the finding of Batelli and Stern, that the red muscle of the hen and the calf are much more active in the oxidation of succinic acid than the pale muscles of these animals; it may be that in pale muscle the whole question of succinic acid metabolism is much less important. Frog muscle certainly contains a succinoxydase, but as the figures given by Thunberg [1909] for the oxidation of succinic acid in the muscle of the frog are obtained from unwashed muscle, while those of Batelli and Stern [1911] for the rabbit, dog, ox, etc. are with washed muscle, the activity of the frog cannot be compared with that of the mammalian muscle. It is hoped that directly comparable figures may be obtained, and also that the succinic acid formation in other red and white muscles may be determined.

The origin of the succinic acid remains obscure. The result with added glutaminic and aspartic acids shows that the tissue possesses the power of deamination of one or both of these amino-acids (whether hydroxyglutaric or succinic acid is formed is, of course, as yet undecided). But the fact that the muscle could form succinic acid from these amino-acids would not necessarily mean that this was the only source of the acid in the muscle. The extraordinary activity of the succinoxydase makes one inclined to believe that

something more important is involved than the oxidative metabolism of these two amino-acids, especially when it is remembered that they have no specific dynamic action [Lusk, 1913; Atkinson and Lusk, 1918].

The similarity, which has been noticed in the effect of pancreatic preparations on the lactic and succinic acid production, might be interpreted as an indication that succinic acid is involved in carbohydrate metabolism. This view would be much strengthened if it were possible to obtain a trypsin-free extract with the property of inhibiting both succinic and lactic acid formation. Work with this object is now in progress. It seems impossible, however, that succinic acid formation can be a reaction necessarily coupled in any way with the production of lactic acid, for it has been shown that lactic acid is still being formed long after the succinic acid has reached its maximum; and the addition of sugar and toluene may affect the production of one acid and not of the other. All that one could suppose would be that the succinic acid formation is a side-reaction or a concurrent reaction, proceeding at a velocity independent of the velocity of the production of lactic acid.

The very low value of the succinic acid maximum might be explained in several ways: (*a*) lack of precursor; (*b*) lack of co-ferment; (*c*) equilibrium point; (*d*) destruction of enzyme, *e.g.* by accumulation of lactic acid. If the precursor be an amino-acid, the low maximum is easily understood, as the amino-acids are brought to the muscle in the blood-stream, and do not accumulate there. The question of the need of a co-ferment has been considered in the paragraph on pancreatic extracts.

In order to test whether the succinic acid maximum obtained is an equilibrium point determined by accumulation of end-product, it would be necessary to remove the succinic acid formed, and see whether more could be produced to take its place. Preliminary experiments, in which the muscle has been placed alternately in atmospheres of nitrogen and oxygen, have been carried out, and the few results obtained so far, seem to show that the maximum can be repeatedly obtained.

Summary.

1. A method has been described for the estimation of succinic acid, but the reservation is made that any hydroxyglutaric acid in the muscle may be included in the figures given for succinic acid.

2. An increased yield of succinic acid is found after anaerobic incubation in phosphate buffer solutions, of p_H 7·0–8·4, in the muscle of the ox, dog, sheep, rabbit, and possibly of the frog.

3. The yield is increased by adding glutaminic and aspartic acids, but not by adding sugar.

4. The yield is markedly decreased by adding certain pancreatic preparations.

5. In the red muscle of the rabbit, the yield is much greater than in the white.

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