CXLVIII. SUCCINOXIDASE.

II. INFLUENCE OF PHOSPHATE AND OTHER FACTORS ON THE ACTION OF THE SUCCINODEHYDROGENASE AND THE FUMARASE OF LIVER AND MUSCLE.

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MUSCLE, liver and other tissues have been shown [Thunberg, 1909; Batelli and Stern, 1911] to contain a very powerful and remarkably specific enzyme, succinoxidase, which, according to Batelli and Stern, converts succinic into optically inactive malic acid. Einbeck [1914] regarded fumaric and not malic acid as the sole product, but later [1919] showed that the enzyme first converts succinic to fumaric acid and then, by a balanced reaction, to a mixture of fumaric (25 %) and malic (75 %) acids. Dakin [1922] showed that fumaric acid, reacting with muscle pulp, formed exclusively *laevo*-malic and not the inactive acid.

A number of attempts have been made to isolate the enzyme. Thus Batelli and Stern extracted it by means of dilute sodium hydroxide but obtained an enzyme of very low activity. Widmark [1918] improved the method by using 1.5 % sodium carbonate. Ohlsson [1921] substituted a *M*/15 solution of disodium hydrogen phosphate and this method has been generally used. Modifications, largely of the method of washing the muscle previous to extraction, have been introduced by Grönwall [1923], Svensson [1923], Sahlin [1924] and finally by Anderson [1927], who extracted the muscle several times with dilute saline before using *M*/15 phosphate, thus obtaining an enzyme extract considerably freer from proteins. All these investigators, however, have followed the course of the action of the enzyme in terms of the reduction times of methylene blue, using the usual Thunberg technique. Recently, Hahn and Haarmann [1927, 1928] and Fischer [1927] have shown that washed muscle, with sufficient methylene blue and sodium succinate, gives rise anaerobically to the same products, fumaric and malic acids, which it produces aerobically, and it seems likely therefore, that the enzyme system is the same in the two types of experiments.

In a recent paper [Clutterbuck, 1927] a polarimetric method was described for the determination of succinic acid in muscle and liver pulp in terms of its conversion into *l*-malic acid. Using this method, it was shown that liver was

eight times as active as the same moist weight of muscle in converting succinic or fumaric acid into malic acid, and cyanide, although completely inhibiting the oxidation or dehydrogenation of succinic acid, had no effect on the conversion of fumaric to malic acid. These experiments emphasised the fact that at least two enzymes were present in the oxidase system, a true succinodehydrogenase converting succinic to fumaric acid and a fumarase, converting fumaric to malic acid. This polarimetric method permits the study of both reactions and of the effect of various factors, other than cyanide, upon them. Also, by its means some preliminary experiments have been carried out, which are designed to discover the best method of separating the two enzymes from tissue and from one another.

SOLUBILITY AND STABILITY OF SUCCINODEHYDROGENASE AND FUMARASE.

One of the most striking differences, apart from the action of cyanide, between the fumarase and dehydrogenase is seen in respect to their solubilities and stabilities. The opalescent enzyme solution obtained by steeping washed rabbit's muscle in phosphate solution and centrifuging readily reduces methylene blue in presence of succinate and absence of oxygen and therefore contains the dehydrogenase. If, however, the solution is shaken a little by hand, the enzyme is completely inactivated. Moreover, when the phosphate enzyme solution or the aqueous extract of muscle is placed in a bottle with sodium succinate solution and rotated, the air being displaced by oxygen as in the experiments previously described [Clutterbuck, 1927], it was found that even the gentle rotational movement necessary to ensure oxygenation, although accompanied by only slight frothing, was sufficient to prevent any oxidation of succinate. During the rotation, however, a clot of coagulated protein formed, but the opalescence only slightly decreased. The clot probably contained the inactivated dehydrogenase, whilst the remaining turbidity was due to the fact that the fumarase protein complex under these conditions is quite stable, and coagulates only to a slight extent. A phosphate enzyme extract, rotated thus with sodium fumarate solution, showed in fact a six times greater fumarase activity than the original muscle pulp. Succinoxidase has also always been regarded as an extremely insoluble enzyme, only elutable by alkaline (phosphate, carbonate, etc.) solutions and after elution being readily precipitable by bringing the p_H of the solution to 6.5. It has been found, however, that although the dehydrogenase could not be detected in the aqueous extracts by our method, owing most probably to its inactivation during rotation, yet active fumarase is readily washed out of muscle by water or dilute (0.25 %) saline and is not precipitated to any great extent at p_H 6.5.

The "active" and "elutable" fumarase of muscle and liver pulp.

A number of experiments were therefore carried out in which the minced liver or muscle pulp (of rabbit) was steeped in an equal weight of water or 0.25 % saline, placed in a fine cloth and the fluid expressed. This process was

repeated 16 times and the extracts combined in fours and the activity determined polarimetrically. In order conveniently to compare the activity of the extracts, the production of 1.22 g. *l*-malic acid in 1 hour at 38° using 500 cc. of 1 % fumaric acid, as sodium salt, is called unit activity. The following results were obtained:

Muscle (150 g.)		Liver (50 g.)	
Saline or aqueous extracts	Fumarase activity units	Saline or aqueous extracts	Fumarase activity units
1-4	1.0	1-4	4.0
4-8	0.5	4-8	2.0
8-12	0.25	8-12	0.5
12-16	0.20	12-16	0.3

It will be seen that the amount of free or "active" enzyme, removed in the aqueous extracts of muscle and liver pulp, has approximately the same activity as the original pulp.

The muscle and liver residues were then steeped, on the one hand, in an equal weight of *M*/15 phosphate for an hour and, on the other, in an equal weight of *M*/15 bicarbonate solution to which a little carbonate had been added to bring the p_H approximately to that of the phosphate, namely about 9, and the extracts were then expressed as before. This process was repeated three times and the activity of each extract was determined. Finally the tissue residue was steeped twice for 24 hours and the activity of the extract found. Using phosphate as the eluting agent, expressing the results in units as above, we obtain the following results:

Muscle (150 g.)		Liver (50 g.)	
Phosphate extracts	Fumarase activity units	Phosphate extracts	Fumarase activity units
1 after 1 hour	4.0	1 after 1 hour	0.25
2 " 1 "	2.0	2 " 1 "	0.25
3 " 1 "	0.6	3 " 1 "	0.25
4 " 24 hours	0.6	4 " 24 hours	1.0
5 " 24 "	0.5	5 " 24 "	0.8

Using bicarbonate as eluting agent the results were similar but the activity was always somewhat smaller.

If we now convert the figures of our previous paper into units, whereas the fumarase activity of 100 g. muscle pulp was 1 unit, that of 100 g. liver pulp was about 8 units. Comparing these figures with those of the two sets of figures above, we obtain the following results.

(1) Liver pulp, weight for weight, has 8 or 10 times as great a fumarase activity as muscle pulp, but this only indicates that the liver pulp contains 8 or 10 times as much free or "active" enzyme as muscle.

(2) An amount of fumarase is removed from muscle and from liver by steeping 8 times in an equal weight of water or saline, approximately equal in activity in each case to the activity of the tissue pulp itself.

(3) After the amount of enzyme, extracted by this treatment with water or saline, has become reduced to a very small amount, steeping in *M*/15

phosphate or in a bicarbonate-carbonate buffer of the same p_H and expressing liberates from the muscle residue a further large amount of enzyme, having in the several extracts six times the activity of the original pulp, whereas similar treatment of the liver residue, the volume of which is however much smaller than the muscle residue, removes only a small amount of enzyme.

(4) The total activity of the water and phosphate extracts of 50 g. liver was approximately equal to the total activity of the extracts of 150 g. muscle.

It is concluded, therefore, that, although in the pulp liver appears 8 or 10 times as active as muscle in converting fumaric to malic acid, yet the total amount of enzyme, free and elutable, of liver is only about three times as much as in muscle, and, whereas almost the whole of the liver fumarase is free and active in the pulp emulsion, only about one-seventh of the total muscle fumarase is free and active, the remainder being readily eluted by alkaline bicarbonate-carbonate or phosphate buffer solution of p_H about 9. Borate buffer at this p_H gelatinised the muscle and no extract could be expressed. Washing the liver or muscle pulp with water or 0.25 % saline removes only the free, active enzyme, *i.e.* removes one-seventh of the total muscle enzyme but almost all of the liver enzyme. Previous workers, using washed muscle, have always employed either pulp in phosphate buffer or a phosphate enzyme extract of muscle and they have, therefore, by their technique, eluted and used in an activated condition that part of the enzyme not removed by washing.

The influence of phosphate on the action of the fumarase of muscle pulp.

That the activity of muscle fumarase is greater in phosphate than in bicarbonate buffer at the same p_H (7.2) is seen from the following experiment in which a sample of muscle (50 g.) was rotated, on the one hand, with fumarate (500 cc. 1 %) as control and, on the other, with fumarate to which had been added phosphate and bicarbonate sufficient to make the concentration 0.05 *M*, the p_H in all three cases being adjusted to 7.2. The following figures were obtained polarimetrically for the amount of *l*-malic acid produced.

Time (hours)	Control. <i>l</i> -Malic g.	+ Phosphate (0.05 <i>M</i>). <i>l</i> -Malic g.	+ Bicarbonate (0.05 <i>M</i>). <i>l</i> -Malic g.
0.5	0.45	1.30	0.60
1.0	0.78	2.22	1.05
1.5	1.26	2.66	1.61
2.0	1.55	2.90	1.97

It would appear therefore that phosphate, in addition to having the power (like bicarbonate) to elute the enzyme in virtue of its alkalinity, has also a direct activating effect on the fumarase. This activating effect was next investigated.

A large number of experiments with muscle pulp and fumarate have now been carried out and it has been found that the initial fumarase activity of the pulp varies considerably from animal to animal and often appears to be

related to the condition of the animal. In all the experiments recorded in this paper the usual apparatus was therefore duplicated, two bottles being rotated at the same speed in the same thermostat, one containing muscle and fumarate as control and the other muscle, fumarate and phosphate or other added substance. The usual 25 cc. sample was taken simultaneously from the two bottles, coagulated by heat and uranium acetate added. It was found that, if the flasks were then allowed to stand for half an hour, 75 cc. water being then added and allowed to stand for a further short period, the occasional difficulty previously reported of obtaining a clear filtrate for polarimetric observation never arose.

When phosphate is added to muscle and liver pulp and rotated with sodium fumarate solution, the rate of conversion to malate is greatly accelerated. That this is not a p_H effect, may be proved by adjusting the p_H of the two bottles to say 7.2 before the experiment when the same phosphate activation is still observed. Moreover, if a sample of the same pulp is caused to react with fumarate on the one hand in phosphate buffer, and on the other in borate buffer solution of the same p_H , whereas in the latter the rate is practically normal (there is actually a slight inhibition), in the phosphate buffer the usual acceleration is obtained. It would appear that the p_H optimum for fumarase covers a fairly wide range from about 6 to 9, and that changes of p_H over this range have relatively a slight effect on the rate of the reaction. The following results are typical examples obtained in a number of experiments using 50 g. minced sterile muscle pulp with 500 cc. 1 % fumaric acid (as sodium salt) rotating at 38° and comparing with a second similar sample of the same muscle and fumarate containing various amounts of added phosphate, the p_H in all cases being adjusted to 7.2. The figures give the *l*-malic acid (g.) produced, as determined polarimetrically.

Time (hours)	0.25	0.50	0.75	1.00	1.50	2.00
	<i>l</i> -Malic produced (g.)					
Control	0.31	0.67	0.97	1.32	1.82	—
+ 0.013 <i>M</i> phosphate	0.43	0.91	1.26	1.52	2.12	—
Increase of <i>l</i> -malic (g.)	0.12	0.24	0.29	0.30	0.30	—
Control	0.50	0.81	1.11	1.46	2.02	—
+ 0.026 <i>M</i> phosphate	0.63	1.14	1.65	2.02	2.64	—
Increase of <i>l</i> -malic (g.)	0.13	0.33	0.54	0.56	0.62	—
Control	0.34	0.61	0.90	1.14	1.55	2.02
+ 0.052 <i>M</i> phosphate	0.78	1.35	1.87	2.28	2.69	2.94
Increase of <i>l</i> -malic (g.)	0.44	0.74	0.97	1.14	1.14	0.92
Control	0.41	0.84	1.16	1.55	2.15	2.62
+ 0.078 <i>M</i> phosphate	0.47	1.03	1.41	2.11	2.82	3.22
Increase of <i>l</i> -malic (g.)	0.06	0.19	0.25	0.56	0.67	0.60
Control	0.40	0.80	1.15	1.50	2.10	—
+ 0.104 <i>M</i> phosphate	0.48	1.00	1.58	2.06	2.67	—
Increase of <i>l</i> -malic (g.)	0.08	0.20	0.43	0.56	0.57	—
Control	0.55	0.93	1.30	1.69	2.34	—
+ 0.156 <i>M</i> phosphate	0.61	1.22	2.00	2.62	3.29	—
Increase of <i>l</i> -malic (g.)	0.06	0.29	0.70	0.93	0.95	—

If the results of two of these experiments are graphed (Fig. 1), namely those with 0.052 *M* phosphate (which shows optimal activity) and with 0.156 *M* phosphate, it is seen that with the former, as also in experiments with smaller amounts of phosphate, the curve with phosphate (*A*) is regular and lies above the control (*B*), whereas with larger than optimal amounts (*C*, *D*)

Fig. 1.

Fig. 2.

there appears at first an inhibition which is only overcome after about half an hour. The control curves for the muscle (*B*, *D*) of different amounts are all similar but vary considerably in steepness, and the activity in those muscles which have been examined in spring and summer appears on the whole to be higher than that of the muscle of animals previously recorded, which were examined in autumn and winter.

The effect of the phosphate concentration is best seen by plotting the concentration of added phosphate against the increased amount of *l*-malic acid found after 1.5 hours (Fig. 2).

From this curve it is seen that the increase in the formation of *l*-malic acid is almost directly proportional to the increase in phosphate concentration until the latter has increased to about 0.05 *M*. This appears to be an optimal concentration and with further increase the curve at first falls and then rises again. It is interesting to note that this optimum is identical with the phosphate optimum obtained by Platt and Dawson [1926] for the action of pancreatic lipase.

Effect of phosphate on the rate of dehydrogenation by muscle pulp.

Since the action of succinodehydrogenase is so very much slower than that of fumarase, it should be possible by carrying out similar experiments, with and without phosphate, replacing fumaric with succinic acid, to detect any effect of phosphate on the rate of dehydrogenation. Rotating 50 g. muscle in 500 cc. of 1 % succinic acid solution (as sodium salt) at 38° in an atmosphere of oxygen as control and a similar suspension containing phosphate (0.05 *M*), no influence on the rate of the dehydrogenation could be detected.

Influence of phosphate on the activity of liver pulp and of aqueous extracts of muscle and liver pulp.

The fumarase of liver pulp is activated by phosphate in the same way as that of muscle pulp. Thus, with one of the most active samples of liver that we have examined, the difference in the activity with and without the optimal amount of added phosphate was of the same order as in the muscle experiments; *e.g.* rotating 15 g. liver with 500 cc. of 1 % fumarate at 38° as control and a similar sample to which phosphate was added to make the concentration 0.05 *M*, the p_H being adjusted in both cases to 7.2, the following results were obtained.

Time (hours)	Control		+ 0.05 <i>M</i> phosphate		Increase of <i>l</i> -malic (g.)
	Rotation	<i>l</i> -Malic (g.)	Rotation	<i>l</i> -Malic (g.)	
0	0	0	0	0	0
0.25	1.02	1.14	1.44	1.61	0.47
0.50	1.74	1.95	2.44	2.73	0.78
0.75	2.10	2.35	3.07	3.44	1.09

Phosphate not only activates the fumarase of the tissue pulps but also activates the free eluted enzyme of the aqueous extracts of the pulps. Thus with 30 g. liver, eight aqueous extracts were combined, made up to 400 cc. and divided into two equal portions. One sample was rotated with fumarate, the final concentration being 1 % as before and the other with fumarate and sufficient phosphate to make the concentration optimal, the final p_H in both cases being 7.2. The results were as follows.

Time (hours)	Control		+ 0.05 M phosphate		Increase of <i>l</i> -malic (g.)
	Rotation	<i>l</i> -Malic (g.)	Rotation	<i>l</i> -Malic (g.)	
0	0	—	—	—	0
0.25	0.72	0.81	1.41	1.58	0.77
0.50	1.25	1.40	2.32	2.60	1.20
0.75	1.65	1.85	2.79	3.12	1.27
1.00	1.98	2.22	3.06	3.42	1.20

Similarly with the enzyme of aqueous extracts of muscle pulp, phosphate activates almost to the same extent as in the pulp experiments.

Influence of temperature on the fumarase activity of liver and muscle pulp.

The fumarase of liver, as we have already shown, is almost entirely in the free active condition and we might expect, therefore, to find that the activity of the enzyme, on rotating with fumarate at different temperatures, would change according to the well-known rule, namely, that for every rise of 10° the rate of reaction would be doubled or trebled. This was found to be the case. Thus with 15 g. of a very active sample of liver the following figures were obtained.

15 g. liver, 500 cc. fumarate, p_H 7.2, temp. 39°		15 g. liver, 500 cc. fumarate, p_H 7.2, temp. 19°	
Time (hours)	<i>l</i> -Malic formed (g.)	Time (hours)	<i>l</i> -Malic formed (g.)
0	0	0	0
0.25	1.00	1	0.96
0.50	1.80	2	1.67
0.75	2.46	3	2.29
1.00	2.84	4	2.83
1.50	3.40	5	3.24
2.00	3.44	6	3.44

The rate at 39° at the beginning of the experiment was almost exactly four times that at 19° but subsequently fell to three times.

With a much less active sample of liver, the activity at 39° was five or six times that at 19° and on plotting the results (see Fig. 3) it is seen that with the less active sample at the lower temperature, there is a tendency for the curve to become more S-shaped. The figures for the less active liver are the following.

Time (hours)	<i>l</i> -Malic formed at p_H 7.2, temp. 39°	Time (hours)	<i>l</i> -Malic formed at p_H 7.2, temp. 19°
0	0	0	0
0.25	0.39	0.5	0.15
0.50	0.90	1.0	0.32
0.75	1.50	1.5	0.52
1.00	2.06	2.0	0.76
1.25	2.50	2.5	1.12
1.50	3.01	3.0	1.58
2.00	3.42	4.0	2.37
2.50	3.46	5.0	2.85
		6.0	3.22
		7.0	3.44

With muscle pulp, however, the influence of temperature appears to be somewhat different. We have already shown that only about one-seventh of the total elutable enzyme of muscle is free and active whilst the rest is adsorbed or in some way held in an inactive form but is elutable by phosphate or

bicarbonate-carbonate buffer of p_H 9.0. Using muscle pulp (50 g.) the activity as is seen from the accompanying figures is only twice as great at 39° as at 19° .

Time (hours)	<i>L</i> -Malic formed at p_H 7.2, temp. 39°	Time (hours)	<i>L</i> -Malic formed at p_H 7.2, temp. 19°
0	0	0	0
0.33	0.60	0.33	0.34
0.66	1.15	0.66	0.60
1.00	1.72	1.00	0.82
1.50	2.22	1.50	1.14
2.00	2.79	2.00	1.46
2.50	2.96	2.50	1.72

Fig. 3.

It is remarkable that the temperature coefficient with muscle pulp is so much smaller. Whether this is an adsorption effect or due to the production of some inhibitory substance at the higher temperature is not clear, but the former view is somewhat supported by the fact that if in place of the muscle pulp we use the aqueous extract of the muscle, then the coefficient is a little larger but even then it does not double every 10° as in the case of the liver enzyme. Thus, eight aqueous extracts of 100 g. muscle were combined, the p_H was adjusted to 7.2, the liquid made up to 800 cc. and divided into two equal portions and rotated with fumarate, one at 39° and the other at 19° . The results were as follows.

Time (hours)	<i>L</i> -Malic formed at 39° (g.)	<i>L</i> -Malic formed at 19° (g.)
0	—	—
0.33	0.47	0.21
0.66	0.86	0.32
1.00	1.28	0.47
1.50	1.50	0.60
2.00	1.79	0.87
2.50	2.22	0.94

At the end of the first hour the rate at 39° was roughly three times that at 19°.

The influence of other factors on fumarase and succinodehydrogenase activity.

Sodium chloride appears to inhibit very considerably the action of succinodehydrogenase, but only slightly the action of fumarase. Thus rotating 15 g. liver with 500 cc. 1 % succinate solution at 38° as control and a similar sample containing in addition 0.9 % sodium chloride, the following figures were obtained.

Time (hours)	Control. l-Malic (g.)	+0.9 % sodium chloride. l-Malic (g.)	Decrease of l-malic (g.)
0	—	—	—
2	0.50	0.22	0.28
4	1.25	0.38	0.87
5	1.60	0.52	1.08

Replacing succinate with fumarate, the inhibition of fumarase by 0.9 % sodium chloride is relatively very small.

Time (hours)	Control. l-Malic (g.)	+0.9 % sodium chloride. l-Malic (g.)	Decrease of l-malic (g.)
0	—	—	—
3	3.81	3.36	0.45

Sodium fluoride, on the other hand, has a well-marked inhibitory effect on fumarase. Thus rotating 50 g. muscle with 500 cc. 1 % fumarate at 38° as control and comparing with a similar sample containing also 1 % sodium fluoride, the following results were obtained.

Time (hours)	Control. l-Malic (g.)	+1 % sodium fluoride. l-Malic (g.)	Decrease of l-malic (g.)
0	—	—	—
0.25	0.28	0.10	0.18
0.50	0.50	0.22	0.28
0.75	0.67	0.29	0.38
1.00	0.91	0.46	0.45
1.50	1.25	0.78	0.47
2.00	1.52	1.08	0.44
3.00	2.11	1.64	0.47

Calcium chloride (4 %) caused a slight inhibition, and calcium nitrate a much greater inhibition, of fumarase activity.

Bile salts (2 %) caused a very slight acceleration.

Presence of fumarase in invertebrate muscle.

Pecten maximus. In his recent investigation on the chemical changes in muscle, Boyland [1928] found that succinic acid was present in extracts of the muscles of several invertebrates, particularly in *Pecten*. It seemed reasonable to expect, therefore, that the enzyme system would also be present. The large adductor muscles of several specimens of *Pecten* were minced, and 100 g. pulp rotated as before with fumarate at 17°. The following results show that fumarase is present in these muscles.

Time (hours)	<i>L</i> -Malic acid (g.)
0	—
1	0.16
2	0.38
3	0.50
4	0.63
5	0.77

Anodonta cygnea. The adductor muscles and the muscular part of the foot of a number of *Anodonta* were minced and 110 g. of the pulp rotated with fumarate at 18°. The muscle appeared about twice as active as the sample of pecten muscle examined above.

Time (hours)	<i>L</i> -Malic acid (g.)
0	—
1	0.26
2	0.59
3	0.98
4	1.37
5	1.77

Presence of fumarase in blood cells.

Defibrinated fresh ox-blood was centrifuged and the corpuscles and serum rotated with fumarate at 37°. The corpuscles showed a small activity but the serum did not appear to contain fumarase. The results with 80 g. corpuscles were as follows.

Time (hours)	<i>L</i> -Malic acid (g.)
0	—
0.5	0.07
1.0	0.11
2.0	0.16
3.0	0.24
4.5	0.44

Preliminary attempts at the isolation of fumarase.

Although liver appears to contain much more fumarase than muscle, the enzyme of the aqueous extract of liver is associated with such a large amount of protein, etc. that liver was regarded as an unsuitable source, and rabbit's muscle has been used in these experiments. The minced muscle (back and leg muscles) was first washed by steeping in an equal bulk of water and expressing 16–20 times. The free enzyme is lost in this process but a very large amount of protein, pigment, lactic acid, extractives, etc. are also got rid of. The washed muscle was then steeped for an hour in *M*/15 phosphate and the extract expressed. The process was repeated and the extracts combined.

When 1 % hydrochloric acid was added little by little to this phosphate extract, a precipitate was thrown down which began to coagulate when the p_H had fallen to 6.5. This precipitate contains the dehydrogenase and was filtered off. The precipitate and filtrate were then rotated in separate bottles with fumarate, the p_H being readjusted to 7.2 in both cases. The precipitate did not contain any fumarase but the filtrate had a powerful fumarase action. Alcohol and acetone added to phosphate extracts at the ordinary temperature gave inactive precipitates. When the phosphate extract is saturated with ammonium sulphate, however, a very active precipitate is obtained. This

precipitate may be dissolved in a little water and dialysed to remove most of the inorganic salts. As dialysis proceeds, some of the material reprecipitates. The contents of the sac were transferred to a measuring cylinder, the p_H adjusted to 7.2 and the material divided into two equal portions, one of which was rotated with fumarate and the other with fumarate and the optimal amount of phosphate. Not only did the control show very considerable fumarase activity but phosphate also showed the usual activating effect.

Similarity of glyoxalase and fumarase.

There is a close resemblance between the properties of fumarase, which adds on the elements of water to fumaric acid, and glyoxalase which adds on the elements of water to glyoxals, *e.g.* with methylglyoxal giving lactic acid.

Thus, glyoxalase was shown by Dakin and Dudley [1913, 1] to be sufficiently finely dispersed to be filtrable through paper without very great loss and fumarase appears to be similarly finely dispersed. Both enzymes are destroyed at some temperature above 48° but below 60°, and an active extract of both may be obtained by precipitating with ammonium sulphate, dissolving in water and dialysing. Both enzymes are eluted from rabbit's muscle by alkaline fluids and are more stable in alkaline solution. Moreover both enzymes are very widely and similarly distributed, the only difference in distribution so far observed being in the case of yeast. Yeast, according to Dakin and Dudley [1913, 1], contains glyoxalase but fresh samples (exposed for sale) on mixing with water and rotating with fumarate did not appear to contain fumarase. Finally the action of pancreatic juice, which contains antiglyoxalase, [Dakin and Dudley, 1913, 2] on the action of fumarase was investigated. The action of fumarase in an aqueous extract of muscle was inhibited but only to a very slight extent, which did not appear comparable with the inhibition of glyoxalase. This is being further investigated.

SUMMARY.

1. The fumarase activity of rabbit's liver pulp is about eight times that of the same moist weight of muscle pulp but this must only be regarded as indicating that liver pulp contains eight times as much free or active enzyme as muscle.

2. An amount of fumarase is removed from muscle and from liver pulp by steeping eight times in an equal weight of water or 0.25 % saline approximately equal in activity in each case to the activity of the tissue pulp itself. Some succinodehydrogenase, which together with fumarase forms the succinoxidase system, is possibly also removed by washing but this could not be detected by the polarimetric method since the dehydrogenase becomes inactivated almost immediately by the gentle rotation necessary for oxygenation.

3. After the amount of enzyme extracted by this process of washing has become very small, steeping the tissue residue in $M/15$ phosphate or in a

bicarbonate-carbonate buffer solution of the same p_H (9) and expressing liberates from the muscle residue an amount of fumarase having about six times the activity of the original pulp, whilst from the liver residue only a small amount is obtained. The total fumarase activity of the aqueous and phosphate extracts of 50 g. rabbit's liver is approximately equal to the total activity of the corresponding extracts of 150 g. of rabbit's muscle.

4. Phosphate not only elutes fumarase in virtue of its alkalinity but also activates it, the optimal phosphate concentration at p_H 7.2 being 0.052 *M*. Phosphate does not appear to activate succinodehydrogenase in the same way as fumarase. Phosphate activates the fumarase of muscle and liver suspensions, of aqueous extracts of these and also of the enzyme preparation obtained by precipitating with ammonium sulphate and removing the salt by dialysis.

5. The temperature coefficient for the fumarase activity of liver pulp is normal (2-2.4) but of muscle pulp is very much lower (1.4). The activity of muscle pulp on reducing the temperature from 39° to 19° only falls to one-half. The coefficient for the activity of an aqueous extract of muscle is nearer normal.

6. Sodium chloride inhibits the action of succinodehydrogenase considerably, but of fumarase only slightly. Sodium fluoride inhibits the action of fumarase considerably.

7. The presence of fumarase has been detected in invertebrate muscle (*Pecten maximus*, *Anodonta cygnea*) but not in baker's yeast. It was present in the corpuscles but absent from the serum of fresh defibrinated blood.

8. A very active fumarase solution is obtained by precipitating the phosphate extract of washed muscle with ammonium sulphate, filtering and dialysing the solution of the precipitate.

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