CLXXXIX. STUDIES IN TISSUE METABOLISM.
V. THE LACTIC DEHYDROGENASES OF YEAST AND HEART-MUSCLE.

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STEPHENSON [1928] and Bernheim [1928] obtained lactic dehydrogenase of *B. coli* and of baker's yeast respectively in solution. Both preparations oxidised lactic acid to pyruvic acid and did not appear to have any coenzyme. Bernheim dialysed his preparations for 7 hours without destroying the activity. One of us [Boyland, E., 1933, 2] found that zymin from baker's yeast could be washed with large quantities of water without loss of lactic dehydrogenase activity [see also Harden and Macfarlane, 1931]. Within the last few years the lactic dehydrogenase of mammalian heart-muscle has been investigated by Szent-Györgyi [1925], who has shown that this enzyme requires a coenzyme, the nature of which he has attempted to determine [Banga *et al.* 1932; Banga and Szent-Györgyi, 1933]. The coenzyme is not adenylpyrophosphate [Banga and Szent-Györgyi, 1932] or vitamin B_1 [Boyland, E., 1933, 1; Birch and Mann, 1934], nor is it identical with glucose dehydrogenase coenzyme of liver (Harrison, personal communication, and observations with a sample kindly supplied by Dr Harrison). Holmberg [1934] found that cozymase or Fiske's adenylpyrophosphate could act as coenzyme for lactate oxidation by heart-muscle in the presence of methylene blue.

Preparation of heart-muscle dehydrogenase in solution.

Szent-Györgyi has always worked with the heart-muscle enzyme in suspension in the form of washed minced muscle, but the enzyme may be obtained in solution. Holmberg [1934] and Birch and Mann [1934] have prepared soluble enzymes from fresh mammalian muscle, Holmberg by extraction of horse skeletal muscle with bicarbonate; Birch and Mann from pig's heart by extraction with alkaline phosphate.

In the present work sheep or bullock hearts fresh from the slaughter-house were freed from fat, finely minced and ground with two volumes of acetone. The acetone was filtered off and the residue treated with another two volumes of acetone, filtered and then dried in a thin layer on filter-paper. The dried heart-muscle obtained in this way was stable for months and after washing with water gave a lactic dehydrogenase preparation as active as the original heart-muscle washed with water. To obtain an extract 15 g. of the acetone-dried heart-muscle are washed with 3 portions of 250 ml. water containing 2 or 3 drops of toluene. The washed material is then suspended in 50 ml. $M/15$ Na_2HPO_4 at 20°. The solid is removed by centrifuging, and the extract containing the enzyme may be clarified by filtration through Kieselguhr. The enzyme can be precipitated by saturation with ammonium sulphate. The extract does not reduce

methylene blue either alone or in the presence of the coenzyme alone (prepared according to Banga *et al.* [1932]) or of lactate alone. With lactate and coenzyme it rapidly reduces methylene blue. The activity of the coenzyme was found to decrease slowly over a period of weeks, even when kept in a desiccator.

The yeast lactic dehydrogenase was made by Bernheim's method [1928], except that the zymin from baker's yeast was well washed with water so as to obviate the necessity for dialysis of the final extract [Boyland, E., 1933, 2][1]. The yeast lactic dehydrogenase in solution is not so stable as that of heart-muscle and during the long dialysis necessary to remove substrates some deterioration of the enzyme must occur.

The p_H *optimum of yeast and heart-muscle lactic dehydrogenase.*

The activity of the preparations was measured by the Thunberg technique. 1–3 ml. of enzyme solution (or 0·5 g. washed acetone heart-muscle) were mixed with $M/15$ buffer, M lithium lactate, and in the case of heart-muscle enzyme 0·5 to 1 mg. of coenzyme preparation in the Thunberg tube. 0·1 ml. of this mixture was taken for colorimetric estimation of the p_H with a B.D.H. capillator. One drop of caprylic alcohol and 0·5 ml. 1/5000 methylene blue were added, and the tube was evacuated with a "Hyvac" pump until the liquid boiled briskly. The tube was then placed in a water-thermostat and the time taken for 90 % decoloration of the methylene blue determined. When the methylene blue was completely decolorised the tube was opened and the p_H again rapidly determined. The p_H values obtained in this way are probably as reliable as determinations with a quinhydrone or hydrogen electrode. A glass electrode was not available, but Dr Morton, of the Chelsea Polytechnic, kindly made determinations with this instrument of zymin suspensions and extracts at about the optimum p_H and found an error of $+0·2$ p_H in each case. A correction of $0·2$ p_H has therefore been applied to the values determined colorimetrically for zymin.

Preliminary experiments had shown that heart-muscle lactic dehydrogenase was most active in alkaline solution. As sodium borate was found to inhibit the enzyme, borate buffers could not be used. From p_H 4·0 to 9·5 a mixture of barbitone [Britton and Robinson, 1931] and phosphates was used. As a check glycine-phosphate-NaOH buffer was used and gave the same results. At p_H 6·0 the heart-muscle enzyme was found to be inactive; from p_H 9·3 to 9·5 the activity was at a maximum (Fig. 1). The relative activity was determined at 18°, 28° and 38° and the results are given in Table I, which also shows the temperature

Table I. *Effect of temperature on heart-muscle extract.*

	Decoloration time (mins.)			Q_{10}	
p_H	18°	28°	38°	18–28°	28–38°
8·0	—	43	19	—	1·8
8·5	55	24	11	2·3	2·2
9·0	30	14	7·5	2·1	1·9

increment (Q_{10}). The relative activity of washed heart-muscle suspension was also determined at different hydrogen ion concentrations at 38°. The same result was obtained for this suspension as for the extract.

[1] We are indebted to the Distillers' Company for supplying the baker's yeast used throughout this work.

Barron and Hastings [1933] found the temperature increment of this enzyme from gonococci to be 2·23. Holmberg [1934] found that his enzyme was much more active at p_H 8·0 than in more acid solution and his times of decoloration of methylene blue were

p_H	6·2	6·6	7·0	7·5	8·0
Time (mins.)	—	32	18	6	4

He does not record results with a solution at a reaction more alkaline than p_H 8·0.

The yeast enzyme was examined as a suspension of washed zymin from baker's yeast and in solution; the results are expressed in Fig. 1. Above p_H 9·0, at which the heart-muscle enzyme was found to have a maximum, the yeast dehydrogenase was almost inactive. In phosphate buffer the suspension of

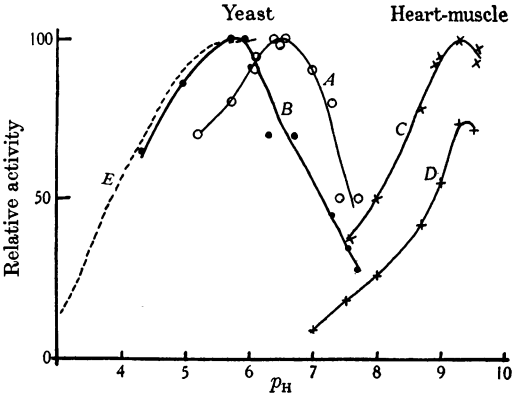

Fig. 1. Relative activities of lactic dehydrogenases of yeast and heart-muscle.

A	o	Zymin extract with lactate.
B	•	Zymin suspension with lactate.
C	×	Heart-muscle extract with lactate.
D	+	Heart-muscle extract with malate.
E	---	Dissociation curve of lactic acid.

washed zymin had a maximum activity at about p_H 5·6, while the optimum p_H for the extract was 6·2 (Fig. 1). This difference may be only apparent as it is possible that the enzyme of the washed zymin suspension is surrounded by protein so that the p_H at the enzyme surface is different from that in the medium. The curve for the suspension approximates to the dissociation curve of lactic acid between p_H 4 and 6.

Table II. *The effect of yeast extract on heart-muscle lactic dehydrogenase.*

Preparation incubated	Time to reduce 1 ml. 1/10,000 methylene blue at 38° mins.
Washed zymin extract with *M*/10 lactate at p_H 6·4	6
,, ,, at p_H 9·3	36
,, ,, after boiling, at p_H 6·4	> 60
Heart-muscle extract at p_H with lactate	>100
,, ,, +1 mg. coenzyme	7
,, ,, +1 ml. washed zymin extract	36
,, ,, +1 ml. boiled washed zymin extract	> 60

The absence of coenzyme from yeast lactic dehydrogenase.

As the yeast enzyme was inactive at the p_H at which that from heart-muscle showed maximum activity, the yeast extract could be tested for coenzyme by comparing its effect with that of the coenzyme preparation at p_H 9·3. The results in Table II show that the yeast lactic dehydrogenase either fresh or inactivated by boiling had no coenzyme activity.

The effect of heart-muscle lactic dehydrogenase on other substrates.

The soluble lactic dehydrogenase of heart-muscle oxidised malate, but not succinate, pyruvate, tartronate, α-glycerophosphate or glucose. It was inhibited strongly by pyruvate and slightly by succinate (Table III).

Table III. *Effect of substrates on lactic dehydrogenase at 38°.*

Heart-muscle extract incubated with	p_H	Time taken to reduce 0·5 ml. 1/5000 methylene blue (mins.) Without coenzyme	With coenzyme
M/20 sodium succinate	6·0	>200	>200
M/20 sodium tartronate	7·0	>200	>200
M/20 sodium pyruvate	8·0	>200	>200
	9·0	>200	>200
M/20 sodium glycerophosphate	7·2	>200	>200
	9·0	>200	About 200
M/20 glue	7·0	>200	,, 200
	9·0	>200	,, 150
M/20 lactate	8·8	>200	10
,, + *M*/10 sodium succinate	8·8	—	14
,, + *M*/750 sodium pyruvate	9·0	—	28
,, + *M*/150 sodium pyruvate	9·0	—	50
,, + *M*/30 sodium pyruvate	9·0	—	85

The inhibitory action of pyruvate is similar to that found by Bernheim [1928] for zymin.

The effect of the heart-muscle extract on sodium malate was determined at different hydrogen ion concentrations as shown in Fig. 1. The optimum p_H was about 9·3, which is the same as that found with lactate, but at p_H 8·4 malate is acted on only at a third of the maximum rate, while at this p_H lactate is oxidised at 70 % of the maximum rate. Even at the optimum p_H malate is dehydrogenated at only about 50–70 % of the rate of lactate. This probably accounts for the fact that the oxidation of malate by heart-muscle has not been more often observed.

Table IV. *Relative rates of reduction of methylene blue by heart-muscle extract in the presence of lactate and malate at* p_H *9·0 at 38°.*

Extract incubated with	Relative activity
M/10 lactate + coenzyme	100
M/10 malate + coenzyme	46
M/5 malate + coenzyme	51
M/10 malate + *M*/10 lactate + coenzyme	82

Holmberg [1934] found that his enzyme preparation partially decolorised methylene blue and completely decolorised thionin in the presence of *l*- and *dl*-malate. We have always observed complete decoloration of methylene blue, even at p_H 7 when the reaction proceeds very slowly.

Discussion.

The difference in the p_H optima of the two enzymes is comparable with the difference in the p_H optima of pepsin and trypsin, which both hydrolyse the peptide linkage. In the case of the peptide-splitting enzymes the p_H-activity curve and the titration curves of their protein substrates approximate to each other. In the case of yeast lactic dehydrogenase the activity increases as the lactic acid dissociation increases, and part of the p_H-activity curve of a washed zymin suspension coincides with the dissociation curve of lactic acid (see Fig. 1). This indicates that the enzyme acts upon charged lactate ions and not upon undissociated molecules.

As the heart-muscle enzyme is active in alkaline solution it must also act upon lactate ions. The p_H-activity curve of this enzyme cannot be explained by the dissociation of the carboxyl group, but it is possible that the hydroxyl group of lactate ions may change in the alkaline solutions in which heart lactic dehydrogenase operates.

Although lactic dehydrogenase reduces methylene blue in the presence of lactate but not in the presence of any other substrate (except malate with heart-muscle enzyme) it has not yet been possible to identify pyruvic acid as a product of the action of the enzyme.

Summary.

1. The lactic dehydrogenases of mammalian heart-muscle and baker's yeast have been prepared in solution.

2. The optimum p_H of the heart-muscle lactic dehydrogenase in solution is about 9·3; the lactic dehydrogenase of yeast has an optimum about p_H 6·4.

3. The yeast enzyme is independent of coenzyme and does not attack malate, while the heart-muscle lactic dehydrogenase requires a coenzyme and oxidises both lactate and malate in alkaline solution.

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