CXV. THE DEHYDROGENASES OF *BACT. COLI*
IV. LACTIC DEHYDROGENASE

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THE disproportionate falling off in the activity of the glucose dehydrogenase of washed suspensions of *Bact. coli* led to the discovery that a coenzyme is concerned in this reaction [Yudkin, 1933]. Evidence has been brought forward to show that this coenzyme is identical with the cozymase of yeast fermentation [Yudkin, 1934]. An investigation of the lactic acid dehydrogenase of suspensions of *Bact. coli* has shown that here, too, the dehydrogenation involves a coenzyme, which is also replaceable by cozymase.

EXPERIMENTAL

(1) *Lactic dehydrogenase of cell suspensions*

The bacteria were grown for about 18 hr. on broth agar, the broth used being a tryptic digest of casein. Washed suspensions in distilled water were made in the usual way by centrifuging. The methylene blue technique as described in the previous papers was used for the estimation of the activity of the enzyme. As substrate for the lactic dehydrogenase 1 ml. $N/10$ sodium lactate was used in the vacuum tube.

Preliminary experiments showed that dilution of the bacterial solution led to a disproportionate fall in the dehydrogenase activity. The addition of a suspension of heated bacteria (100° for 10 min.) increased the activity to the value calculable from the degree of dilution (Tables I and II).

Table I. *Existence of coenzyme of cellular lactic dehydrogenase*

	Reduction time min.
Bacteria	>120
Bacteria + lactate	10·5
Bacteria diluted 1/4 + lactate	92
Bacteria diluted 1/4 + heated bacteria + lactate	42
Bacteria diluted 1/4 + heated bacteria	>120

The effect of the coenzyme (heated bacteria) on a series of dilutions is shown in Table II. The activity (reciprocal of the reduction time) for the undiluted suspension is given an arbitrary value of 100. It is seen that the product of activity and dilution, which should be constant on dilution, falls off rapidly unless the coenzyme is present.

The concentration of the coenzyme necessary for the activation of the lactic dehydrogenase is of the same order as that necessary for the activation of glucose dehydrogenase (Table III).

Table II. *Effect of dilution on activity of cellular lactic dehydrogenase*

Each tube contains sodium lactate

Concentration of suspension	Without heated bacteria: Reduction time min.	Without heated bacteria: Activity × dilution	With heated bacteria: Reduction time min.	With heated bacteria: Activity × dilution
1	5·25	100	—	—
1/2	10·5	100	10·5	100
1/4	23	92	23	92
1/6	38·5	81	30	105
1/8	55	76	44	96
1/10	77	68	51	103
1/12	115	61	65	97
1/14	165	49	79	92
1/20	About 7 hr.	(20)	110	95
1/24	>7 hr.	?	135	93
1/30	∞	0	180	87

Table III

Each tube contains glucose (1) or sodium lactate (2)

	Reduction time (min.): (1) Glucose dehydrogenase	Reduction time (min.): (2) Lactic dehydrogenase
Bacterial suspension	37	100
Bacterial suspension + 1 ml. heated bacteria	16·5	33
Bacterial suspension + 0·3 ml. heated bacteria	15·5	33
Bacterial suspension + 0·1 ml. heated bacteria	16	38
Bacterial suspension + 0·03 ml. heated bacteria	31	82

As with glucose dehydrogenase, activation of the diluted cellular lactic dehydrogenase is brought about by cozymase (Table IV).

Table IV. *Cozymase as cellular lactic dehydrogenase coenzyme*

1 ml. cozymase contains about 30,000 ACo units

	Reduction time min.
Bacterial suspension	80
Bacterial suspension + heated bacteria	48
Bacterial suspension + 1 ml. cozymase	46
Bacterial suspension + 0·1 ml. cozymase	47
Bacterial suspension + 0·05 ml. cozymase	65
Bacterial suspension + 0·03 ml. cozymase	80

Each tube contains sodium lactate.

It has already been shown [Yudkin, 1934] that preparations of heated bacteria are able to reactivate apozymase of yeast; that is, they contain cozymase. It seems very probable therefore that the coenzyme of lactic dehydrogenase in *Bact. coli* suspensions, as that of glucose dehydrogenase, is identical with cozymase.

(2) *Soluble lactic dehydrogenase*

The cell-free preparation of lactic dehydrogenase was next investigated. The enzyme was prepared from washed suspensions of *Bact. coli* by the method described by Stephenson [1928]. The activity of the enzyme falls off slightly on dilution and the diluted enzyme is activated by the heated preparation, by a heated cell suspension and by cozymase (Table V).

Table V. *Existence of coenzyme of soluble lactic dehydrogenase*

Each tube contains sodium lactate.

	Reduction time min.
Soluble enzyme ("preparation")	8
Soluble enzyme + heated preparation	8
Soluble enzyme diluted 1/8	120
Soluble enzyme diluted 1/8 + heated preparation	56
Soluble enzyme diluted 1/8 + heated bacteria	60
Soluble enzyme diluted 1/8 + cozymase	62

Control experiments showed that the effect of the heated preparation was not due to the presence in it of substances capable of acting as hydrogen donators or to the presence of the fluoride which is used in preparing the soluble enzyme.

The heated preparation contains "cozymase" since it is able to activate both the lactic acid and glucose dehydrogenases of bacterial suspensions (Table VI).

Table VI. *Presence of "cozymase" in heated soluble enzyme preparations*

	Reduction time min.
Glucose dehydrogenase:	
Bacteria + glucose	33
Bacteria + glucose + heated preparation	12·5
Lactic acid dehydrogenase:	
Bacteria + lactate	>120
Bacteria + lactate + heated preparation	42

Experiment further showed that the effects of a heated preparation of soluble lactic dehydrogenase, heated bacteria and cozymase are not additive, either for the soluble enzyme or for the lactic acid dehydrogenase of the intact cells. This is strong evidence for the identity with cozymase of the coenzymes of both enzymes.

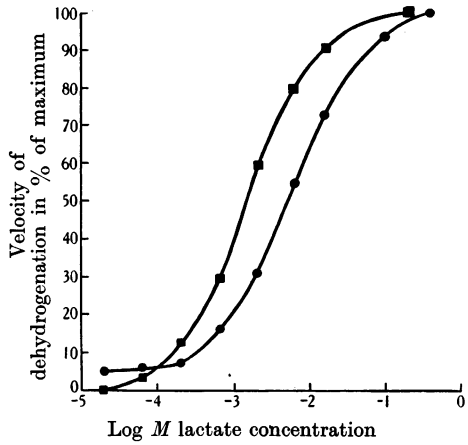

Fig. 1. Affinities of lactic dehydrogenase.
•—• Of intact cells. ■—■ Of cell-free preparation.

(3) *Affinities of the dehydrogenases*

Certain small quantitative differences in the behaviours of soluble lactic dehydrogenase and intact cells acting on lactate suggested that there might be

other and more fundamental differences between the enzymes. It therefore seemed of interest to measure their affinities for their substrate. The result of a typical experiment showing the relation between the activities of the enzymes and the substrate concentration is given in Fig. 1. It will be seen that there are some minor differences in the two curves. For example, the pK_m of the soluble preparation is about 2·8, that of the enzyme of the intact cells about 2·3. These differences, although constantly obtained, are quantitatively not sufficient for any definite conclusions to be drawn with regard to the enzymes.

Summary

1. Dilution of suspensions of *Bact. coli* results in a considerable falling off in activity of lactic acid dehydrogenase, which has been shown to be due to the presence of a heat-stable coenzyme.

2. The coenzyme is replaceable by the cozymase of yeast fermentation.

3. Similar results were obtained with the cell-free preparation of lactic dehydrogenase of Stephenson.

4. These findings recall the results previously obtained with the glucose dehydrogenase of *Bact. coli*.

5. The affinities for lactate of the lactic dehydrogenase of intact cells and the soluble preparation show slight but constant differences.

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