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KINETICS OF LACTIC DEHYDROGENASES OF *SCHISTOSOMA MANSONI* AND OF RABBIT MUSCLE*

BY

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It has been shown that in *Schistosoma mansoni* glycolysis proceeds at an exceedingly rapid rate (Bueding, 1950) and that this process is more critical for the survival of the parasite than is respiration (Bueding and Peters, 1951; Bueding, Peters, Koletsky, and Moore, 1953). Although glycolytic enzymes might catalyse the same reactions in the host as those in the parasite their nature might be different. Such differences would be of importance for the development of chemotherapeutic agents. They would afford opportunities to select specific inhibitors against enzymes of the parasite which would be without effect on those of the host. In an attempt to investigate such a possibility the kinetics of lactic dehydrogenase of *Schistosoma mansoni* were compared with those of lactic dehydrogenase of rabbit muscle.

METHODS

Adult worms were dissected from the mesenteric and portal veins of mice six to eight weeks after these animals had been infected with cercariae of *Schistosoma mansoni*. All subsequent operations were carried out at temperatures varying between 0 and 2° C. The worms were cut with fine scissors in distilled water (1 ml. of water was used per 100 pairs of worms). The process of cutting required two minutes. The suspension containing the worm fragments was stirred for five minutes in a beaker surrounded with cracked ice. This extract was then centrifuged for five minutes at 4,000 r.p.m., and 0.1 ml. of phosphate gel (dry weight per ml.: 1.4 mg.) was added per ml. of supernatant fluid. After stirring, this mixture was centrifuged for 10 minutes at 4,000 r.p.m. Most of the enzymatic activity was then absorbed by the use of 0.1 ml. of calcium phosphate gel (dry weight per ml.: 14 mg.) per ml. of solution. After centrifuging for 10 minutes at 4,000 r.p.m. the residue was washed with 0.01 M glycylglycine buffer (*p*H 7.5). Elution of the enzyme was carried out with a volume of solution containing glycylglycine buffer (0.5 M; *p*H 7.5) and potassium chloride (0.5 M) equivalent to one-half of the original extract. Sucrose was added to this eluate (final concentration: 32%) because this ensured the stability of the enzyme when stored in the frozen state. In a number of experiments a homogenate was used. This was prepared by homogenizing the worms in glycylglycine buffer (0.01 M; *p*H 7.5). After centrifugation for 10 minutes at 4,000 r.p.m. sucrose (final concentration: 32%) was added to the supernatant fluid which was used as the source of the enzyme.

The mammalian enzyme was prepared by the method of Kornberg and Pricer (1951). Reduced diphosphopyridine nucleotide ($DPNH_2$)† was prepared according to Gutcho and Stewart (1948), and obtained as a dry powder according to Ohlmeyer (1938). Enzymatic activity was measured by a spectrophotometric procedure described by Kornberg and Pricer (1951). This method is based on the change in the optical density at a wavelength of 340 mμ as a result of the oxidation or reduction of diphosphopyridine nucleotide (DPN)† during the following reaction:

$$\text{Pyruvate} + DPNH_2 \rightleftarrows \text{lactate} + DPN$$

All measurements were made in a Beckman spectrophotometer in an air-conditioned room at 26° C. Under optimal conditions the activity of the enzyme was proportional to its concentration. The latter was adjusted so that under optimal conditions 0.013 to 0.016 μM of DPN was reduced or oxidized in one minute. The total volume of the reaction mixture was 1 ml. Reduction of DPN was recorded over a period of two minutes, and oxidation of DPN for one minute. When tested under optimal conditions, the specific lactic dehydrogenase activity of homogenates of schistosomes was two to three times lower than that of alkaline extracts of rabbit or rat muscle.

Lithium-*l*(+)lactate was prepared as follows: A commercial preparation of zinc-*l*(+)lactate (City Chemical Corporation) was dissolved in distilled water (16 ml. per g. of salt). The solution was run through a cation exchange resin (Dowex 50). The eluate,

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† In this paper the following abbreviations are used: DPN—oxidized diphosphopyridine nucleotide; $DPNH_2$—reduced diphosphopyridine nucleotide.

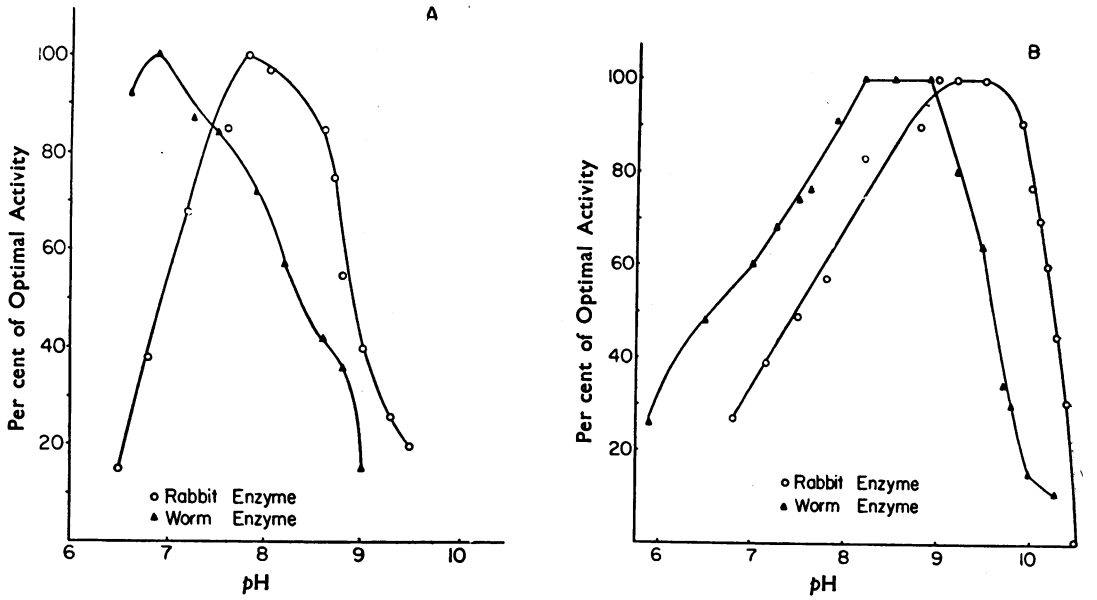


FIG. 1.—Effect of *p*H on the activities of the lactic dehydrogenases of schistosomes and of rabbit muscle. Abscissae: *p*H. Ordinates: Percentage of optimal activity.

FIG. 1A.—Reduction of pyruvate.

FIG. 1B.—Oxidation of lactate.

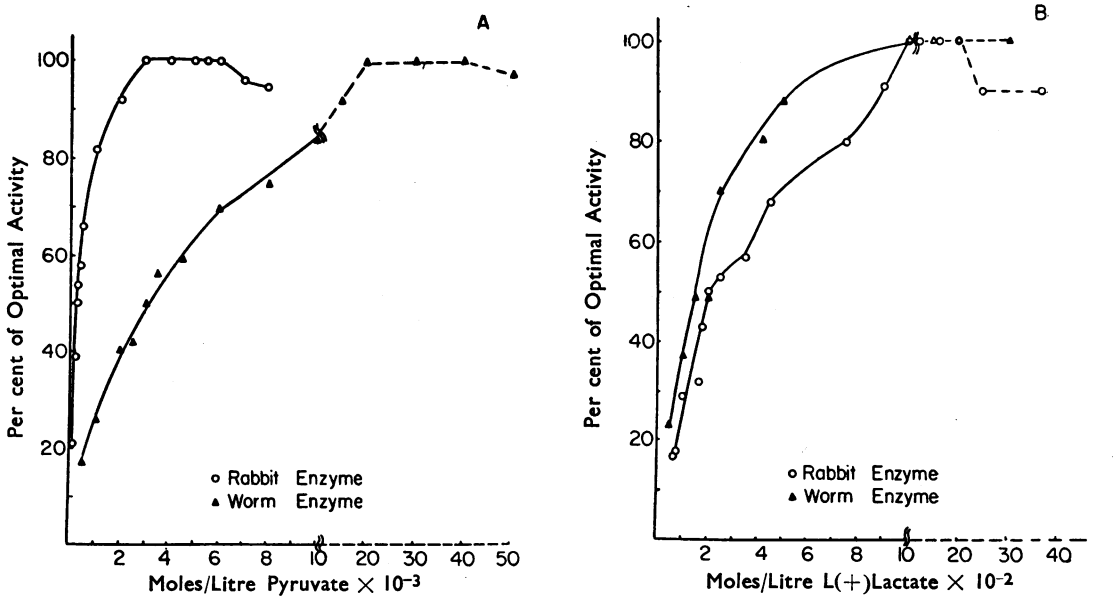
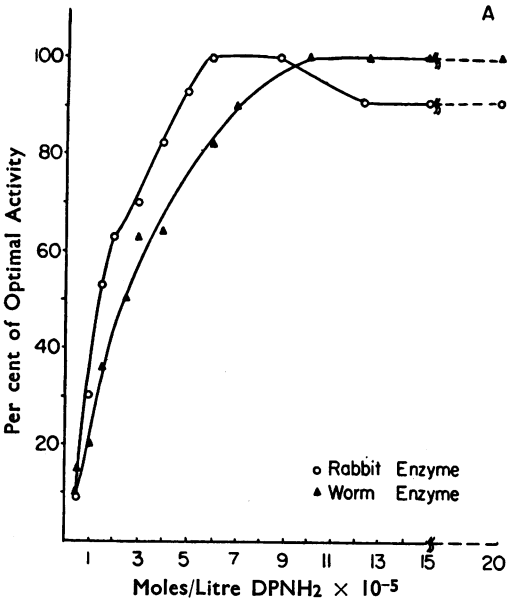


FIG. 2.—Effect of substrate concentration on the activities of the lactic dehydrogenases of schistosomes and of rabbit muscle. Ordinates: Percentage of optimal activity.

FIG. 2A.—Reduction of pyruvate. Abscissa: Molar concentration of pyruvate.

FIG. 2B.—Oxidation of lactate. Abscissa: Molar concentration of *l*(+)lactate.



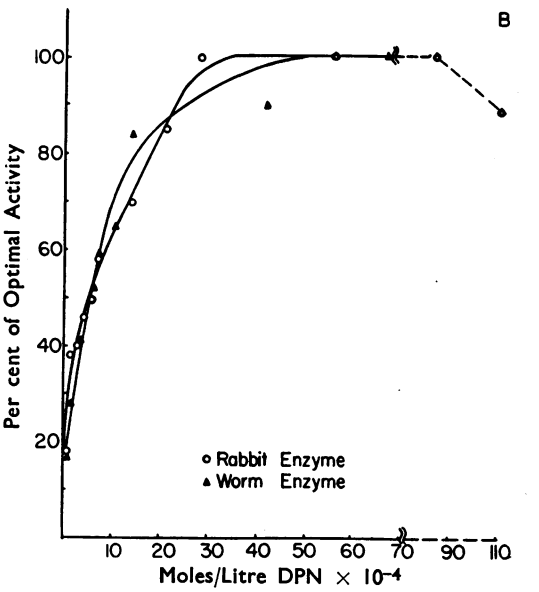


FIG. 3.—Effect of coenzyme concentration on the activities of the lactic dehydrogenases of *Schistosoma mansoni* and of rabbit muscle. Ordinates: Percentage of optimal activity.

FIG. 3A.—Reduction of pyruvate. Abscissa: Molar concentration of $DPNH_2$.

FIG. 3B.—Oxidation of lactate. Abscissa: Molar concentration of DPN.

which was free of zinc, was concentrated *in vacuo* and neutralized with lithium carbonate. Optical rotation of this solution revealed the absence of *d*(−) isomer. The latter was prepared according to Herzog and Slansky (1911). In the kinetic studies the *l*(+) lactate was used exclusively because no reduction of DPN occurred when *d*(−) lactate was used as a substrate.

RESULTS

Effect of pH.—The activities of both the schistosome and the mammalian enzymes were tested at different hydrogen ion concentrations in the presence of optimal concentrations of the substrates. Measurements of the rate of reduction of pyruvate to lactate revealed differences in the effect of *p*H on the activity of the two enzymes. The optimal activity of the worm enzyme was at *p*H 6.9 and that of the mammalian enzyme at *p*H 7.8 (Fig. 1A). The activity of the schistosome enzyme was tested also in the presence of phosphate buffer. Under these conditions optimal activity was seen even at lower *p*H levels. The optimal range for the oxidation of lactate to pyruvate was between *p*H 8.2 and 8.9 for the schistosome enzyme and between *p*H 9.0 and 9.5 for the mammalian enzyme (Fig. 1B). Therefore, the *p*H optima for the schistosome enzyme were significantly lower in either direction of the reaction.

Effect of Substrate Concentrations.—The effects of concentrations of $DPNH_2$ and pyruvate on one hand, and of DPN and lactate on the other, were determined for both enzymes. The results are shown graphically in Figs. 2 and 3. Table I summarizes the optimal concentrations and the Michaelis–Menten (1931) constants (K_M) of the substrates for both enzymes. It should be noticed that the dissociation constants and the optimal concentrations for lactate are identical for both enzymes. These two values were, however, six to twelve times higher for the worm enzymes with pyruvate, and slightly higher for the same enzyme with DPN and $DPNH_2$.

TABLE I

KINETIC DATA FOR THE LACTIC DEHYDROGENASES OF RABBIT MUSCLE AND OF *SCHISTOSOMA MANSONI*

All values are expressed as molar concentrations

Reaction	Substrate	Worm Enzyme		Rabbit Enzyme	
		Optimal Concentration	K_M	Optimal Concentration	K_M
Pyruvate→ lactate	$DPNH_2$	10×10^{-5}	$2{\cdot}5 \times 10^{-5}$	6×10^{-5}	$1{\cdot}5 \times 10^{-5}$
,, ,,	Pyruvate	20×10^{-3}	$3{\cdot}0 \times 10^{-3}$	3×10^{-3}	$0{\cdot}25 \times 10^{-3}$
Lactate→ pyruvate	DPN	56×10^{-4}	$5{\cdot}95 \times 10^{-4}$	28×10^{-4}	$5{\cdot}6 \times 10^{-4}$
,, ,,	*l*(+) lactate	10×10^{-2}	$2{\cdot}0 \times 10^{-2}$	10×10^{-2}	$2{\cdot}0 \times 10^{-2}$

Discussion

The differences in the kinetics of lactic dehydrogenase from rabbit muscle and from schistosomes suggest that the two enzymes are not identical, although they catalyse the same reaction. Similar kinetic differences between the enzymes of *Schistosoma mansoni* and those catalysing the same reaction in its mammalian host have been observed with hexokinase (Bueding and MacKinnon, 1953) and acetylcholine esterase (Bueding, 1952). It should be noted that the optimal substrate concentrations and the dissociation constants for the enzymes of schistosomes are usually higher than those for the host. This may be explained by a greater availability of substrate in the environment of the parasite, and may be related to a process of biochemical adaptation in an environment of high and relatively constant substrate concentration.

Summary

The kinetics of lactic dehydrogenase of *Schistosoma mansoni* are compared with those of lactic dehydrogenase of rabbit muscle. Differences in the *p*H optima and in some dissociation constants suggest that these two enzymes are not identical.

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