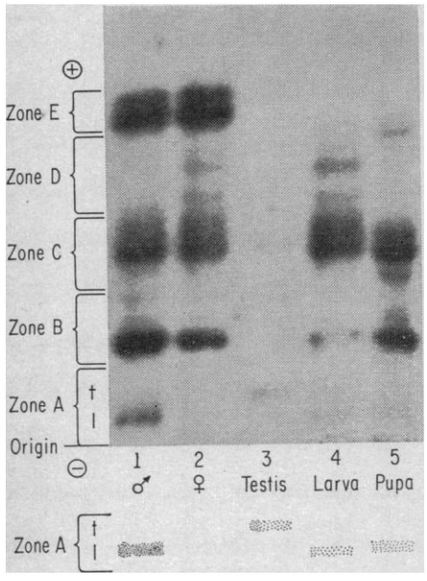

Fig. 1. Starch-gel electrophoretic pattern of hexokinases in homogenates of *Drosophila melanogaster* at different stages of development, and in testes of adult males. The diagram shows the positions of Hex-t and Hex-l bands present in zone A.

tested separately. At this point a new band was discovered which we call Hex-t. The newly discovered band, which migrated slightly faster than Hex-1 (Fig. 1), was found only in the testes, while the original sex-associated band was present only in the accessory gland. Testes from XO males and *tra/tra* females also had Hex-t activity. Ovaries concentrated from as many as 20 females showed neither Hex-t nor Hex-1 activity.

Larvae and pupae were tested to establish the time during development at which these two bands were first detectable. In homogenates of individual third-instar larvae and pupae, a weak Hex-1 band was present in both males and females. Females as well as males continued to show Hex-1 activity through the pupal period and as late as 2 days after hatching. However, the activity in larvae, pupae, and young adult females was never as great as that in adult males. Hex-1 activity could not be localized to a particular organ or region in larvae and pupae or in young, dissected females. Hex-t activity was not observed in whole larvae or in testes dissected from larvae, but was first observable in testes of individuals in the early pupal stage. The relative amounts of protein in larval and adult testes were determined, and the results indicated that more than enough material should have been present in the number of larval testes used to show activity if the specific hexokinase activities in larval and adult testes were comparable.

The occurrence of testis-specific LDH's in various organisms has been described (*6*) and the occurrence of a testis-specfiic hexokinase in the rat has been reported (*16*). The LDH testis-specific activity reported in mammals appears to be correlated in some way with the production of mature and functional sperm. Since it is known that sperm from XO males are not motile (*17*), the fact that XO males showed Hex-t activity would indicate that this band is not associated with sperm motility. Testes dissected from *tra/tra* females were examined and no mature sperm were found, indicating that testes do not need to contain mature sperm in order to show Hex-t activity. This does not rule out the possibility that the Hex-t band is in some way associated with spermatogenesis, perhaps at some earlier stage. Hex-t activity is first noted in the early pupal stage at approximately the same time as mature sperm appears. The band might also be related to the elongation of the testis which begins at this time.

During the course of this study, electrophoretic variation was noted in the Hex-1 band as well as in others. Homogenates of whole flies and testes of *D. pseudoobscura* were examined. The pattern of hexokinase bands was markedly different from that of *D. melanogaster*, and no sex-associated or testes-related variations were observed.

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Rabbit Lactate Dehydrogenase Isozymes: Effect of pH on Activity

Abstract. *The ability of lactate dehydrogenase isozyme five from rabbit skeletal muscle to catalyze pyruvate reduction is extremely sensitive to changes of* p*H within the range of 6.2 to 7.8. The activity of lactate dehydrogenase isozyme one from rabbit heart is virtually unaffected by changes of* p*H within the same range. Isozyme five is activated by low concentrations of oxalacetate and inhibited by higher concentrations, but other substrates of the citric acid cycle have no effect.*

The traditional interpretation of the effect of *p*H on enzyme activity is that a *p*H optimum is caused by ionization of a group in the region of the active center on the enzyme. If the enzyme has no *p*H optimum, it is assumed that no ionizable group is involved in its active center. Thus Winer and Schwert (*1*), in studying the influence of *p*H on the kinetics of lactate dehydrogenase from beef heart, concluded that the imidazolium ring of histidine must be protonated for the conversion of pyruvate to lactate and unprotonated for the conversion of lactate to pyruvate. A number of enzymes undergo changes in conformation when exposed to certain small molecules, and the manifestation of a *p*H optimum by an enzyme could be the result of such an exposure. In these cases, the active sites of the enzymes are not necessarily directly involved; for example, the catalytic site of bovine L-glutamate dehydrogenase has a *p*H optimum of around 9; this optimum is apparent only if the native conformation of the enzyme is stabilized by either methyl mercuric hydroxide or adenosine diphosphate (*2*). Interactions between subunits may be additional determining factors not directly involving the active sites, as is the case with hemoglobin and aspartate transcarbamylase (*3*).

I have studied the effect of *p*H on the activity of lactate dehydrogenase

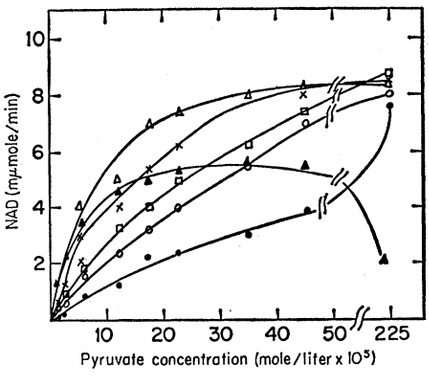

Fig. 1. Effect of *p*H on LDH-5 activity. Assays were performed at 37°C in a volume of 2 ml containing $2.24 \times 10^{-5}M$ NADH in $0.03M$ sodium phosphate buffer at the indicated *p*H values. Concentration of LDH-5 was 2.6×10^{-10} mole/liter. Pyruvate concentrations were as indicated. The *p*H values represented by the symbols are: △, 6.5; x, 7.0; □, 7.2; ○, 7.4; ●, 7.8; ▲, 6.2.

five (LDH-5) from rabbit skeletal-muscle and rabbit heart lactate dehydrogenase one (LDH-1) in vitro. The results account for the previously reported "activation" of LDH-5 by intermediates of the citric acid cycle, which is now shown to be an effect of *p*H (see *4*). Still the effect of *p*H may not be directly on the active site but, rather, may be indirect effecting a change in molecular conformation.

The enzymes were prepared and assayed as previously described (*4*). All assays were carried out at 37°C, and $0.03M$ sodium phosphate buffer was used. The concentration of reduced nicotinamide-adenine dinucleotide was 2.24×10^{-5} mole/liter. The *p*H of each reaction mixture was measured with a Beckman model G *p*H meter.

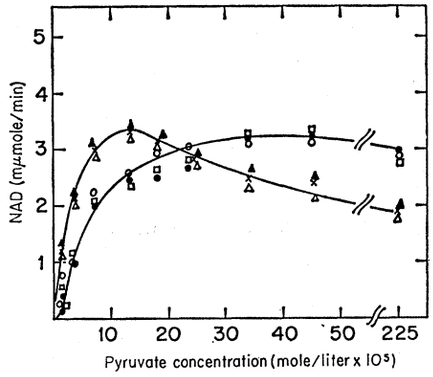

Fig. 2. Effect of *p*H on LDH-1 activity. Assays were performed as indicated in Fig. 1. Concentration of LDH-1 was 2.0×10^{-10} mole/liter. The *p*H values represented by the symbols are: ▲, 6.2; △, 6.5; x, 6.8; □, 7.2; ○, 7.4; ●, 7.8.

Figure 1 shows the effect of *p*H on the pyruvate saturation curve of LDH-5. At *p*H 6.5 and *p*H 7.0 maximum activity is attained at low concentrations of pyruvate and maintained even at the highest concentration of pyruvate used. At *p*H's 7.2, 7.4, and 7.8 essentially the same maximum activity is reached, but only at the higher concentrations of pyruvate. Thus, for LDH-5 in the range of *p*H 6.5 to 7.8, the substrate concentration necessary for half the maximum velocity (K_m) to be reached is dependent on *p*H, whereas the maximum velocity (V_{max}) is unaffected by *p*H. There appears to be a sharp transition in the behavior of LDH-5 between *p*H 6.2 and 6.5. At *p*H 6.2, the kinetics of LDH-5 resemble those of LDH-1 (Fig. 2)—that is, there is a low turnover number, maximum activity reached at a low concentration of pyruvate, and inhibition at high concentrations of pyruvate. Vesell has recently reported that human LDH-5 behaves in a similar manner with respect to lactate inhibition (*5*). The altered activity of LDH-5 at low *p*H is apparently due to denaturation, for, if the enzyme is incubated at the low *p*H for 3 minutes and then assayed at *p*H 7.4, it does not attain the activity it normally would have at *p*H 7.4 (Fig. 3). The assays were conducted for a 3-minute period. The activity of LDH-1 as seen in Fig. 2 is divided into two distinct groups. The group active at the higher *p*H valves is much less sensitive to inhibition by pyruvate than is the group active at lower *p*H's. These data confirm recent observations that differences between LDH-5 and LDH-1, with respect to substrate inhibition, diminish appreciably when the reactions carried out at near physiological conditions of *p*H and temperature (*6*).

For LDH-5 there is about a 70 percent increase in activity within the range of *p*H 7.0 to 7.4 (Fig. 3). It was reported (*4*) that LDH-5, but not LDH-1, was activated by seven substrates of the citric acid cycle, as well as by aspartic and glutamic acids. It is now clear that this activation was not due to the specific acids used (with exception of oxalacetic acid) but to a change in *p*H. The *p*H of the reaction mixtures in the presence of the various acids was a value between 6.8 and 7.2 rather than the presumed value of 7.4. In the range of *p*H 6.8 to 7.4 there is essentially no change in the activity of LDH-1 (Fig. 3), thus accounting for the fact that no activation was observed with this isozyme in the presence of the substrates of the citric acid cycle.

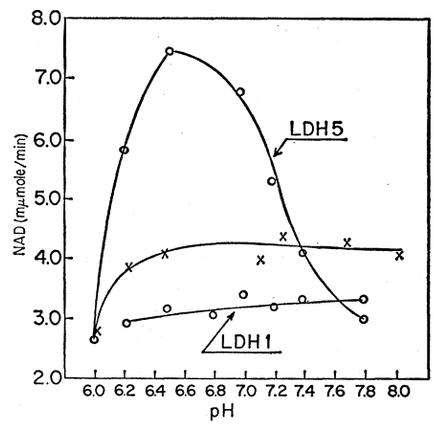

Fig. 3. Effect of *p*H on LDH-5 and LDH-1 activity. Assays were performed as indicated in Figs. 1 and 2. In the curve delineated by (x) all assays were performed with LDH-5 at *p*H 7.4 after the enzyme had first been incubated for 3 minutes at the indicated *p*H. Pyruvate concentration was 22.4×10^{-5} mole/liter.

It was also reported (*4*) that LDH-5 was activated by low concentrations of the pyruvate analogue oxaloacetate but was competitively inhibited by higher concentrations. This effect was in contrast to that on LDH-1, which was inhibited by all concentrations of oxalacetate tested. These observations have been confirmed in this series of investigations. The effect of oxalacetate on the activity of LDH-5 was similar to the effects of maleate and succinate on aspartate transcarbamylase (*3*). Using the pyruvate analogue oxamic acid Hochachka has made similar observations on LDH of goldfish muscle (*7*).

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