

A NOTE ON THE MECHANISM OF ACTION OF RABBIT MUSCLE
LACTATE DEHYDROGENASE*

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In the course of a series of experiments on the effect of deuterium on rabbit muscle lactate dehydrogenase, we have obtained some data that have a bearing on the reaction mechanism of this enzyme. When the transferable hydrogen of DPNH is replaced by deuterium to form α -DPND, appreciable changes are produced in all four constants of the rate equation (Dalziel, 1957):

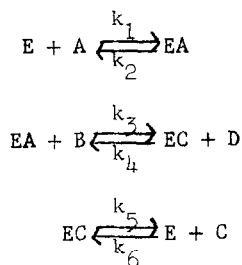
$$\frac{E_0}{v} = \phi_0 + \frac{\phi_1}{A} + \frac{\phi_2}{B} + \frac{\phi_{12}}{AB}$$

where v is the initial velocity, E_0 , A , and B are the initial concentrations of the enzyme, coenzyme, and substrate, respectively, and the ϕ 's represent various combinations of rate constants, the significances of which depend on the reaction mechanism. The effects observed with α -DPND are shown in Table 1; particular attention is called to the ratio ϕ_{0H}/ϕ_{0D} .

Zewe and Fromm (1962) on the basis of product-inhibition studies have suggested that rabbit muscle lactate dehydrogenase obeys the Theorell-Chance mechanism, an ordered binding of coenzyme and substrate without

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formation of kinetically significant ternary complexes:



where C and D represent the coenzyme and substrate products.

For the Theorell-Chance mechanism, ϕ_0 is equal to $1/k_5$. Baker and Mahler (1962) have pointed out that the ratio of ϕ_0 (DPNH)/ ϕ_0 (α -DPND) would be unity; i.e., there would be no isotope effect, since the hydrogen (or deuterium) would have been previously transferred at step 2 of the reaction sequence. Since the value observed is significantly different from unity, it would appear that an important requirement for the mechanism proposed by Zewe and Fromm is not satisfied. A mechanism involving one or more ternary complexes, as postulated by Takenaka and Schwert (1957) for beef heart lactate

TABLE 1
Effect of Replacement of DPNH by α -DPND on Rate Constants
for Rabbit Muscle Lactate Dehydrogenase

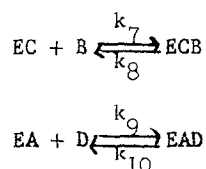
| Constant | Ratio, ϕ_H/ϕ_D , \pm S.D. |
|-------------|-------------------------------------|
| ϕ_0 | 0.71 ± 0.11 |
| ϕ_1 | 2.50 ± 0.29 |
| ϕ_2 | 0.58 ± 0.09 |
| ϕ_{12} | 0.33 ± 0.06 |

These data are based on results of experiments involving 181 combinations of DPNH and pyruvate concentrations, and 86 of α -DPND and pyruvate. Experimental methods are described elsewhere (Thomson, Bray, and Bummert, 1962).

dehydrogenase, or one of the modifications of the Theorell-Chance mechanism discussed by Mahler *et al.* (1962), would be more likely.

Although the ϕ_0 values were calculated by the method of Dalziel (1957) and thus represent an extrapolation from a series of values which themselves are extrapolations, the ratio can also be estimated by assaying the enzyme in the presence of near-saturation levels of coenzyme and substrate. Under these conditions, $v_{\text{DPND}}/v_{\text{DPNH}} \sim \phi_0(\text{DPND})/\phi_0(\text{DPNH})$, and the ratio observed by this direct comparison was 0.73.

Zewe and Fromm (1962) suggested that secondary reactions occurred, with the formation of inactive ternary complexes:



It may be pointed out that conventional steady-state treatment of the Zewe-Fromm scheme with C present does not yield the equation (number 8) that they presented, but rather one in which $1/k_5$ is multiplied by $(1+k_7\text{B}/k_8)$. Their equation can be derived by treating C not as a product, but as an inhibitor which forms a complex EC that is different from the EC produced from EA + B.

REFERENCES

- Baker, R. H., Jr., and Mahler, H. R., *Biochemistry*, 1, 35 (1962).
 Dalziel, K., *Acta Chem. Scand.*, 11, 1706 (1957).
 Mahler, H. R., Baker, R. H., Jr., and Shiner, V. R., Jr., *Biochemistry*, 1, 47 (1962).
 Takenaka, Y., and Schwert, G. W., *J. Biol. Chem.*, 223, 157 (1956).
 Thomson, J. F., Bray, D. A., and Bummert, J. J., *Biochem. Pharmacol.*, in press.
 Zewe, V., and Fromm, H. J., *J. Biol. Chem.*, 237, 1668 (1962).