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Pyruvate Inhibition of Lactate Dehydrogenase Activity in Human Tissue Extracts

Abstract. *Pyruvate inhibition of lactate dehydrogenase activity of crude human tissue extracts has been studied at both 25° and 37°C. The lactate dehydrogenase activity of tissues containing predominantly the slower moving isoenzymes is inhibited significantly less than that of other tissues. These findings are not in accordance with some recently reported.*

Variations in the catalytic response towards pyruvate of human and rabbit lactate dehydrogenase isoenzymes were first described by Plagemann *et al.* (*1*), who showed that the enzyme from both human and rabbit heart muscle was inhibited at much lower concentrations than the enzyme from human or rabbit liver. Similar observations have been described in relation to other species (*2*, *3*). A report (*4*), however, has indicated that the lactate dehydrogenase (LDH) activities of whole human tissue extracts behave more similarly toward increasing concentration of pyruvate and lactate than would be expected from the differences in substrate inhibition of the individual isoenzymes LDH-1 and LDH-5. It was pointed out that even these differences were abolished at 37°C.

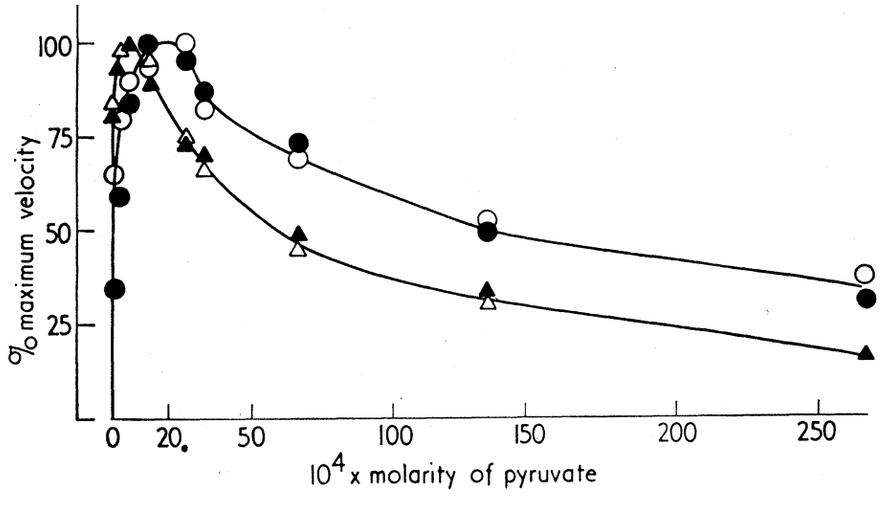

Fig. 1. Pyruvate inhibition of lactate dehydrogenase activity of human tissue extracts at 25° and 37°C (○, liver 25°C; ●, liver 37°C; △, heart 25°C; ▲, heart 37°C).

It has been suggested that the variations in substrate inhibition of the lactate dehydrogenase isoenzymes may be related to their metabolic function (*3*, *5–7*). Those tissues containing a preponderance of LDH-1 usually function under aerobic conditions, whereas those containing a preponderance of LDH-5 function under relatively anaerobic conditions. Both the pyruvate concentration at which the enzyme is most active and the concentration at which the enzyme is strongly inhibited are much lower for LDH-1 than for LDH-5 (*1*, *3*).

Although temperature dependence of the pyruvate inhibition of lactate dehydrogenase from rabbit tissues has been reported (*8*), observations on the lactate dehydrogenase activity of extracts of amphibian tissues suggest that there is little change in the overall shape of each of the substrate inhibition curves in the temperature range 11° to 50°C, although the optimum pyruvate concentration may be increased as much as tenfold (*7*). These observations and our own knowledge of the kinetic properties of human tissue lactate dehydrogenases (*6*) led us to reexamine the pyruvate inhibition of lactate dehydrogenase activity of human tissues to see whether the phenomena reported by Vesell (*4*) could be confirmed.

Using saline extracts of human tissues, we have examined the pyruvate inhibition of lactate dehydrogenase activity at both 25° and 37°C. The tissues studied included liver, skeletal muscle, heart, kidney, erythrocytes, and spleen. Extracts were prepared from such tissues obtained within 24 hours of death by homogenizing with approximately five parts of physiological saline, in an electrically driven ground-glass homogenizer. The homogenates were centrifuged at 100,000*g* for 20 minutes, and the supernatants were assayed spectrophotometrically for LDH activity by measuring the rate of change in optical density at 340 mμ. The reaction mixture contained 1 ml of 0.1*M* tris-HCl

buffer *p*H 7.4, 0.2 ml of 2 m*M* $NADH_2$, 0.5 ml of dilute tissue extract and pyruvate concentrations varying from 3.3×10^{-4} to 3.3×10^{-2} molar. The volume of the assay mixture was in all cases made up to a total volume of 3 ml with distilled water. The tissue extracts were diluted with 0.1 percent bovine albumin in 0.1*M* tris-HCl buffer, *p*H 7.4, so that they gave an initial fall in optical density of 0.050 to 0.060 per minute when the pyruvate concentration in the cuvette was 0.33 m*M*. The LDH activity of each extract was estimated at the end of each set of experiments to ascertain that the enzyme activity at 0.33 m*M* pyruvate remained constant throughout. Measurements were made at both 25° and 37°C with a Unicam SP 800 spectrophotometer with the SP 820 and SP 825 attachments. The water-jacketed cell holder was maintained at the required temperature by circulating water from a thermostatically controlled water bath. All solutions were kept in the water bath before they were transferred to the cuvettes. The same tris-HCl buffer was used for the measurements at both temperatures. This gave *p*H measurements of 7.4 at 25°C and 7.2 at 37°C. We think it unlikely that these differences bear any real significance in regard to possible interpretations of our results, since Vesell himself (*4*) admits that there were *p*H variations of as much as 0.3 units during his own observations. We have found that the *p*H did not vary at all significantly during our own estimations. The isoenzyme patterns of the tissue extracts were examined by starch-gel electrophoresis (*9*) to confirm that the patterns were normal.

The LDH activities of saline extracts of a variety of human tissues have shown differences toward increasing concentrations of pyruvate at both 25° and 37°C (Figs. 1 and 2). Those tissues containing a preponderance of the faster moving isoenzymes, such as heart and kidney, have shown inhibition at much lower concentrations than liver or skeletal muscle, which contain much more of the slower moving isoenzymes. Extracts of human spleen, which contain mostly those isoenzymes of intermediate mobility, have given a pyruvate inhibition response intermediate between that of heart and liver at both 25° and 37°C.

The optimum pyruvate concentration for those tissues containing predominantly the slower moving isoenzymes differs at both 25° and 37°C from that of those tissues containing the faster moving isoenzymes. This finding was expected in that different Michaelis constants for LDH have been found with both saline extracts of human tissues (*10*) and purified lactate dehydrogenase isoenzymes (*1*, *11*). The Michaelis constants for lactate dehydrogenase with pyruvate and lactate as substrate are temperature dependent (*8*, *12*), and it appears that with the purified isoenzymes of human and rabbit heart and skeletal muscle the optimum pyruvate concentration becomes lower as the temperature is lowered (*8*). The effect of temperature on pyruvate inhibition of LDH-5 has not been described over quite the same range of pyruvate concentrations as that of LDH-1, but the behavior of each isoenzyme is markedly different at 6° and 40°C. Moreover, there is still a significant difference between the pyruvate inhibition of LDH-1 and LDH-5 at 40°C. This is contrary to the statement made by Vesell (*4*).

Our own results with human tissues show that temperature changes in the range 25° to 37°C do not have much effect on the overall shapes of the pyruvate inhibition curves of lactate dehydrogenase in human tissue extracts (Fig. 2). These results are in agreement with those found with amphibian tissues where temperature changes in the range 11° to 50°C did not appear to alter the shapes of the substrate inhibition curves (*7*). We have found differences in the ascending portions of the curves at 25° and 37°C, the differences indicating that the isoenzymes have higher Michaelis constants at higher temperatures. This is in agree-

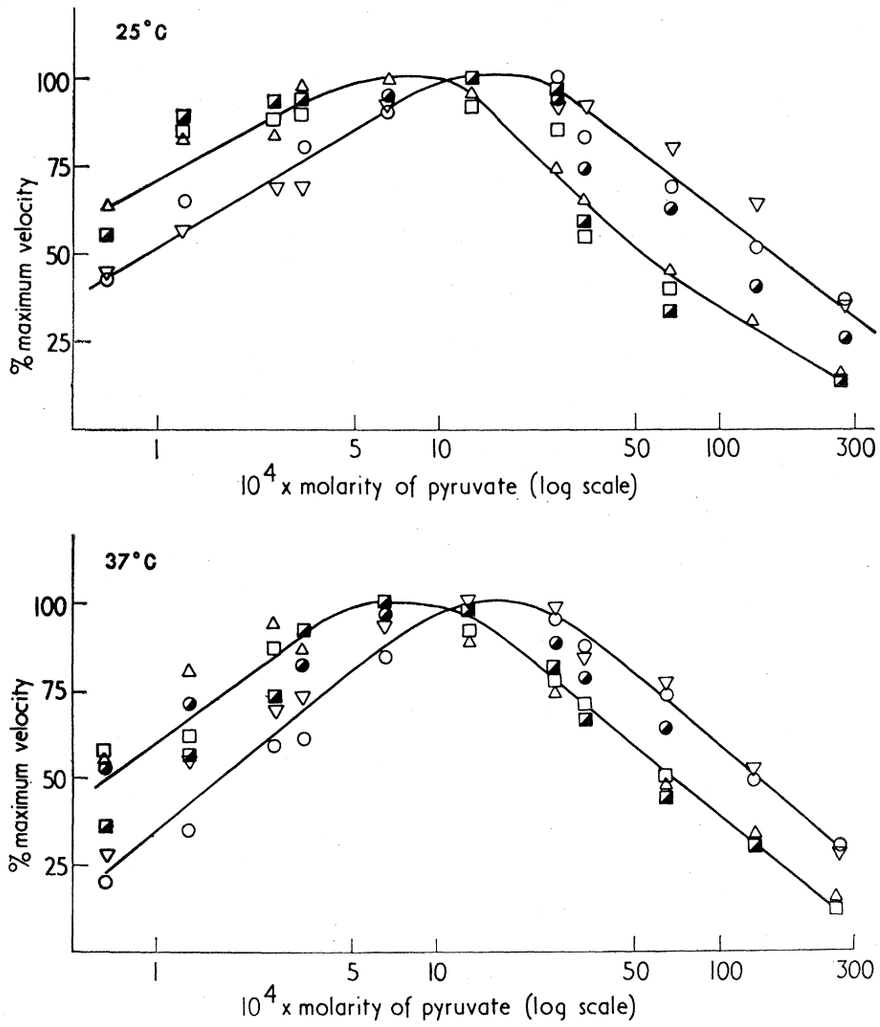

Fig. 2. Pyruvate inhibition of lactate dehydrogenase activity at 25°C (top) and 37°C (bottom) (○, liver; △, heart; ▽, skeletal muscle; ◪, kidney; □, erythrocytes; ◕, spleen).

ment with the results of Hakala and co-workers (*12*), who have shown that the K_m of beef heart lactate dehydrogenase is doubled in the range 25° to 35°C.

The ratio between the enzyme reaction rates in 0.33 m*M* and 10 m*M* pyruvate has been taken as characteristic of the heart (H) and muscle (M) types of lactate dehydrogenase; tissues of the H-type giving values between 2.2 and 4.0 and those of the M-type values below 2.0 (*3, 6, 7*). Our results give an average value for erythrocytes, heart extracts, and kidney extracts of 2.8 at 25°C and 2.5 at 37°C and for liver and skeletal muscle extract values of 1.2 at 25°C and 1.1 at 37°C.

Our results offer confirmation that the response of tissue lactate dehydrogenase activity toward increasing pyruvate concentration at both 25° and 37°C can be used as an indication of whether a particular tissue contains more of the faster or slower moving lactate dehydrogenase isoenzymes. The assertion of Vesell (*4*) that the total activity of tissue lactate dehydrogenase does not retain the characteristics of the activity of individual isoenzymes with respect to substrate inhibition phenomena is definitely not confirmed by our observations.

The similarities in the behavior of tissue extracts towards pyruvate which have been described by Vesell have been attributed by him to the presence of sufficient amounts of the substrate-sensitive isoenzymes in all tissues to render the total lactate dehydrogenase activity subject to the same degree of inhibition (*4*). We have, however, shown that tissues which contain relatively large amounts of the slowest moving isoenzymes are inhibited to a significantly lesser degree than tissues which contain predominantly faster moving isoenzymes. At 25°C and 10 m*M* pyruvate we have found approximately 65 and 45 percent inhibition respectively with heart and liver extracts. Under these conditions of temperature and substrate concentration, Vessel obtained 75 percent inhibition in both cases. We consider that our own findings correlate quite closely with what could be expected from the known percentage concentration of each of the isoenzymes present in the extracts.

From the results described by Vesell (*4*) it would appear that the pyruvate inhibition of heart extracts is more similar to LDH-1 than the pyruvate inhibition of liver extracts is to LDH-5. In the same way the behavior of purified LDH-1 at 20°C does not appear to be significantly different from that of LDH-1 at 37°C, whereas the behavior of purified LDH-5 appears markedly temperature dependent (*4*). This would seem to imply that Vesell's findings might conceivably reflect instability of LDH-5 in his whole homogenates and purified enzyme preparations rather than an increased sensitivity to pyruvate. We are otherwise at a loss to explain why his findings with tissue homogenates differ from our own. We admit that Vesell prepared homogenates in a Waring Blendor, centrifuged them at 15,000*g* for 1 hour and presumably used undiluted homogenate in the assay, in which he also used phosphate buffer and $1 \times 10^{-4}M$ $NADH_2$. Conceivably these differences in technique could be responsible for the observed discrepancies between our findings, but we do not believe this is the case.

It would appear, therefore, that at present there is insufficient evidence to warrant the suggestion that the kinetic properties in vitro of lactate dehydrogenase from crude human tissue extracts cannot be correlated with the distribution of isoenzymes and their possible metabolic role.

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Release of Catecholamines and Specific Protein from Adrenal Glands

Abstract. *Rabbit antiserums directed against a purified protein obtained from the catecholamine storage vesicles of the adrenal medulla of the cow were used to study the mechanism of secretion from the adrenal medulla. During secretion evoked by acetylcholine in isolated perfused adrenal glands, catecholamines and the specific intravesicular protein were released in relative amounts comparable to those present in the intact gland. Thus catecholamines are apparently released from the storage vesicles by a process resembling reverse pinocytosis.*

Adrenaline and noradrenaline are released from the adrenal medulla in response to nerve stimulation or to perfusion with acetylcholine and other secretagogues. There is evidence (*1, 2*) that the catecholamines which are stored in intracellular vesicles are secreted from these vesicles directly to the exterior of the cell by a process resembling "reverse pinocytosis." This evidence is based on (i) electron microscopy and (ii) observations that, during stimulated secretion, the ratio of the quantity of catecholamines to that of adenine nucleotides in the perfusate was similar to that in the intact storage vesicles. We have obtained further evidence to support this mechanism by measuring the amounts of catecholamines and a specific intravesicular protein released during stimulation and then comparing the ratios of these compounds in the perfusates to the ratios of the amounts in intact vesicles. Banks and Helle (*3*) have recently reported that this protein was present in perfusates of isolated glands during stimulated secretion but was not detectable by Ouchterlony analysis during the resting period.

The major soluble protein component of the catecholamine storage vesicles can be readily purified on diethylaminoethyl (DEAE) cellulose and Sephadex G-200 (*4*). Rabbit antiserums were prepared by immunization with the purified protein in complete Freund's adjuvant. The purified protein showed a