acid and 0.02M acetate, buffered at pH 5.5 and 5.0. Unlike the hydrogen peroxide decomposition, this reaction proceeded both in the presence and absence of added metal ions. The rate at −11° exceeded that at +1°C under all of these conditions. At pH 7.2, all of the ascorbate oxidations occurred more rapidly than at lower pH levels, the reaction in ice being too rapid to give satisfactory kinetics under the conditions used.

At least two chemical species participate in each of the reactions reported here. The reactants, including molecular oxygen in the case of ascorbic acid oxidation, are clearly brought by freezing into an environment conducive to reaction. Whether this environment is the ice lattice or liquid microinclusions is not clear. However, the kinetic data appear to contradict a mechanism based upon the concentration increases resulting from phase separation.

The types of chemical reactions known to be capable of proceeding at significant rates in ice now include hydrolysis (*1*, *3*), dehydration (*2*), aminolysis (*4–6*), peptide formation (*9*), oxidation, and peroxide decomposition. In view of the facility with which these reactions occur, and as a result of Urey's postulate of a low-temperature origin of the earth (*10*), there is reason to question the assumption that water present only as frost or ice precludes prebiotic chemical evolution. It is evident, for example, that the combination of restricted diffusion and low temperature fails to slow down a number of reactions of biochemical importance. Second, one of the conditions regarded as essential for prebiotic interactions is concentration of various precursor molecules (*11*); the process of freezing could accomplish this concentration. Third, a frozen environment suggests limitations to the assumption that once living organisms have appeared they would consume biochemicals arising *de novo*, thereby perpetuating existing characteristics (L-amino acids, and so forth). In ice, newly formed chemical structures would probably be sequestered from living organisms and might evolve to considerable complexity.

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Lactate Dehydrogenase Isozymes: Substrate Inhibition in Various Human Tissues

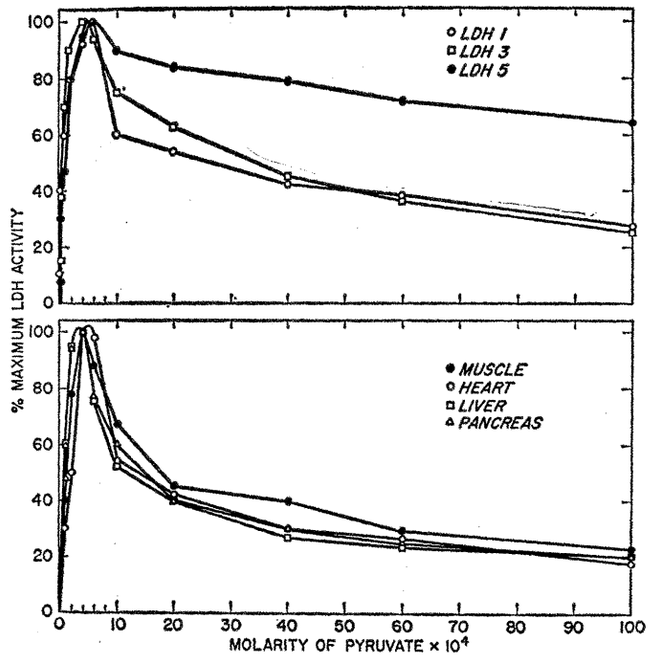
Abstract. *The total lactate dehydrogenase (LDH) in whole homogenates of various human tissues reacts more similarly toward pyruvate and lactate at 25°C than expected from the marked differences in substrate inhibition at this temperature between isolated, purified LDH-1, and LDH-5. At 37°C, LDH-5 closely resembles LDH-1 in extent of inhibition by substrate. These results are incompatible with the theory that differences in degree of isozyme inhibition by substrate have resulted in predominance of LDH-5 in anaerobic tissues and predominance of LDH-1 in aerobic tissues.*

Two principal theories have been proposed to elucidate the functional significance of LDH isozymes. Based on inhibition at 25°C of isolated LDH-1, purified by concentrations of pyruvate to which LDH-5 is resistant (*1*), the first theory states that LDH-5 predominates in anaerobically metabolizing tissues because, in high pyruvate concentrations, it functions more efficiently than does LDH-1 (*2*). By contrast, LDH-1 is claimed as the predominant isozyme in aerobic tissues where high substrate concentrations do not develop (*2*). The second theory, consistent with the first but more general, maintains that individual isozymes fulfill distinctive roles by virtue of being situated in various regions of a cell. Subcellular localization of isozymes would depend on their different kinetic properties, as well as on the metabolic organization of the particles with which they associate (*3*). Recently various isozymic forms have been demonstrated to be subject to feedback control by compounds in divergent metabolic pathways (*4*).

The purpose of this report is to describe the observation that the total LDH activity in whole homogenates of several human tissues behaves even at the unphysiologic temperature of 25°C in a qualitatively and quantitatively similar fashion toward substrate. These tissues do not reveal the marked differences in substrate inhibition expected and predicted by the first theory in view of the dissimilarity at 25°C between isolated, purified LDH-1 and LDH-5. These kinetic studies performed on whole homogenates have obvious limitations from which investigations on isolated, purified isozymes do not suffer. However, it is shown that isolated, purified LDH-1 and LDH-5 at the physiologic temperature of 37°C exhibit similar kinetic behavior on exposure to increasing concentrations of substrate.

Human heart, psoas muscle, liver, and pancreas, obtained within 12 hours of death, were homogenized in a Waring blendor at 4°C (one part of tissue to five parts of phosphate buffer pH 7.4, 0.4M). The homogenates were centrifuged at 15,000g for 1 hour; the supernatant was assayed spectrophotometrically at 340 mμ for LDH activity, both by oxidation and reduction of the coenzyme at 25°C. Between 2 and 5 μl of homogenate provided sufficient activity when assayed with varying concentrations of pyruvate (4×10^{-5} to $4 \times 10^{-2}M$) and lactate (1×10^{-4} to $3.6 \times 10^{-1}M$). The volume of the assay mixture was kept constant at 3 ml by appropriate additions of phosphate buffer (pH 7.4, 0.4M).

The substrate solutions were adjusted to pH 7.4 before addition to the cuvettes. Nicotineamideadenine di-



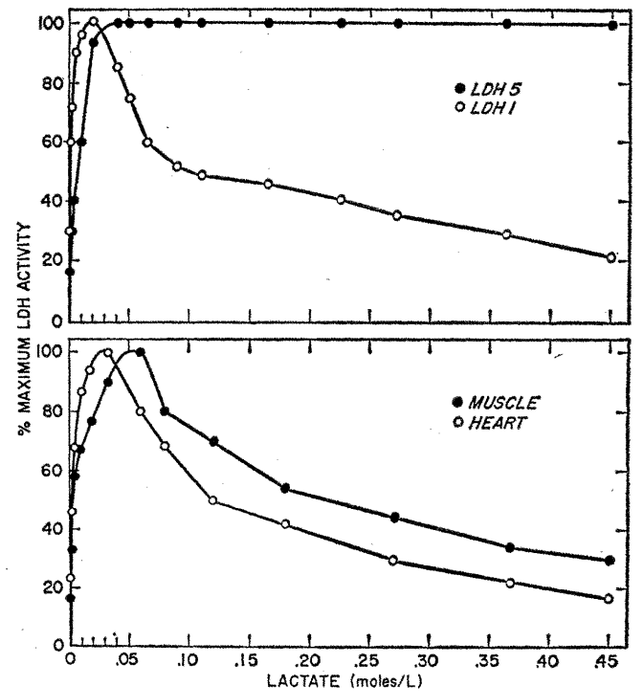


Fig. 1 (left). Activities of isolated LDH isozymes (top) and of various tissue homogenates (bottom) in the presence of increasing concentrations of sodium pyruvate at 20°C. Fig. 2 (right). Activities of LDH-1 and LDH-5 (top) and of human skeletal muscle and heart homogenates (bottom) in the presence of increasing concentrations of sodium lactate at 25°C.

nucleotide (NAD) ($10^{-3}M$) or its reduced form (NADH) ($10^{-4}M$) was added to the cuvettes for determination of activity with lactate or pyruvate, respectively. The *p*H determined after completion of the reaction did not vary by more than ±0.3 from the initial value. The LDH-1 and LDH-5 were prepared from human heart and psoas muscle homogenate, respectively, by starch-block electrophoresis (*5*). The LDH-3 was obtained by starch-block electrophoresis of homogenates of human kidney. Activities at 37°C were determined after incubation of the isozymes and reagents in a water bath for 30 minutes. Measurements were made in a Gilford automatic recorder attached to a Beckman DU spectrophotometer maintained at 37°C by circulating water.

Whole homogenates of skeletal muscle, heart, liver, and pancreas react similarly at 25°C to pyruvate, although distribution of total LDH activity among the five isozymes varies greatly among these four tissues (Fig. 1). Approximately 30 percent of the total LDH activity of skeletal muscle resides in LDH-5, 20 percent each in LDH-2, -3, and -4 (*6*); by contrast human heart contains approximately 73 percent of its total activity in LDH-1 (*5*).

At 25°C, LDH-5 resists the high pyruvate concentrations to which LDH-1 and -3 are sensitive (Fig. 1, top). Figure 1 (bottom) shows that all of the tissue homogenates behave more like LDH-1 and -3 than like LDH-5; they are inhibited to an even greater extent by increasing pyruvate concentrations than are LDH-1 and LDH-3. In four separate experiments skeletal-muscle homogenates consistently exhibit slightly greater resistance to pyruvate than do the other tissue homogenates. Figure 2 (bottom) reveals that, at 25°C, homogenates of skeletal muscle and heart resemble LDH-1 more than LDH-5 (Fig. 2, top) in reactivity toward increasing lactate concentrations. However, at high lactate concentrations muscle homogenates in four separate experiments consistently retain 10 to 20 percent more of their activity than do heart homogenates. With lactate as substrate LDH-1 is inhibited to a slightly greater extent than the tissue homogenates.

None of the tissues, even those exhibiting appreciable quantities of LDH-5, retain the marked resistance to pyruvate or lactate inhibition characteristic of LDH-5. This observation probably reflects the fact that most tissues contain significant concentrations of all five isozymes, rather than exclusively LDH-1 or LDH-5 (*5*, *6*). Thus, while the LDH-5 of a tissue may resist substrate inhibition, its appreciable complement of LDH isozymes 1–4 renders the total activity subject to inhibition. Sufficient amounts of these substrate-sensitive isozymes are present in the tissues tested to prevent development of marked differences on exposure to increasing substrate concentrations.

This interpretation is strengthened by calculations of expected pyruvate inhibition of muscle homogenates based on pyruvate inhibition of individual isozymes (Fig. 1, top) and the known isozyme composition of human skeletal muscle (*5*, *6*). If the total LDH activity of muscle homogenates consists of approximately 10 percent LDH-1, 20 percent each for LDH-2, -3, and -4, and 30 percent LDH-5 (*5*, *6*), then for each of the last five points on the pyruvate inhibition curve (Fig. 1, bottom) there would be expected 74, 62, 57, 49, and 34 percent activity, whereas there was obtained 67, 45, 40, 29, and 22 percent activity; differences between predicted and experimental values for these 5 points range from 7 to 20 percent. Assumptions for calculation of the ex-

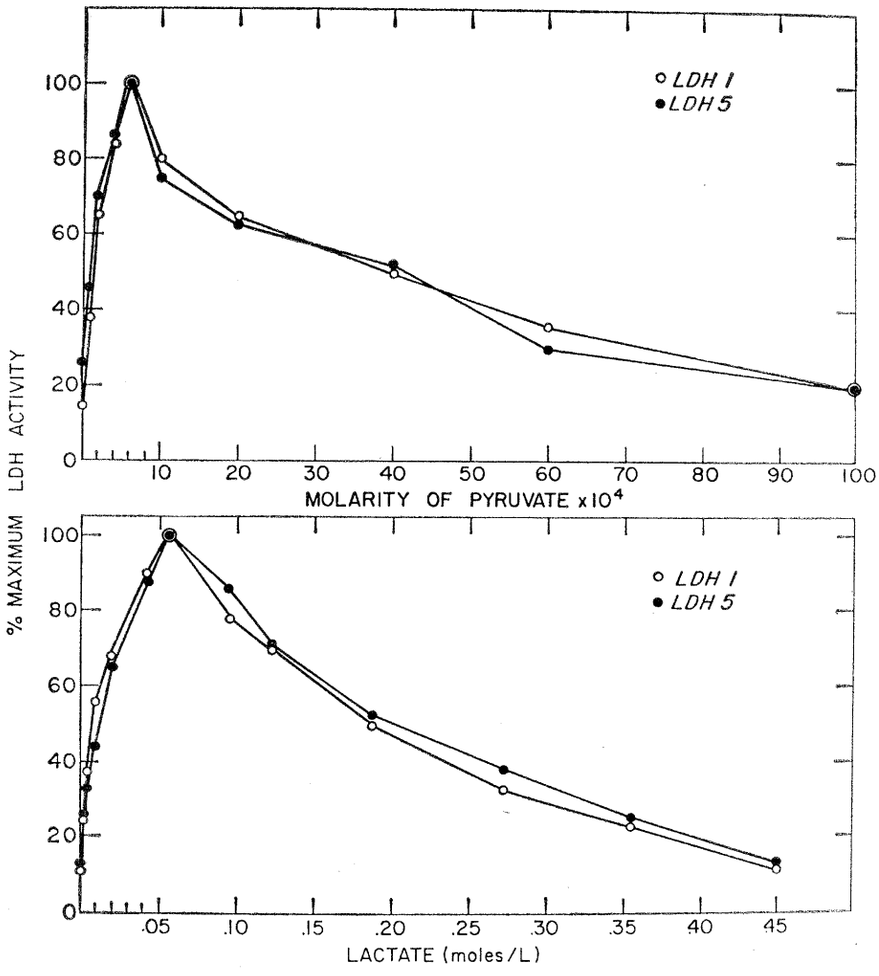


Fig. 3. Activities of LDH-1 and LDH-5 in the presence of increasing substrate concentrations at 37°C.

pected values are that LDH-2 resembles LDH-1 and LDH-3 and that LDH-4 is intermediate between LDH-3 and LDH-5 in reactivity toward pyruvate.

Similarity of the substrate-inhibition curves of various tissues at 25°C and particularly the close resemblance in behavior between LDH-1 and LDH-5 at 37°C (Fig. 3) to increasing substrate concentrations raise questions concerning the biological significance of theories based on differences obtained with the isolated, purified isozymes at 25°C. Measurement of lactate and pyruvate concentrations in various tissues under what have been termed highly anaerobic conditions would help answer this question, but these determinations have not been reported in conjunction with the aforementioned theory (*2*).

Normal concentrations of pyruvate ($10^{-4}M$) and lactate ($10^{-3}M$) in human plasma fall on the ascending portions of the curves shown in Figs. 1 and 2, rather than at their peaks or descending parts (*7*). If these amounts of substrate are never exceeded in vivo, differences, at the unphysiologic temperature of 25°C, between LDH-1 and LDH-5 in degree of inhibition at high substrate concentrations apparently have little biological significance. Concentrations of these substrates in tissues or in subcellular particles may theoretically be expected to exceed blood levels; however, experimental determinations of lactate in muscle and plasma after exercise suggest that lactate is freely diffusable, since concentrations of lactic acid become even higher in plasma than in muscle (*8*). Elevations of blood pyruvate after exercise or in pathological conditions resemble those of lactate, but are smaller, presumably because pyruvate, unlike lactate, has several alternative metabolic pathways (*7*).

A division of tissues according to relative dependence on aerobic or anaerobic metabolism appeared initially to correspond to tissue LDH isozyme patterns, that is, to predominance of LDH-1 (heart) or LDH-5 (skeletal muscle). However, several highly anaerobic tissues, including the mature fiber cells of the bovine lens and mature human erythrocytes, contained mainly anodal isozymes and had negligible amounts of LDH-5 (*5, 9*). Other evidence adduced to support the theory is largely circumstantial and involves shifts in isozyme pattern toward LDH-5 in such diverse environments as exist in tissue culture, neoplasia, hormonal stimulation of uterus and phylogenetic evolution of muscle (*2, 10*). In certain of these situations, the shift to LDH-5 occurs concomitantly with development of what has been termed increased anaerobic metabolism.

Even at 25°C, the total LDH activity in homogenates of a variety of human tissues reacts more similarly toward substrate than would have been expected from the behavior at this temperature of isolated, purified LDH-1 and LDH-5. The total LDH activity of tissues then reacts, not as a mixture retaining the characteristics in vitro of either LDH-1 or LDH-5 at the unphysiologic temperature of 25°C, but rather as a mixture containing all five isozymes in fairly high concentrations. It appears unwarranted to attribute the distribution of isozyme patterns among tissues to differences in activity between isolated, purified LDH-1 and LDH-5 in the presence of increasing substrate concentrations, since at physiologic temperature isolated, purified LDH-5 resembles LDH-1 in this respect. Identical observations have previously been made at 40°C (*11*).

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Fluorescence Quantum Yield Measurements: Vitamin B_6 Compounds

Abstract. *Quantum yields for pyridoxal, pyridoxamine, and pyridoxamine-5-phosphate at 25°C in neutral aqueous solution were 0.048, 0.11, and 0.14, respectively. The fluorescence efficiencies were highly temperature-dependent, and the compounds were extremely sensitive to light. There were marked discrepancies between the values obtained in this study for quantum yield and fluorescence polarization of pyridoxamine-5-phosphate and the values recently reported by others.*

It is desirable, when studying the fluorescence of solutions, to be able to measure absolute quantum yield (Q) conveniently and accurately. The quantum yield, Q, is the ratio of the number of photons emitted to the number absorbed. Spectrophotofluorometers have been calibrated by a number of methods (*1–3*) which make it possible to obtain corrected emission spectra with such instruments. The Aminco-Bowman spectrophotofluorometer, patterned after an instrument designed by Bowman *et al.* (*4*), has been used to measure the fluorescence quantum yields of a number of organic compounds by comparison of their corrected emission spectra with that of a fluorescent standard, and these values agreed closely with published data (*5*). However, it was surprising that reliable quantum-yield data obtained under well-defined conditions were available for so few compounds. Fluorescence data are incomplete without knowledge of the quantum yields, since this parameter is a measure of the strength of emission and is somewhat analogous to the extinction coefficient in absorption spectroscopy.

The quantum yields of compounds of the vitamin B_6 group, namely, pyridoxal, pyridoxamine, and pyridoxamine-5-phosphate (PMP) were measured. The rationale for undertaking this study is as follows. The binding of coenzymes such as diphosphopyridine nucleotide to proteins has been successfully studied by fluorimetry (*6*), and Undenfriend suggested that fluorescence techniques could also be used to study combination of vitamin B_6 coenzymes with proteins (*7*, p. 213). However, such a proposal is hampered by the fact that the emissions of free vitamin B_6 compounds have been poorly characterized; although a series of papers by Churchich on this subject has recently appeared (*8–10*). Measurements by Churchich of the quantum yield of PMP with an instrument described by Weber and Young (*11*) gave Q equal to 0.55 in neutral aqueous solution at 25°C, and this figure for quantum yield was used in the calculation of the fluorescence-lifetime and "radiationless" energy-transfer properties of PMP (*9–10*). However, there are no reports giving the impression that PMP possesses as high a fluorescence efficiency as quinine sulfate—one of the most fluorescent laboratory compounds—which has been shown to have a quantum yield of 0.55 at 25°C (*12*). Furthermore, the value of Q equal to 0.55 for PMP was inconsistent with the value of 0.11 reported for this compound (*8*).

I have measured fluorescence quantum yields with an Aminco-Bowman spectrophotofluorometer fitted with an Osram XBO-150 xenon arc lamp and an RCA 1P28 multiplier phototube (*13*). Calibration of the exciting light source was carried out with solutions of Rhodamine B (3 g/liter in ethylene glycol) or 1-dimethylaminonaphthalene-5-sulfonate (0.01M in 0.1M $NaHCO_3$) as fluorescence screens by the method of Melhuish (*2*), which requires the use of an adapter (*14*) to allow fluorescence to be observed from the side of the cuvette which faces the light source. Calibration of the detector system was accomplished with a standard tungsten lamp obtained from the U.S. National Bureau of Standards (*1*) or with the aforesaid xenon lamp after it had been calibrated (*2*).

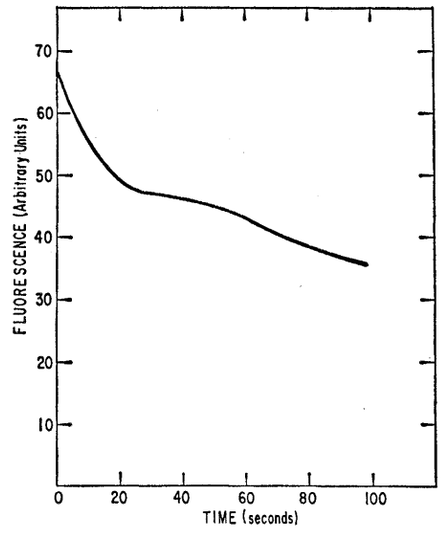

Fig. 1. Photodegradation of pyridoxamine-5-phosphate, $3 \times 10^{-6}M$, in 0.05M potassium phosphate buffer, pH 7.0. Illumination at 326 mμ in a microcuvette in the Aminco-Bowman spectrophotofluorometer. Temperature, 8°C.

Polarization of fluorescence was measured with polarized exciting light; light-stable, ultraviolet, polarizing filters were employed (*13*). Optical densities were determined on a Cary model 11 spectrophotometer. All reagents were obtained from commercial sources (*15*). Pyridoxal, pyridoxamine,

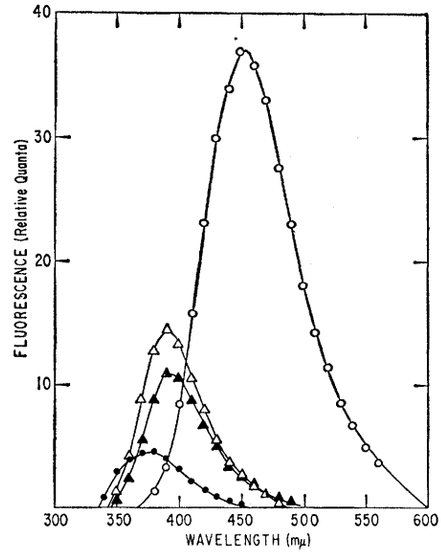

Fig. 2. Corrected emission spectra for quinine in 1N H_2SO_4 excited at 348 mμ (○), pyridoxal excited at 315 mμ (●), pyridoxamine excited at 324 mμ (▲), and PMP excited at 326 mμ (△). Solvent for the vitamin B_6 compounds was 0.05M potassium phosphate, pH 7.0. Areas beneath the curves are proportional to the absolute quantum yield.