key-kidney antigen and to Fortner-tumor antigen in 2 weeks, indicating that development in hamsters of antibody to LCM is unrelated to the development of tumors.

No evidence of LCM has been observed in any hamsters bearing the Fortner fibrosarcomas. Newborn hamsters inoculated with cell transplants shortly after birth develop visible tumors in 7 to 10 days; the tumors enlarge rapidly and most animals die with massive tumors (25- to 50-mm) in 14 to 31 days. Weanlings develop palpable tumors within 10 to 12 days after receiving implants; none died after 2 months of progressive growth of tumors.

The data indicate that the Fortner fibrosarcoma No. 2 is contaminated with LCM virus and that presence of this virus in tumor transplants is responsible for the antibody response observed in tumor-bearing hamsters. Preliminary studies reveal that serum and organs (brains, lungs, liver, spleen, and kidney) from tumor-bearing hamsters that received implants during the first 24 hours of life also contain LCM virus. Consequently transplantation of tumor fragments from hamster to hamster results in a generalized infection and is simply a mode of inoculating virus. Development of high-titer complement-fixing antibody in weanling hamsters within 2 weeks of inoculation with cell-free tumor extracts corroborates this point.

Weanling hamsters bearing transplanted tumors induced by the Schmidt-Ruppin strain of Rous-sarcoma virus and an avian adenovirus–like agent (chicken-embryo lethal-orphan virus, CELO) (*10*) were housed in the same room with the animals bearing Fortner-fibrosarcoma No. 2 tumors. A number of the weanlings developed LCM-antibody titers of 1:80 or greater. Both the Rous and the CELO tumors were known to be free of LCM virus in earlier passages. One CELO-tumor antigen reacted at a 1:32 dilution with specific hamster antiserum to LCM. Also a number of hamsters bearing various primary or transplanted tumors (free of LCM in earlier passages), and housed in nearby facilities serviced by the same personnel, have developed complement-fixing antibody to LCM. Such data suggest that infected hamsters shed LCM virus and that a colony of susceptible hamsters may become contaminated with LCM by exposure to fomites.

Several weeks after the hamsters bearing Fortner-fibrosarcoma No. 2 tumors were introduced into the animal colony, personnel working with these animals developed influenza-like illnesses and serological evidence of LCM infection. This is further evidence of environmental contamination and indicates hazards that may attend work with transplantable rodent tumors, even when such transplanted tumors are derived from spontaneous or chemically induced neoplasms. The Fortner fibrosarcoma No. 2 had been contaminated by LCM while it was carried in certain other laboratories working on LCM infection in mice. Tumor samples received from one such laboratory were free of LCM virus and antigen.

Sabin (*11*) has shown that serological reactions to isoantigens in transplanted tumors may resemble the virus-specific reactions described by Huebner *et al.* (*1*). Our results demonstrate that a contaminating virus also may be a source of misleading results. Awareness of possible contamination of hamster tumors by LCM is also important for the protection of personnel. Presence of LCM virus in tumors has one possibly useful aspect: since the titer of LCM-complement-fixing antigen is superior to that obtained in other systems, the tumor extracts may be useful as potent and cheap diagnostic reagents.

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Rabbit Muscle Lactate Dehydrogenase 5; A Regulatory Enzyme

Abstract. *Lactate dehydrogenase isozyme 5 from rabbit skeletal muscle is activated by citrate,* cis*-aconitate, isocitrate, α-ketoglutarate, succinate, fumarate, malate, aspartate, and glutamate. In the presence of these activators the shape of the pyruvate saturation curve is changed from sigmoid to hyperbolic. Lactate dehydrogenase isozyme 1 from rabbit heart gives a hyperbolic pyruvate saturation curve and is not activated by these compounds. Oxalacetate is a competitive inhibitor of both isozyme 5 and isozyme 1 but at low concentration it activates the former. These results indicate that lactate dehydrogenase isozyme 5 from rabbit skeletal muscle is an allosteric protein and a regulatory enzyme, while lactate dehydrogenase isozyme 1 from rabbit heart is apparently neither.*

A regulatory enzyme is one which takes part in the intracellular control of metabolic pathways. Evidence is accumulating that such enzymes have a number of characteristics in common; for example, (i) they are composed of subunits; (ii) the substrate saturation curves under certain conditions are sigmoid shaped; and (iii) they undergo conformational changes when exposed to "effectors" (*1*). The effectors may be activators or inhibitors and are bound to the enzyme at a site distinctly different from the substrate site. This type of molecular alteration has been termed an allosteric transition (*2*) and, as pointed out by Umbarger (*3*), is a special case of the "induced-fit" hypothesis of Koshland (*4*). Aspartate transcarbamylase is a classic example of a regulatory enzyme (*5–7*).

The subunit nature of various lactate dehydrogenases has been well documented (*8*). This report will present evidence that lactate dehydrogenase isozyme 5 (LDH 5) from rabbit skeletal muscle has a sigmoid-shaped substrate saturation curve which becomes hyperbolic in the presence of a number of effectors. The results suggest that this isozyme can be classified as a regulatory enzyme.

Twice recrystallized rabbit muscle LDH, which contains all five isozymes, was purchased from Worthington Biochemical Corporation. Pure LDH 5 was obtained from this prepa-

ration by chromatography on diethylaminoethyl Sephadex (*9*). Isozyme 1 was prepared from rabbit heart by the method of Wachsmuth and Pfleiderer (*9*). The bands were identified electrophoretically on polyacrylamide gels at *p*H 10.0. Assays were performed by measuring the rate of change of the 340 mμ absorption due to oxidation of reduced nicotinamide adenine dinucleotide (NADH) in a Beckman DU spectrophotometer at *p*H 7.4 and at 28° or 37°C. Protein determinations were based on a molar extinction coefficient for rabbit muscle LDH of 1.24×10^5 at 280 mμ (*10*). Enzyme dilutions were made with "Microcap" micropipettes obtained from Kensington Scientific Corporation.

The rate of the LDH 5–catalyzed conversion of pyruvate and NADH to lactate and nicotinamide adenine dinucleotide (NAD) is increased by seven citric acid cycle intermediates as well as by aspartic and glutamic acids which are directly converted to citric acid cycle intermediates. The activation is accompanied by a lowering of the apparent Michaelis constant (K_m) for pyruvate (Table 1). No significant change was noted in maximum velocity of conversion (V_{max}) values. No other amino acids had any effect on the reaction. These same compounds had little effect on the reaction catalyzed by LDH 1. Oxalacetate, the remaining citric acid cycle substrate, is an analogue of pyruvate and is a competitive inhibitor of LDH 5 at concentrations greater than $7 \times 10^{-4}M$. However, oxalacetate activates LDH 5 at lower concentrations. In contrast, oxalacetate, at all concentrations, inhibits LDH 1 (Fig. 1). Gerhart and Pardee (*6*) have made similar observations with aspartate transcarbamylase, using the aspartate analogues maleate and succinate. They have interpreted their data as being consistent with the idea that binding of a ligand at the substrate site at low substrate concentration, whether substrate or substrate analogue, brings about a molecular alteration leading to exposure of new substrate sites and to increased enzyme activity. As the substrate concentration is increased, the analogues compete for the more available substrate sites, and the result is decreased enzyme activity. My results thus suggested that the isozyme was capable of undergoing a conformational change and that the activators of LDH 5 were binding at an effector site.

Figure 2 is a pyruvate saturation curve and reveals that the curve for LDH 5 alone is slightly but definitely sigmoid shaped, whereas the curve in the presence of one of the activators, in this case *cis*-aconitate, is hyperbolic. The figure also shows that the curve for LDH 1 is hyperbolic.

Table 1. Effect of LDH 5 activators on apparent K_m for pyruvate. Initial reaction velocities were determined in 2-ml reaction mixtures containing $2.24 \times 10^{-5}M$ NADH; pyruvate concentration varied between $1 \times 10^{-5}M$ and $5 \times 10^{-4}M$; $1.5 \times 10^{-3}M$ activator; $20 \times 10^{-11}M$ LDH 5; 0.05*M* sodium phosphate buffer; *p*H 7.4 at 37°C. The K_m value were determined by the Lineweaver-Burk method with a least-squares program in an IBM 1620 computer to obtain the slopes and intercepts.

Compound added	$10^{-4} K_m$
Aspartate	1.29
α-Ketoglutarate	2.11
Succinate	1.46
Glutamate	2.46
Fumarate	1.35
cis-Aconitate	4.40
Malate	1.12
Isocitrate	3.31
Citrate	1.20
None	9.60

The other activators gave curves similar to that shown for LDH 5 and *cis*-aconitate, that is, their presence in the assay mixture resulted in a change in the curve from sigmoid to hyperbolic. These results further suggested that LDH 5 was capable of undergoing an allosteric transition. Gerhart and Pardee (*5*) found that they could destroy the allosteric site for cytidine triphosphate on aspartate transcarbamylase, without affecting the catalytic activity, by heating the enzyme at 60°C for 4 minutes. Heating LDH 5 for 3 minutes at 60°C completely destroyed the catalytic activity, but heating at 40°C for 3 minutes resulted in a desensitization of the enzyme toward the activators, while the catalytic activity remained unchanged (Fig. 3). Isozyme 1 is stable when heated at 60°C for 3 minutes. The assays for the data presented in Fig. 3 were carried out at 28°C, where the sigmoid shape of the LDH 5 substrate saturation curve is not as pronounced as it is at 37°C. The enzyme LDH 5 becomes desensitized toward the activators by standing in ice for 3 hours at a concentration of $4 \times 10^{-7}M$, while at a concentration of $5 \times 10^{-5}M$ (7 mg/ml) the enzyme retains full catalytic activity but is desensitized in about 2 weeks even in the cold room. Thus, rabbit muscle LDH 5 appears to be an allosteric protein while rabbit heart LDH 1 most likely is not.

Kaplan and his group (*11*) have

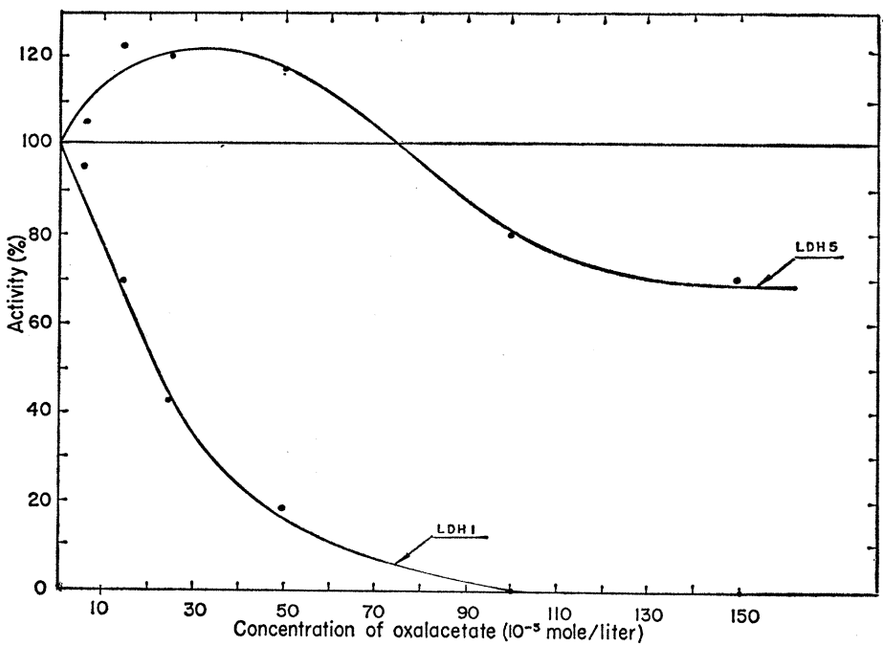

Fig. 1. Effect of oxalacetate on LDH activity. Assays were performed in a volume of 2 ml containing $2.24 \times 10^{-5}M$ NADH in 0.05*M* sodium phosphate buffer, *p*H 7.4 at 37°C. For LDH 5 the pyruvate concentration was $6.7 \times 10^{-5}M$, which was 19 percent of the saturating concentration. For LDH 1 the pyruvate concentration was $2.2 \times 10^{-5}M$, which was 35 percent of the saturating concentration. Enzyme concentrations were, for LDH 5, $20 \times 10^{-11}M$; for LDH 1, $7 \times 10^{-11}M$. Oxalacetate concentrations were as indicated.

previously proposed that LDH 5 and LDH 1 have significantly different functional roles. Their proposal was based on the fact that pyruvate concentrations which are subsaturating for LDH 5 are drastically inhibiting for LDH 1. The work here reported supports this proposal. Isozyme 5 is by far the most prevalent isozyme in skeletal muscle. It is a well-known fact that lactate accumulates in these muscles during violent exercise, when the oxygen supply becomes limiting. It is no doubt true that under these conditions the lactate accumulation may be due in part to increased NADH levels, but the activation of LDH 5 by the citric acid cycle substrates is probably a contributing factor. On the other hand, LDH 1 is the major molecular form in cardiac muscle, which fact, in view of the present observations, is not unexpected. A constant supply of energy is required by the heart, and it is furnished mainly by the adenosine triphosphate produced as a result of the oxidation of citric acid cycle substrates. In the heart these substrates do not feed back and limit their own concentration by activating LDH as they do in skeletal muscle.

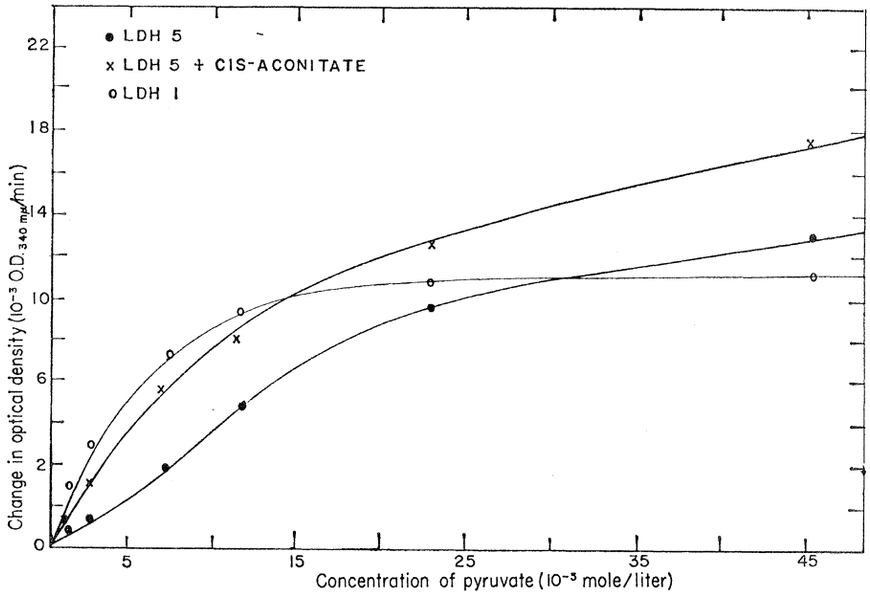

Fig. 2. Effect of activators on LDH 5 kinetics. The reaction mixtures were contained in 2 ml; $2.24 \times 10^{-5}M$ NADH and $1.5 \times 10^{-3}M$ *cis*-aconitate where used (see Table 1 for other activators which gave similar results); pyruvate concentrations as indicated; LDH 5, $20 \times 10^{-11}M$; LDH 1, $7 \times 10^{-11}M$; $0.05M$ sodium phosphate buffer, *p*H 7.4. Reactions were run at 37°C.

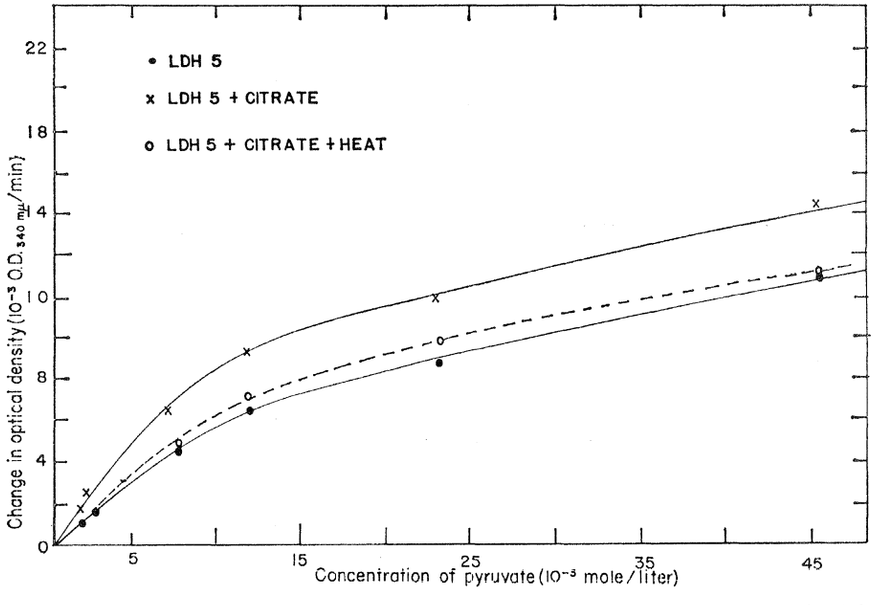

Fig. 3. Effect of heating on LDH 5 kinetics. The reaction mixtures were contained in 2 ml; $2.24 \times 10^{-5}M$ NADH and $1.5 \times 10^{-3}M$ citrate where used; pyruvate concentration as indicated; $0.05M$ sodium phosphate buffer, *p*H 7.4; LDH 5, $20 \times 10^{-11}M$. Reactions were run at 28°C. In the heated samples the enzyme at $4.2 \times 10^{-7}M$ was heated in a water bath at 40°C for 3 minutes, then diluted for the assay in the presence or absence of citrate. The assay in the absence of citrate gave the same curve as the unheated enzyme.

Another metabolic consequence of the LDH 5 behavior here reported would be a marked advantage of anaerobic glycolysis over aerobic respiration, since any activation of LDH would result in less pyruvate being available for conversion to acetylcoenzyme A and more NAD being available to spark glycolysis through the reaction catalyzed by triose phosphate dehydrogenase. Thus, when the citric acid cycle substrates act to reduce the concentration of a substance which is one of their precursors, they are in effect controlling their own concentrations, a simple case of feedback control by an "end product" of a biosynthetic pathway.

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