

# Lactic/Malic Dehydrogenase Quotients During Transformation of Fibroblasts into Cartilage and Bone<sup>1</sup> (35527)

A. H. REDDI AND CHARLES HUGGINS

*The Ben May Laboratory for Cancer Research, The University of Chicago, Chicago, Illinois 60637*

Two of the pyridine nucleotide-linked dehydrogenases in rat mammary glands exceed other dehydrogenases in this tissue in their activity. Moreover, the concentrations of these enzymes, lactic dehydrogenase (LDH) and malic dehydrogenase (MDH), form characteristic patterns (1), respectively, in normal hyperplastic gestational mammary glands and in mammary cancer. MDH has the greater activity in the normal mammary hyperplasias of pregnancy and lactation; whereas, LDH occupies the first rank in mammary cancer. We find it useful to relate the activity of LDH of tissues in terms of MDH measured concurrently. In the present work, LDH/MDH quotients were determined for tissues of normal rats and also during the transformation of fibroblasts brought about by transplants of the acid-insoluble residue of bone (2) which initiate a chain reaction (3).

*Material and Methods.* Male and female rats of Long and Evans strain were studied exclusively. The animals have been bred *inter se* for 12 years in our closed colony.

*Enzyme assays and units.* The rats were decapitated; the tissues were excised rapidly, cleansed, weighed, and homogenized in an ice-cold solution of 0.15 *N* NaCl containing 0.003 *M* NaHCO<sub>3</sub>; the enzyme solutions were kept cold thereafter until assayed. The homogenate was centrifuged at 11,000*g* for 15 min and the supernatant was removed for enzyme assay. The initial (1 min) velocity of oxidation of reduced nicotinamide-adenine dinucleotide (NADH) was measured at 340

*mμ* in a Beckman Model DU spectrophotometer at 25°.

The concentrations of LDH and MDH in the supernatant solutions were measured concurrently. The substrate for LDH was 0.003 *M* sodium pyruvate dissolved in 0.0833 *M* Tris buffer, pH 7.4, containing 0.0001 *M* NADH. The substrate for malic dehydrogenase was 0.001 *M* sodium oxalacetate dissolved in 0.0833 *M* Tris buffer, pH 7.4, containing 0.0001 *M* NADH. One unit of LDH or MDH is defined as the enzyme activity which oxidized 1 *μ*mole of NADH in 1 min (initial velocity) under the stated conditions. The content of alkaline phosphomonoesterase in grafts was determined; 1 unit was defined as the enzyme activity which liberated 1 *μ*mole of *p*-nitrophenol/0.5 hr under the stated conditions (4). The units are expressed in terms of 1 g of tissue (wet wt).

*Transplants of bone matrix.* We prepared a dry powder of acid-insoluble residue of rat bone which was extracted with water-saturated phenol followed by alcohol and ethyl ether; the powder was <200 *μ* in size. Allogeneic transplants (5) of the bone matrix, 10–20 mg, were made in prepared subcutaneous pockets of rats (age 25–30 days); there were 4–6 grafts in each recipient. The day of transplantation is denoted day 0.

At harvest, the grafts were excised, freed from adherent tissues, weighed, and prepared for enzyme assay as we have described for the normal tissues.

*Results. Reproducibility of methods.* Ten samples of liver from an adult male rat were assayed consecutively for LDH and MDH. The results were: LDH 346.2 ± 24 units; MDH 398.1 ± 24 units/g. The reproducibility of the method was within 5–7% of the mean values.

<sup>1</sup> Supported by grants from the American Cancer Society, The Jane Coffin Childs Memorial Fund for Medical Research, and the U.S. Public Health Service, National Institutes of Health (No. CA 11603).

TABLE I. Lactic and Malic Dehydrogenase Levels and Quotients in Tissues of Normal Rats.

Tissue	LDH (units/g)	MDH (units/g)	$Q \frac{\text{LDH}}{\text{MDH}}$
I Cartilage, articular	32.5 ± 18 <sup>a</sup>	19.0 ± 8	2.12 ± 0.52
II Bone marrow	44.6 ± 8.4	24.4 ± 5.0	1.80 ± 0.32
III Quadriceps muscle	443 ± 114	314 ± 70	1.44 ± 0.34
IV Tooth pulp	60.3 ± 7	57.5 ± 6	1.05 ± 0.14
V Cartilage, ensiform	44.0 ± 12	43.7 ± 7	1.03 ± 0.32
VI Liver	346.2 ± 24	398.1 ± 24	0.95 ± 0.19
VII Ileum	119 ± 24	145 ± 20	0.82 ± 0.12
VIII Fascia	14.6 ± 3	18.9 ± 5	0.77 ± 0.14
IX Lung	77.3 ± 19	128 ± 39	0.60 ± 0.21
X Bone	0.7 ± 0.6	1.4 ± 0.8	0.50 ± 0.30
XI Testis	40.6 ± 4	90.3 ± 23	0.50 ± 0.09
XII Myocardium	277 ± 59	661 ± 97	0.42 ± 0.05
XIII Diaphragm	175 ± 32	413 ± 58	0.42 ± 0.03
XIV Cerebrum	55.8 ± 20	158 ± 53	0.40 ± 0.14
XV Kidney cortex	131 ± 35	424 ± 71	0.31 ± 0.08
XVI Dentin	0.3 ± 0.1	1.5 ± 0.8	0.25 ± 0.09

<sup>a</sup> Mean ± standard deviation of the mean.

*Enzymes in tissues of normal rat.* Levels of LDH and MDH (Table I) were determined on the cytosol of 16 tissues of 8 normal rats. The highest level of LDH was found in skeletal muscle (III) followed by liver (VI). The highest level of MDH was found in myocardium (XII) followed by kidney cortex (XV) and liver (VI). The levels of LDH and MDH were very low in cortex of femur (X) and in dentin (XVI). Fascia (VIII) was obtained from lower back and lower leg.

The  $Q \text{ LDH/MDH}$  was greater than 1 in 5 tissues (I–V); the biggest quotient was found in articular cartilage (I).  $Q \text{ LDH/MDH}$  was less than 1 in 11 tissues (VI–XVI). The quotient of ensiform cartilage (V) was lower than that of the cartilage (I) of the hip joint.

*Enzymes in grafts of bone matrix.* Allogeneic transplants of powdered demineralised bone to the subcutaneous tissue of rats induced large amounts of cartilage, which later disappeared to be superseded by bone (2, 3). Cartilage was present in large amounts from days 7–11; and  $Q \text{ LDH/MDH}$  exceeded 1 in this period (Fig. 1). Soon after day 11 cartilage largely vanished from the grafts whereas large quantities of bone appeared:  $Q \text{ LDH/MDH}$  was less than 1 for day 12–29 (Fig. 1). Alkaline phosphatase content

reached its highest level in the grafts on day 12 (Fig. 1).

Throughout the period of observation of the grafts, days 7–28, MDH remained at a rather steady level; the change in  $Q \text{ LDH/MDH}$  from  $>1$  to  $<1$  was due to the decline of LDH (Fig. 2).

*Discussion.* It is useful to relate the concentration of LDH in tissues in terms of MDH when both enzymes are assayed concurrently on the same solution of tissue con-

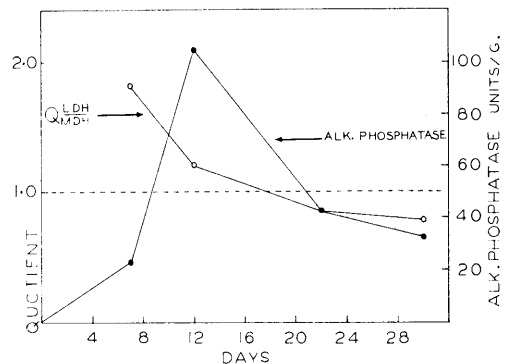


FIG. 1. Level of alkaline phosphomonoesterase and  $Q \text{ LDH/MDH}$  in subcutaneous transplants of demineralized bone powder. One days 7–11, large quantities of cartilage were present; on days 12–28 large amounts of bone were observed.

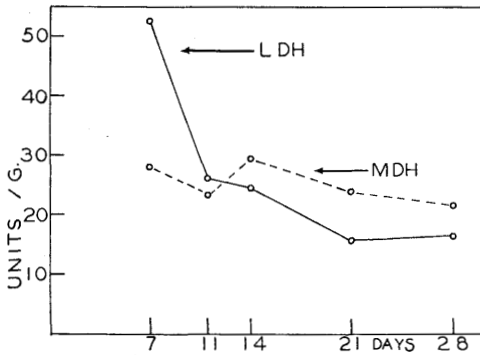


FIG. 2. Levels of LDH and MDH in subcutaneous transplants of demineralized bone powder: Cartilage was present in large amounts on days 7-11; bone appeared on day 11 and large quantities were found on days 14-28.

stituents. The methods are rapid, simple and reproducible. Meister (6) found that the levels of LDH in malignant tumors of rodents are frequently higher than those in the tissues of origin. With respect to neoplasms of the rat we obtained the following quotients: induced mammary cancer (1), 1.54; mammary fibroadenoma,  $0.52 \pm 0.05$ .

But  $Q$  LDH/MDH  $>1$  is not an exclusive attribute of malignant tumors. A quotient  $>1$  was found in five normal tissues of the rat. It was significant that the quotient, LDH/MDH, in cartilage was  $>1$ ; whereas, in bone it was  $<1$ .

Two of the major events in the chain reac-

tion (3) of transformation of fibroblasts of adult rats by acid-insoluble residues of bone are the consecutive formation of cartilage followed by bone. In the present experiments it was found that these consecutive events were characterized by profound differences in the grafts characterized by a decrease of the quotient, lactic/malic dehydrogenases. It is interesting to note that the  $Q$  LDH/MDH values during the successive appearance of cartilage and bone correlated well with the values for, respectively, articular cartilage and bone in normal rats (Table I).

*Summary.* The concurrent assay of lactic and malic dehydrogenase in the cytosol of homogenized tissues is a simple and useful device in studying the biochemistry of transformation of fibroblasts from fascia into cartilage, followed by bone. The quotient, LDH/MDH, in cartilage exceeds 1 whereas in fascia and bone it is less than 1.

1. Rees, E. D., and Huggins, C., *Cancer Res.* **20**, 963 (1960).
2. Urist, M. R., *Science* **150**, 893 (1965).
3. Huggins, C., and Urist, M. R., *Science* **167**, 896 (1970).
4. Huggins, C., and Morii, S., *J. Exp. Med.* **114**, 741 (1961).
5. Huggins, C., Wiseman, S., and Reddi, A. H., *J. Exp. Med.* **132**, 1250 (1970).
6. Meister, A., *J. Nat. Cancer Inst.* **10**, 1263 (1950).

Received Dec. 6, 1970. P.S.E.B.M., 1971, Vol. 137.