ISOZYMES OF LACTIC DEHYDROGENASE IN HUMAN TISSUES*

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The heterogeneity of lactic dehydrogenase (LDH) activity in human serum and erythrocytes has been observed in several laboratories (1–7). If each of the electrophoretically-distinguishable enzymes in serum with LDH activity were derived from different tissues, in disease states involving a single organ the normal pattern of serum LDH activity would be altered according to the type of LDH released from the affected organ. This hypothesis was supported by analysis of sera from patients with myocardial infarction and leukemia (1–7). The results suggested that several tissues had characteristic electrophoretic distributions of lactic dehydrogenase activity. It was concluded that the patterns of serum LDH activity obtained by electrophoresis furnished more information regarding the site of pathology than did an examination of the LDH activity of whole serum (1–3). Furthermore, when several tissues were affected in a generalized disease process, as in hemorrhagic or endotoxic shock, it was found that all of the peaks of LDH activity in serum were elevated (8, 9), in contrast to the selective elevation of certain peaks in conditions involving individual tissues. The term isozyme was proposed by Markert and Møller to refer to the electrophoretically-distinguishable enzymes with similar substrate specificities (6). The present study demonstrates the heterogeneity of LDH activity in several human tissues and correlates the electrophoretic patterns of LDH activity obtained in tissues with those of sera in certain disease states.

MATERIALS AND METHODS

Four g of heart, of kidney, of liver and of skeletal muscle were obtained within 12 hours after the death of 8 patients from the following conditions: carcinoma of the cervix, bladder, breast, or kidney, brain tumor, diabetic gangrene of right foot, rheumatic heart disease and mitral stenosis, and rheumatic heart disease and aortic stenosis. No metastases to the liver, heart or kidney occurred in the cancer group. The tissues were washed thoroughly in cold normal saline to remove the majority of red cells and were rinsed in cold water to lyse any remaining erythrocytes. Homogenates were prepared in 10 ml of barbital buffer, pH 8.6, ionic strength 0.1, using a mortar and pestle and then submitting the tissues to further grinding in a Potter-Elvehjem motor-driven tissue homogenizer. Centrifugation of these extracts for 40 minutes at 15,000 G produced a supernatant fluid which was decanted and diluted with an equal volume of barbital buffer, pH 8.6. Four ml of the diluted supernatant was separated electrophoretically on a starch supporting medium in barbital buffer, pH 8.6, with an ionic strength of 0.1. After electrophoresis at 4° C for 36 hours at 400 v and 120 ma, each of the sections of the starch block was cut into 0.5 inch segments, the protein eluted with 5.0 ml barbital buffer and its concentration determined (10). An aliquot from each eluate was then assayed spectrophotometrically for LDH activity (11). Between 85 and 98 per cent of the LDH activity of the homogenate was recovered from the block following electrophoresis. Each eluate was incubated for 20 minutes at room temperature with 0.2 ml of 0.003 M diphosphopyridine nucleotide reduced (DPNH) and 2.5 ml of barbital buffer, pH 8.6, ionic strength 0.1. The mixture was transferred to a Beckman cuvet of 1 cm path length; 0.1 ml of 0.001 M sodium pyruvate was added, and the decrease in absorption at 340 mμ was measured in the Beckman DU spectrophotometer. Readings were obtained every 30 seconds for 3 minutes. One unit of dehydrogenase activity was defined as a decrease in the optical density at 340 mμ of 0.001 per minute.

In several experiments, a hemolysate prepared from human red cells (12) was fractionated electrophoretically and the eluates were assayed for LDH activity.

Human white cells were obtained from 40 ml of heparinized blood by the method described by Hirsch and Church (13). The LDH activity was extracted from this purified preparation of leukocytes by freezing in acetone and dry ice, followed by rapid thawing. The white blood cell extract was separated electrophoretically and the eluates were assayed for LDH activity.

Serum (5 ml each) from a patient with hepatitis and from a healthy control were separated simultaneously on a starch block.

The pH optimum for each peak of LDH activity was determined. An aliquot from the tube containing the

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maximal LDH activity in each peak was removed and added to barbital buffers, varying in pH from 7.4 to 9.2, all at 0.1 ionic strength. LDH activity was then reassayed as described above.

The Michaelis-Menten constants (Km) of lactic dehydrogenase were determined for each of the 5 peaks of LDH activity. One-thousandfold variations in the final concentration of sodium lactate (from 1.6×10^{-3} moles to 1.6 moles, pH 7.0, ionic strength 0.1) were employed. Diphosphopyridine nucleotide (DPN) was used (0.3 ml of a 0.05 M solution adjusted to pH 7.5) (14). Barbital buffer, pH 8.6, ionic strength 0.1, was added to an aliquot from the tubes with peak LDH activities. The final pH was 8.6 in a constant volume of 3.0 ml. The mixture was assayed for LDH activity and the Km calculated according to the method of Lineweaver and Burk (15).

RESULTS

Five electrophoretically-distinguishable peaks of LDH activity were observed in the homogenates of human tissues. According to the convention of earlier publications (2–4) enumeration will be made from the slower to the more rapidly migrating peaks. Figure 1 relates the mobility of these activity peaks to the electrophoretic pattern of serum proteins. Peaks 1 and 2 migrated with the mobility of γ-globulin, peak 3 with the mobility of β-globulin, peak 4 with the mobility of α_2-globulin and peak 5 with a mobility between α_1-globulin and albumin. Serum from a patient with hepatitis exhibited all five activity peaks; normally, peak 1 was present only in trace amounts and peak 2 contained less of the total activity, as shown by the control serum in Figure 1. Not all five activity peaks appeared in each of the tissues. Liver exhibited five peaks, whereas kidney, red cells and white cells revealed four, and heart and skeletal muscle had three peaks. The percentage of the total LDH activity found in each peak was relatively constant, as indicated by the values in Table I. Furthermore,

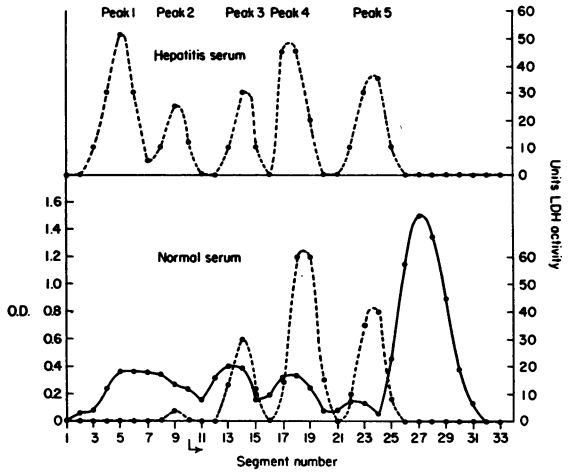

FIG. 1. DISTRIBUTION OF LACTIC DEHYDROGENASE ACTIVITY IN NORMAL HUMAN SERUM AND IN SERUM FROM A PATIENT WITH HEPATITIS. The lower curves show the electrophoretic pattern of normal human serum in the solid line and the distribution of lactic dehydrogenase activity of the same serum in broken lines. The upper curve shows the LDH activity in the serum from a patient with hepatitis separated on the same starch block. Note the appearance of peak 1 in this serum and the increased activity in peak 2, as compared with the normal serum below.

each tissue had a characteristic distribution of LDH activity. Most of the LDH activity in heart appeared in peak 5 with a smaller component in peak 4, whereas in liver the majority of the activity was found in peaks 1 to 3, and in skeletal muscle the largest portion of LDH activity was in peak 1. In kidney homogenate most of the LDH activity was divided between peaks 4 and 5, and in leukocyte extract peak 4 contained the majority of the LDH activity (Table I). The mobility of each of the activity peaks in one tissue resembled the mobility of the corresponding peaks in other tissues, as shown in Figure 2. Figure 3 also illustrates the similarity in mobility of activity

TABLE I

Lactic dehydrogenase activity in fractions of human tissues with standard deviations

Tissue	No. of cases	Per cent total activity				
		Peak 1	Peak 2	Peak 3	Peak 4	Peak 5
Heart	8			3 ± 2.5	24 ± 4.7	73 ± 5.6
Kidney	8		2 ± 2.9	11 ± 8.1	45 ± 5.2	42 ± 6.9
Liver	8	37 ± 9.6	24 ± 11.9	27 ± 7.2	8 ± 8.9	4 ± 6.6
Skeletal muscle	4	78 ± 8.95	17 ± 8.6	5 ± 4.2		
Hemolysate	5		1 ± 1.8	12 ± 2.9	44 ± 4.3	43 ± 2.2
White blood cells	5		6 ± 3.6	33 ± 6.1	49 ± 5.1	12 ± 3.6

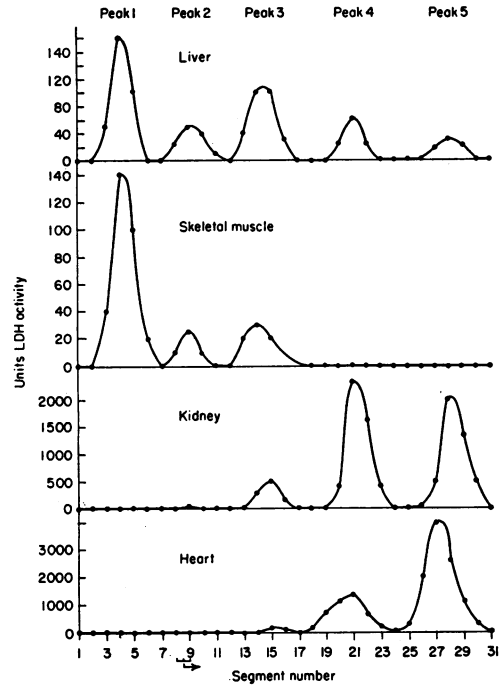

FIG. 2. DISTRIBUTION OF LACTIC DEHYDROGENASE ACTIVITY IN HOMOGENATES OF HUMAN HEART, LIVER, KIDNEY AND SKELETAL MUSCLE. These tissue homogenates were separated electrophoretically on the same starch block and are derived post mortem from a patient with carcinoma of the bladder.

peaks in one tissue to corresponding peaks in another tissue.

The pH optima of the 5 peaks of LDH activity were compared. Table II shows the close resemblance of these pH optima, which ranged from 7.8 to 8.5. The differences in pH optima among the five peaks of LDH activity were not statistically significant.

TABLE II

pH optima of five peaks of LDH activity

	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5
Heart			8.0	8.1	8.2
Hemolysate			8.0	8.2	8.5
Kidney			7.8	8.1	8.3
Liver	8.3	8.3			
Skeletal muscle	8.2	8.2			

TABLE III

Michaelis Menten constants (lactate) $\times 10^{-5}$ moles per L for 5 peaks of LDH activity

	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5
Heart			1.9	1.5	1.2
Kidney			1.0	2.0	1.0
Liver	4.4	4.8			
Skeletal muscle	5.6	4.2			

The Michaelis-Menten constants (Km) were calculated for the five activity peaks. The values obtained (Table III) revealed no significant differences among peaks 3, 4 and 5. The Km of peaks 1 and 2 (4.2 to 5.6 $\times 10^{-5}$ moles) were

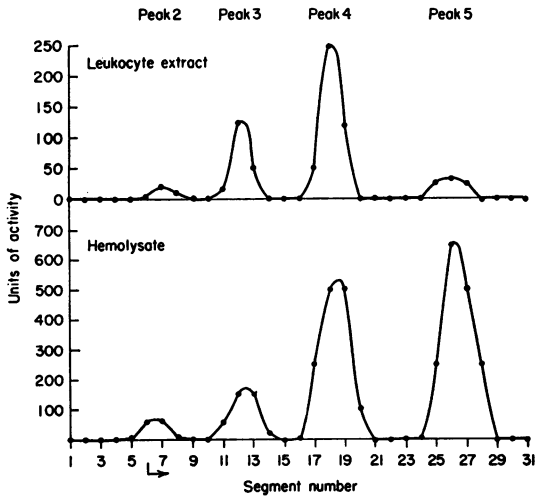

FIG. 3. DISTRIBUTION OF LACTIC DEHYDROGENASE ACTIVITY IN NORMAL HUMAN HEMOLYSATE AND LEUKOCYTE EXTRACT.

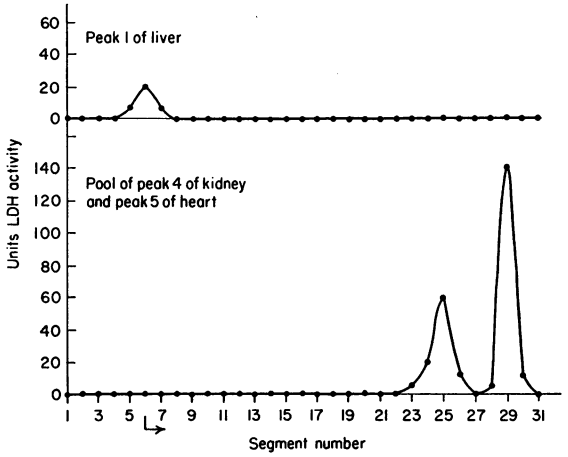

FIG. 4. THE PATTERNS OBTAINED WHEN PEAKS ISOLATED FROM A STARCH BLOCK WERE RERUN ON A SECOND BLOCK. The upper curve represents peak 1 of liver homogenate rerun on a second block. The lower curve represents a rerun of a pool of peak 4 from kidney homogenate and of peak 5 from heart homogenate on a second block. Note that when these peaks are rerun they do not alter in mobility or give rise to peaks not present in the initial run.

greater than the Km of peaks 3, 4 and 5 (1.0 to 2.0×10^{-5} moles).

Peak 5 from heart homogenate was pooled with peak 4 from kidney homogenate; the mixture was rerun on a second starch block under similar conditions, and the peaks had the same mobilities as in the starting material (Figure 4). Peak 1 from liver homogenate was rerun under identical conditions and no alterations in mobility from the starting material were observed (Figure 4).

DISCUSSION

Each of the homogenates of human tissue examined electrophoretically exhibited heterogeneity of LDH activity. Furthermore, each tissue had a constant number of activity peaks and a characteristic distribution of LDH activity in these peaks. The major portion of the LDH activity in heart homogenate was in peak 5 with a minor component in peak 4. Liver homogenate had the highest activity in peaks 1, 2 and 3, whereas most of the LDH activity of leukocytes resided in peak 4 with minor components in peaks 3 and 5. From analysis of alterations in the peaks of serum LDH activity in disease states, this type of distribution in tissues had been anticipated (1), from the hypothesis of enzyme release from damaged organs. In the sera of patients with myocardial infarction, a selective elevation of the LDH peaks with mobilities of α_1- and α_2-globulins was observed (1–3), and the present study reveals that these are the locations of LDH activity in heart homogenate. The report of an elevated peak of LDH activity in the α_2-globulin in certain leukemic sera (1) correlates with the observation that the majority of LDH activity in leukocytes is contained in this activity peak. The elevation of the LDH activity peak in the β-globulin and the appearance of a large activity peak in the γ-globulin in plasma from a patient with hepatitis are in harmony with the observation that the majority of LDH activity in liver homogenate resides in peaks with similar mobilities. Analagous to the appearance of a large LDH activity peak in serum during hepatitis is the appearance of additional peaks of leucine aminopeptidase activity in serum during liver disease (16). Both observations provide further support for the hypothesis that elevations in serum enzyme activity during several disease states are attributable in part to the liberation of enzymes from damaged organs.

Data of Hess (5) and Hill (17) are in accord with the reports of alterations in activity peaks of serum in cases of myocardial infarction and leukemia. However, Hill, employing curtain electrophoresis and a different method of obtaining leukocytes (17), localized the majority of LDH activity in leukocytes and erythrocytes in peak 3, whereas our data indicate that the majority of leukocyte LDH activity is contained in peak 4 and that peaks 4 and 5 contain most of the erythrocyte LDH activity. Wieme, utilizing agar gel electrophoresis, revealed 5 peaks of LDH activity in human serum (7). Peaks 1 and 2 were not constantly present and combined represented approximately 10 per cent of the total activity, the majority of the activity being distributed among peaks 3, 4 and 5. Wieme found that in patients with hepatitis, peaks 1 and 2 contained most of the serum LDH activity (7). This observation is confirmed in the present paper.

There are many examples of different molecular forms of enzymes in one organism and even in one tissue. Wieland and Pfleiderer observed several electrophoretically-distinguishable enzymes with LDH activity in several organs of the rat (18). The heterogeneity of pepsin (19), chymotrypsin (20), cytochrome C (21), ribonuclease (22), lysozyme (23), enolase (24), alkaline phosphatase (25), leucine aminopeptidase (16), ceruloplasmin (26), and cholinesterase (27) has been demonstrated. Emphasizing the similarities in substrate specificities of several enzymes previously shown to be heterogeneous, Markert and Møller proposed the useful term *isozyme* (6). They clearly distinguished isozymes, which exhibit similar substrate specificities and which usually resemble one another in several additional physicochemical characteristics, from different enzymes with broad substrate specificities which may overlap. Since comparison of the physicochemical factors in a group of electrophoretically-distinguishable enzymes which react with a common substrate is often difficult, caution must be exercised to avoid calling different enzymes with overlapping substrate specificities isozymes.

It appears unlikely that the differences in electrophoretic mobility of the LDH activity peaks are attributable to aggregation. Patterns similar

to those obtained on a starch block, which separates proteins primarily by charge, are obtained on a starch gel (7), which separates protein according to molecular weight and shape as well as according to charge. Further evidence against aggregation is the monodispersion of whole serum LDH activity in the ultracentrifuge (28).

Studies of pH optima and Km are usually sufficient to distinguish between the active centers of different enzymes. The differences in Km of peaks 1 and 2 from peaks 3, 4 and 5 suggest that the active centers of peaks 1 and 2 differ from those of peaks 3, 4 and 5. It is anticipated that further differences between each of the 5 isozymes will be established in the future. Previous studies showed that the inhibition behavior of peaks 3, 4 and 5 of hemolysate was identical (2). The possibility arises that the active centers engaged in catalyzing the pyruvate-lactate reaction in the five LDH isozymes are similar. The variations in electrophoretic mobility of the five LDH isozymes may be explained by postulating structural differences in the enzyme other than at the active center. These differences might determine the location of an enzyme within the cell (29) and could function in binding the enzyme to a particular organelle within a specialized cell. It is conceivable that one gene controls the catalytic site and that other genes or the chemical environment of the cell determine the attachment of the enzyme (29). This hypothesis may be applicable to the species differences in insulin, ACTH and ribonuclease (30). Recent studies of polymorphism in pseudocholinesterase reveal that the mutant enzyme differs from the normal type in pH optima and in affinity for different substrates, and that the mutant enzyme fails to react with acetylcholine (31). Here, the gene probably determines the polypeptide chain of the catalytic active center of the enzyme.

During the preparation of this manuscript, two extensive studies were published on fractionation of LDH activity in mammalian tissues employing starch gel electrophoresis (32, 33). Although the gel technique yielded results similar to those of the present study, detailed quantitative comparison of the results is rendered difficult by trailing of LDH activity on the starch gel in some of the experiments (32).

SUMMARY

1. Heterogeneity of lactic dehydrogenase (LDH) activity in the electrophoretically separated homogenates of human heart, kidney, liver, skeletal muscle, and extracts of leukocytes and erythrocytes is described. Five peaks of LDH activity were found in liver; four in kidney, leukocytes and erythrocytes, and three in heart and skeletal muscle. Each tissue exhibited a characteristic distribution of LDH activity: liver and skeletal muscle had the greatest activity in peaks 1, 2 and 3; whereas heart, hemolysate and kidney had most activity in peaks 4 and 5. Leukocytes and serum showed highest activity in peak 3.

2. The electrophoretic patterns of LDH activity in human tissues correlated with alterations in serum activity in disease states. In the serum from a case of hepatitis, a large peak of LDH activity is described which is present only in trace amounts in normal serum. This activity peak had the same mobility as the peak in liver with greatest LDH activity.

3. These observations suggest that assay of the LDH activity in the fractions of electrophoretically separated serum permits a greater specificity in localizing pathology than does assay of whole serum LDH activity.

4. Studies of Michaelis-Menten constants of the five peaks of LDH activity show peaks 1 and 2 to differ from peaks 3, 4 and 5. The possibility that the active centers of peaks 1 and 2 are different from the active centers of peaks 3, 4 and 5 is discussed.

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