Electrophoretic Fractionation of Serum
Lactic Dehydrogenase *

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SUMMARY

A reproducible method for the electrophoretic separation of human serum into fractions with lactic dehydrogenase (LDH) activity has been described. Fifty-eight sera from apparently healthy and diseased individuals were fractionated.

Continuous-flow paper electrophoresis has been used to separate human blood serum and extracts of the formed elements of blood into several fractions exhibiting lactic dehydrogenase (LDH) activity (1, 2, 4). Fractionation patterns obtained by this procedure have been shown to differ from normal in certain diseases. The pattern differences were not attributable to the appearance of new fractions but to changes in the distribution of the activity among the separable fractions.

The present study is a continuation of the investigation of electrophoretic fractionation and the interrelationship of fractions.

MATERIALS AND METHODS

Serum was fractionated at room temperature in the Spinco Model CP continuous-flow paper electrophoresis unit in the presence of 0.02 ionic strength barbital buffer, pH 8.6. A current of 30 milliamperes at 450 volts was employed. The speed of sample delivery was regulated so that 1.5 to 2.0 ml. of serum required about 18 hours for fractionation. Fractions were collected from the time of the first appearance of protein at the drip points until about 6 hours after the serum sample had been completely introduced into the system. All tubes were assayed for LDH activity, and the total activity for each tube was determined. The above method was reproducible, and about 98 per cent of the LDH activity introduced was recovered.

Under these conditions serum from an apparently healthy individual gave four fractions with LDH activity (1, 2, 4). These fractions, designated I, II, III, and IV, were associated with the following protein fractions: I, γ-globulins; II, β-globulins; III, α1-globulins; and IV, α2-globulin-albumin. In earlier fractionation studies (1, 4) diluted serum was dialyzed against barbital buffer before fractionation. This procedure was found to give variable results for fractions I and II dependent on length of time of dialysis or of standing after dilution, even at 4° C. Several samples diluted 18 hours before fractionation lost all fraction I activity and as much as one-fourth of the activity of fraction II. Losses sustained in these labile components were minimized by fractionation of the serum undiluted, or immediately after dilution. All results in this investigation were obtained by analysis of 1.5 to 2 ml. of fresh, undiluted, undialyzed serum.

Lactic dehydrogenase activity was determined by a modification of the spectrophotometric method previously described by Hill and Levi (3). In the modified method 0.1 to 0.3-ml. samples were assayed from each tube dependent on the activity of the original serum. The reaction was initiated by the addition of an assay mixture containing 0.1 ml. of 0.025 M sodium pyruvate (in water), 2.5 ml. of 0.1 M phosphate buffer (pH 7.8), and 0.4 ml. of dihydrodiphosphopyridine nucleotide (DPNH) (1 mg/ml water). The reaction mixture was incubated at 37° C. for 30 minutes and the activity determined as previously described (3). LDH activities are expressed as μg DPNH oxidized/min/ml of original serum. The volume of solution in each tube was noted and the total activity per tube calculated.

RESULTS

Chart 1 shows the electrophoretic profile of serum from an apparently healthy individual. The
percentage values represent the per cent of the total activity found in each fraction. The total activity represents the summation of the activity of all tubes.

Serum patterns were obtained by determining the relative proportions of the total activity found in individual fractions. Chart 2 shows three types of patterns which have been obtained. A noticeable difference between the patterns is seen in the ratio between fractions IV and III and has been designated the IV/III ratio. The ratio of fractions I and II differs, especially in pattern 3, but this ratio is not always reproducible because of the lower reliability of the assay of fraction I, which usually has very low activity as compared with the other fractions. In the fractionation of low-activity serum this can be especially significant. Pattern 1 has a IV/III ratio of approximately 1.0; pattern 2, less than 1.0; and pattern 3, greater than 1.0.

Pattern 1 is from the electrophoretic profile shown in Chart 1 and was obtained from the serum of an apparently healthy individual whose serum LDH activity was in the normal range. The percentage activity of fractions IV and III is approximately the same, and the IV/III ratio is 1.01.

Pattern 2 is from the serum of an untreated patient with chronic granulocytic leukemia whose total serum LDH activity was approximately 9 times normal. The increase of serum LDH activity in leukemic patients is not accompanied by the appearance of any new fractions or increase in a single fraction but rather is reflected in a rise of all four fractions. The IV/III ratio was much lower than the ratio from a normal pattern. When a leukemic patient showed improvement with treatment, the serum LDH decreased and the IV/III ratio increased. The change in ratio is chiefly attributable to an increase in fraction IV.

Pattern 3 is from a patient with cancer of the ovary and widespread metastases whose serum LDH was about 5 times normal. This type of pattern was found for the sera of six patients, and the ratio varied from 1.15 to 2.00. Two patients had lymphosarcoma, two sarcoma, and two metastatic cancer, one of the testes, the other of the ovary. All were in an advanced state of their disease. In all cases the serum LDH activity was elevated, with the increase predominantly in fractions III and IV. Fraction I was absent or extremely low in activity, and fraction II was similar in activity to II of serum from a healthy individual. A water extract of homogenized human heart muscle (obtained at autopsy) had this type of pattern (IV/III ratio of 3.8) with about 75 per cent of the total activity in fraction IV. This resembles the pattern of serum from patients with myocardial infarction as reported by Vesell and Bearn (5).

The results of a number of fractionations of sera from apparently healthy and leukemic individuals are shown in Chart 3. Several of the sera were run in duplicate or triplicate with results reproducible to 0.03 for the IV/III ratio. The healthy individuals had ratios very close to 1.0, and there was very little variation. There was a wide variation in the leukemic ratios. All the untreated leukemic patients had values less than 0.45. Some of the treated leukemias had values below this ratio, but these patients had advanced disease or were not responding favorably.
to therapy. All patients in the treated group had elevated serum LDH activity. In the group in remission five patients were under treatment and had normal serum LDH activity. One of these with a ratio of 0.72 had never shown a total serum LDH above the normal range. The patient with a ratio comparable to that of a healthy individual was diagnosed as a chronic lymphocytic leukemic, was under treatment, and had a total serum LDH in the normal range. No correlation has been noted as yet between leukocyte count and electrophoretic pattern, nor has any difference been noted in granulocytic and lymphocytic leukemias.

Chart 4 shows that leukemias are not the only neoplastic diseases that give a low IV/III ratio. Five sera from patients with metastatic cancer of the breast gave ratios varying from 0.30 to 0.62. Six out of seven patients with Hodgkin's disease gave low ratios. The other patient had a normal child with aplastic anemia and the other an adult with pulmonary emphysema. The sera from two patients with multiple myeloma gave five fractions, the extra fraction close to fraction I and associated with the γ-globulins. The IV/III ratios were similar to those found for treated leukemias.

DISCUSSION

Electrophoretic fractionation of serum has provided a tool for a more critical examination of serum LDH than is obtained with a single assay of total activity. Although two sera may have similarly elevated serum LDH activity, their fractionation patterns may differ considerably. Several leukemic patients in remission with serum LDH activity in the normal range gave patterns similar to the leukemic pattern rather than to the pattern for healthy individuals. The similarity of patterns for myocardial infarction and human

![Diagram]

**Chart 3.**—IV/III ratios of sera from apparently healthy and leukemic individuals. The healthy individuals had LDH values in the range of 190 to 210 μg DPNH oxidized per min. per ml. serum. The LDH range for untreated leukemic patients was 500 to 4500, for treated leukemic patients 450 to 1500, and for patients in remission 140 to 210. See text.

![Diagram]

**Chart 4.**—IV/III ratios of sera from apparently healthy individuals and patients with metastatic cancer of the breast and Hodgkin's disease. The ratios for healthy individuals are those shown in Chart 3. The LDH range for patients with metastatic cancer of the breast was 230 to 4600, and for Hodgkin's disease 180 to 460. See text.
heart muscle extract and the patterns of leukemia and platelets (and other formed elements of blood) (1) suggest that electrophoretic fractionation procedures may be helpful in the investigation of the source of LDH in cases in which it is elevated in the serum. Wieme (6) described an electrophoretic separation on gel for the study of enzyme fractions in biological fluids and found that several diseases gave different LDH patterns. The two most active fractions in the serum pattern for patients with myocardial infarction were also the predominant fractions of cardiac muscle fluid; the two predominant fractions found in serum patterns from patients with hepatitis also were the main fractions of liver fluid.

REFERENCES
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