procedure described earlier(1), it was found in replicate experiments that as much as 200 μg per ml of CS alone had no inhibitory action on the growth of strain B103; INH alone inhibited in a dose of 5 to 10 μg per ml; and with the mixture, concentrations in the proportion of 100 μg CS and 10 μg INH per ml gave an inhibition endpoint the same as with INH alone. Similar experiments with 24-day growth of 3 other strains, 198ARB, H37Rv, and BCG, although demonstrating variations in response to CS alone (inhibition with 200, 20, 10 μg, respectively) were similar in their inhibition results with INH (5 to 10 μg) and with the mixture (10 μg INH). One may conclude from these *in vitro* tests that at least the presence of CS under the conditions used did not interfere with the inhibitory action of INH.

Summary. The acute toxicity of isoniazid (INH) in DBA mice by gavage, $LD_{50} = 5$ mg/20 g mouse, may be reduced by simultaneous administration of the antibiotic cycloserine (CS). Each of 22 mice survived a single dose of 8 mg INH with 75 mg CS (0.4 g and 3.75 g/kg respectively), or of 10 mg INH with 100 mg CS (0.5 g and 5.0 g/kg respectively). On repeated semi-weekly administration for 10 weeks, 19 of 26 mice, 73%, survived the latter mixture. Significant amounts of INH were detected in the blood plasma 72 hours after the 20th dose. In *in vitro* bacteriostatic tests with 4 strains of *Mycobacterium tuberculosis*, CS did not interfere with the activity of the INH.

Since submission of this manuscript for publication, a statement in a technical bulletin indicated that in clinical use "toxicity (of CS) is decreased and its efficacy increased" when administered with INH. (*Phys. Bull.*, 1956, v21, 227).

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Received October 22, 1956. P.S.E.B.M., 1957, v94.

Localization of Lactic Acid Dehydrogenase Activity in Serum Fractions. (22866)

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The enzyme lactic acid dehydrogenase (LDH) is known to catalyze the conversion of pyruvic acid to lactic acid in the presence of reduced diphosphopyridine nucleotide (DPNH)(1,2). More recently Vallee has shown that LDH is a zinc protein(3). Increased interest in the level of LDH in various disease states has followed reports that in patients with myocardial infarction and leukemia the serum level of LDH is increased significantly(4,5,6,7).

The main purpose of this paper is to describe 3 separate peaks of LDH activity in the electrophoretically separated fractions of the serum proteins.

Methods and materials. Twelve subjects, 3 with leukemia, 2 with myocardial infarction, 2 normal individuals and 5 patients with such various diseases as cirrhosis of liver, hemochromatosis and Wilson's disease, were employed. Three to 5 ml of fresh, non-hemolyzed serum were separated by zone electrophoresis using a starch or polyvinyl supporting medium in barbital buffer pH 8.6 ($\pi/2$

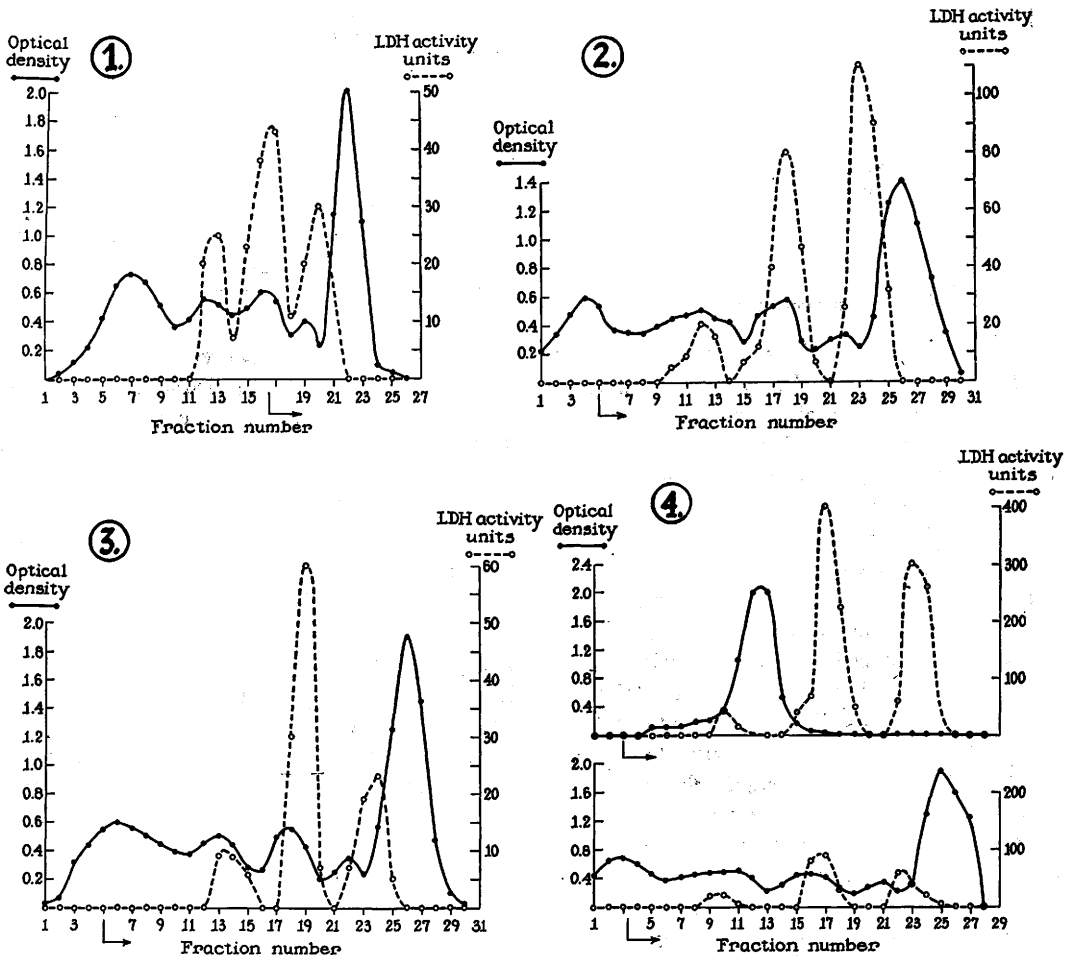

FIG. 1. Curves showing distribution of lactic acid dehydrogenase in normal human serum.

FIG. 2. Distribution of lactic acid dehydrogenase in serum of patient with myocardial infarction.

FIG. 3. Distribution of lactic acid dehydrogenase in patient suffering from myelogenous leukemia.

FIG. 4. Upper curve represents distribution of lactic acid dehydrogenase in hemolysate from human red cells. Lower curve represents the localization of lactic acid dehydrogenase of the serum from the same patient separated under identical conditions on the same starch block.

$= 0.1$). After electrophoresis the starch or polyvinyl block was cut into half-inch segments, protein was eluted and its concentration determined by modification of Folin-Cioculteau procedure(8). An aliquot of each eluate was then analyzed for LDH activity by the method of Wroblewski and LaDue(4) which depends on oxidation of the coenzyme DPNH to DPN according to the equation

$$\text{Pyruvate} + \text{DPNH} \underset{\text{Lactic acid dehydrogenase}}{\rightleftharpoons} \text{Lactate} + \text{DPN}$$

Sixty to 90% of the LDH activity of the original serum was recovered after electrophoresis and elution. To obtain maximum activity of the fractions complete recovery was not always attempted. However, if larger volumes of eluate were used, recoveries up to 90% could be obtained. A water flow experiment showed that all the LDH migrated with the protein band, none being differentially absorbed by starch. In several experiments rabbit serum and hemolysate from human red cells were separated electrophoretically and assayed for LDH activity.

Results. Each of the 12 sera separated

TABLE I. LDH Activity in Serum Fractions.

	No. of cases	Total activity units	% activity in serum fraction β	α_2	α_1	α_2/α_1
Controls	6	284 (193–370)	19 (12–23)	47 (43–55)	34 (31–37)	1.5 (1.3–1.6)
Myocardial infarction	2	626	10	38	52	0.7
		1892*	4	35	61	0.6
Leukemia	3	500	18	57	25	2.4
		180	13	57	30	1.9
		198	17	55	28	2.0

* Extensive infarction (fatal).

electrophoretically and assayed for LDH activity revealed 3 distinct loci of activity (Fig. 1). One peak of activity was found in the β-globulin; a second peak of activity was found in the α_2-globulin; and the third occurred between the α_1-globulin and the albumin, hereafter referred to as the α_1 peak. Addition of excess ionic zinc to the serum prior to electrophoresis did not alter the pattern of LDH activity obtained. Since it is known that red cells are particularly rich in the enzyme LDH special care was taken to avoid excessive hemolysis. Presence of trace hemolysis, however, was not found to increase the serum LDH activity significantly. Dialysis of the serum prior to electrophoretic separation likewise did not alter the results obtained.

In the 6 control subjects each peak of LDH contained a relatively constant percentage of the total LDH activity found in the serum. Nineteen % of total LDH activity was found in the β-globulin, 48% in the α_2-globulin and 33% in the α_1 peak. The variability of the percentages found in the 3 fractions on the control subjects is shown in Table I. The greatest variation appeared in the β peak. In control subjects the ratio between the LDH activity in the α_2 peak and the α_1 peak was approximately 1.5.

In sera obtained from 2 cases of myocardial infarction in which total LDH activity was raised the α_1 activity peak was increased becoming larger than the α_2 peak (Fig. 2), and thus the α_2/α_1 activity ratio decreased. Three cases of leukemia, 2 myelogenous and one lymphatic were studied. In the patient with lymphatic leukemia and the patient with myelogenous leukemia in whom the white count was not raised, no significant increase in serum LDH was observed. However, one patient with myelogenous leukemia, in whom the white count at time of examination was 33,000, showed an increased serum LDH activity. Despite the fact that only one of the 3 cases showed an absolute rise in LDH activity all showed a significant increase in the α_2 activity peak relative to the α_1 peak and thus the α_2/α_1 ratio became greater (Table I).

When a hemolysate from human red cells was separated electrophoretically 3 peaks of LDH activity were identified. These 3 activity peaks approximately corresponded in their electrophoretic mobility and their relative concentrations to those found in serum. From the point of view of the isolation and purification of LDH it is of interest that the LDH activity protein ratio was greatly increased in the hemolysate compared to that found in serum. In the red cell hemolysate both the α_1 and α_2 activity peaks occurred in regions where the protein concentration was extremely low (Fig. 4). Considerably greater LDH activity was found in the hemolysate than in the serum.

In contrast to human serum, rabbit serum separated electrophoretically revealed two principal peaks of LDH activity. One of these occurred in the α_2-globulin region while the second and larger peak appeared in the α_1-globulin region.

In view of the finding that LDH is a zinc enzyme a study of the localization of radioactive zinc in the various serum proteins was carried out. Radioactive zinc was added to human and rabbit serum *in vitro* and electrophoresis performed. In both species 2 peaks of radioactivity were observed. In man the larger peak occurred in the slow moving part of the albumin (in some experiments coin-

ciding with the albumin peak) and the smaller peak migrated in the a_2-globulin region, in a position similar to the a_2 peak of LDH. Details of these experiments will form the substance of a separate communication.

Discussion. Since previous investigators have concluded that measurement of LDH activity in serum, based on change in optical density of the coenzyme is specific (2,4,5,6,7) the finding of 3 distinctly defined regions of LDH activity in serum fractions was a somewhat unexpected observation.

The rise in serum LDH reported in patients with myocardial infarction(4,5,7) was confirmed in the present study. However, it was of interest that the increase in LDH activity was restricted, in large part, to a rise in the a_1 peak with a consequent decrease in the a_2/a_1 ratio. In the 3 cases of leukemia although the total LDH activity of serum was raised in only one instance, thus confirming the inconstancy of an elevated serum LDH in leukemia(7), an increase in the a_2 peak relative to the a_1 peak occurred in all cases and the a_2/a_1 activity ratio was significantly raised. It thus appears that considerably more information can be obtained concerning LDH activity in disease states when fractionation is carried out than when the measurement of LDH activity is confined to whole serum.

The possibility that the 3 activity peaks might correspond to different forms of LDH elaborated from different sites is tentatively raised. It should be emphasized, however, that thus far the 3 peaks are demonstrably similar only in that they all exhibit LDH activity. More work on the detailed kinetics of the enzymatic reaction in the 3 peaks is required before it can be concluded that they do not represent different enzymes which react with a common substrate. It is of interest that Bajusz and Kovary(9) in investigating serum dehydrogenase by an entirely different method found 3 distinct spots on filter paper. In these experiments an indicator dye (2,3,5-triphenyltetrazolium chloride) was used. Unfortunately, details of the experiments are lacking.

The distribution of radioactive zinc in the serum fractions suggests that in both the rabbit and man, zinc is located principally in two electrophoretic regions. Since LDH contains zinc as an integral part of its molecule it was of interest that the a_2 radioactivity peak coincided approximately with the LDH peak in the a_2 region. However, further experiments are necessary to establish a definite relationship since the a_2 fraction is known to be a mixture of many proteins.

Summary. 1. Using the method of Wroblewski and LaDue the LDH activity in serum has been localized to 3 electrophoretic regions. In 6 control subjects approximately 48% of the LDH activity in serum was present in the a_2-globulin, 33% between the a_1-globulin and albumin and 19% in the β-globulin. 2. Three peaks corresponding approximately to those found in serum were observed in the hemolysate from human red cells. In the 2 cases of myocardial infarction in which the total LDH activity was increased the a_1 activity peak was differentially raised while in 3 cases of leukemia, although the total activity was increased in only one instance, the percentage of the total LDH activity in the a_2 peak was uniformly increased. 3. The results obtained suggest that an examination of the LDH activity in the different serum fractions may provide a more sensitive index of alterations of LDH activity than examination of the total serum concentration of the enzyme. 4. The distinguishing chemical and physical properties of the enzymes in the three peaks, in addition to the observed electrophoretic difference, remain to be studied.

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