LACTIC DEHYDROGENASE ISOENZYMES IN BLOOD SERUM AFTER STORAGE AT DIFFERENT TEMPERATURES

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SUMMARY

LDH isoenzymes differ considerably in "cold stability". At temperatures below zero fraction 4 is the least stable, then fraction 5, then fractions 3 and 2; fraction 1 is very stable in the cold. At room temperature total LDH activity as well as the isoenzyme pattern remain unchanged for about 10 days. Sera in which LDH activity and LDH isoenzymes have to be studied should be stored at room temperature but not in the refrigerator.

Lactic dehydrogenase activity in human blood serum is reported to remain constant if the serum has been stored at room temperature for some days[1,2] or even for a week[3]. In the refrigerator the enzyme was found to be stable for periods from one night to several weeks[1-5]. In the frozen state no significant losses were found after one week to one month[3-5]. Unlike all these authors who found a high stability of LDH, SÜDHOF AND WÖTZEL[7] observed a rapid decline in activity in the course of 48 h; at that time nearly 40% of the initial activity had been lost if the serum had been stored at room temperature or at +4° and about 30% after storage at −20°.

Very little is known about the stability of the 5 isoenzymes. WIEME[2] reports slight modifications in *some* sera; they mainly concerned the slowest migrating fractions.

While studying the diagnostic value of LDH isoenzymes, we wanted to make sure that a serum which had been stored for some days in the usual way (*i.e.* in the refrigerator) or which had been sent by post would show the same isoenzyme pattern as when examined freshly. In a number of normal sera we found only a slight decrease in total LDH activity in the first week; the relative percentages of the 5 fractions remained constant. In the course of 6 weeks, however, the total activity fell markedly and at the end of this period fractions 4 and 5 had disappeared almost completely, whereas fraction 3 was reduced to about half its initial value. There was only a slight difference between samples stored in the freezing compartment of the refrigerator (± −10°), in the refrigerator itself (0–+4°) or at room temperature (16–22°). At that time we believed that these differences fell within the experimental errors.

In the sera from 2 cases of hepatitis, however, we found a rapid decrease in total LDH activity in the samples stored at −10°, and a less rapid decrease at 0–4°. In the samples stored at room temperature the activity remained virtually

constant for a period of 7 days. The electrophoretic patterns of these sera revealed that the loss of activity at low temperatures was confined to the (elevated) fraction 5, which had disappeared completely after 7 days at −10°, and to fraction 4, which was no longer visible after 4 days at that temperature.

In another pathological serum (mononucleosis) that was stored at room temperature we observed a gradual disappearance of the slowest fractions; after 10 weeks, fractions 3 and 2 were also lost; the total activity at that time was only 70 Units, its initial value being 1040 Units. At 37°, fraction 5 was found to decrease very quickly. Details will be published elsewhere[8].

In the meantime we were often surprised by the poor reproducibility of the isoenzyme patterns in some sera that were studied on consecutive days; this, however, did not apply to sera from cases of myocardial infarction or of pernicious anemia, but appeared to be confined to sera in which the slowest fractions were elevated (hepatitis). We therefore decided to study the behaviour of the 5 LDH isoenzymes in a serum which is particularly suited for this kind of investigation, *viz.* serum from cord blood in which the slowest fractions are much higher than in sera from adult people.

MATERIAL AND METHODS

Cord blood was allowed to clot and the serum divided into 30 tubes, 10 of which were stored at about −10°, 10 at 0–+4° and 10 at room temperature. During the first week, the total LDH activity was determined and electrophoresis carried out daily; the tubes from which the sera had been taken were then discarded. Later on, the interval between determinations was greater and we had to put back the tubes at the same temperature and use serum from the same tubes again. In some tubes that were left at room temperature, bacterial contamination became visible after two weeks; sera that were very turbid were discarded; the others were centrifuged before use. The sera that were stored at −10° appeared not to be frozen.

Total activity was determined 10 times, and electrophoresis was carried out 13 times in the course of a month. All determinations were done in duplicate.

Total activity was determined by the method of HENRY *et al.*[9] at room temperature; a conversion factor, taken from their paper, was used to express the measured activity in Units at 25°.

LDH electrophoresis in agar gel was carried out at constant temperature (16–18°); 1.5 μl of serum (or 2.5 in the later experiments when the activity had become lower) were run for 9 min; the applied voltage was 200 V, and the distance between the agar bridges was 6.5 cm. After electrophoresis a second agar layer, containing phosphate buffer pH 7.4, lactate, cyanide, NBT and phenazinemethosulphate was poured on to the slide which was then incubated at 37° for 1 h. After fixation and drying the 5 areas of activity were scanned, the peaks cut out and weighed and their relative surface percentages calculated. This method is essentially that of WIEME *et al.*[10], but the concentrations of lactate, NBT and PMS were higher.

RESULTS

In the fresh serum the total activity was 380 Units; the relative surface percentages were 26 for fraction 1 (the fastest), 34.5 for 2; 19 for 3; 12 for 4, and 9 for 5.

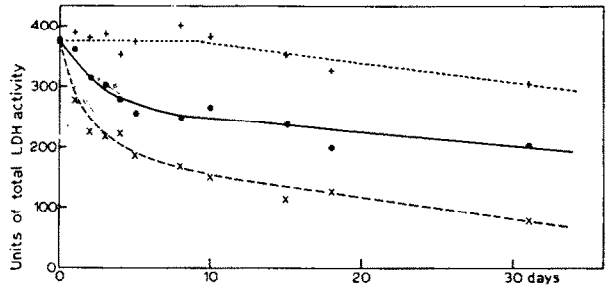

Fig. 1. Total LDH activity in a serum after storage at room temperature (+ + + + +), at 0–+4° (. . . . .) and at −10° (× × × × ×).

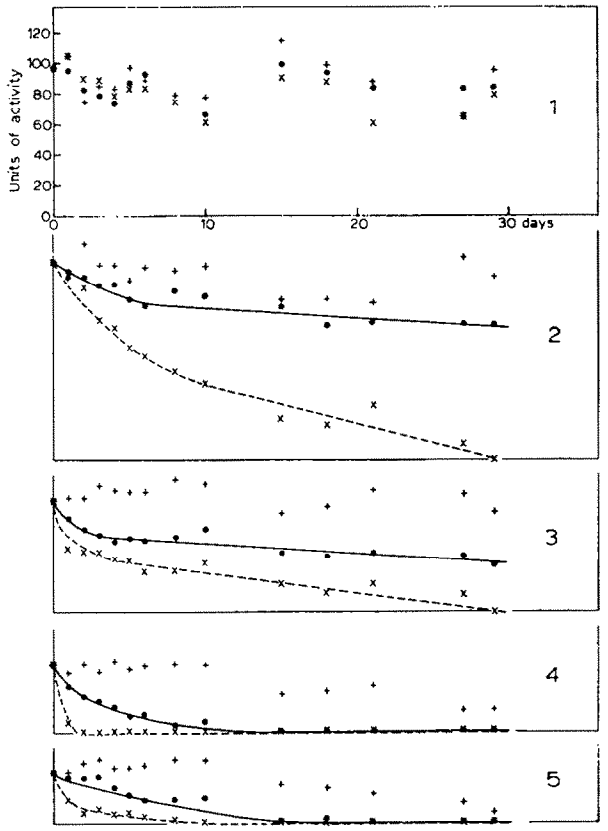

Fig. 2. Activity of the 5 LDH isoenzymes after storage at room temperature (+ + + + +), at 0–+4° (. . . . .) and at −10° (× × × × ×).

From Fig. 1 it can be seen that at room temperature total activity remains constant for about 10 days; after that time there is a slight decrease. At 0–4° activity falls rather rapidly during the first week and more slowly in the 3 following weeks. The samples stored at −10° show the same picture but activity decreases more rapidly and to a higher degree.

From the electrophoretic experiments it appeared that the loss of activity is very different for the 5 fractions. The activity of these fractions was expressed in "Units" which were calculated in the following way. The total activity on the days when electrophoresis was carried out was read from Fig. 1 and multiplied by 0.01 times the relative surface percentage of each fraction. We are aware that the numbers of Units obtained in this way are not the actual numbers of enzyme activity, but for comparing the degree of loss of activity in the 5 isoenzymes they are useful. The results are given in Fig. 2. The following conclusions may be drawn:

(1) Fraction 1 remains virtually constant in the course of one month at these 3 temperatures.

(2) At room temperature fractions 2–5 show no alteration for about 10 days; thereafter there is a slight decrease in fractions 2 and 3 and a pronounced decrease in fractions 4 and 5.

(3) At −10° there is a very rapid loss of activity in fraction 4, which disappears completely after 2 days. Fraction 5 disappears more slowly in about 8 days. Fractions 3 and 2 have lost half of their activity after 8 days and are no longer visible after one month; at that time only fraction 1 is left and has retained its initial activity.

(4) At 0–4° the same picture is seen as at −10° but to a lesser degree.

TABLE I

TOTAL LDH ACTIVITY AND RELATIVE PERCENTAGES OF THE ISOENZYMES IN A SERUM AFTER STORAGE FOR 2 DAYS

	Total activity (Units)	*Fraction 1* (%)	*Fraction 2* (%)	*Fraction 3* (%)	*Fraction 4* (%)	*Fraction 5* (%)
Fresh serum	380	26	34.5	19	12	9
After 2 days (room temp.)	380	20	38	19.5	12	10.5
After 2 days (0–+4°)	315	27	38.5	17	8	9.5
After 2 days (−10°)	225	36	46	15.5	0	3

DISCUSSION

The differences between the 5 LDH isoenzymes in heat stability[11] find their counterpart in differences in cold stability. At high temperatures, however, even at 37°, fraction 5 is most rapidly inactivated, whereas in the cold fraction 4 is the least stable.

We do not know whether fractions 4 and 5 from other sources than cord blood and hepatitis blood are as rapidly destroyed at low temperatures; this is now under investigation. It is common practice in the clinical laboratory to store samples of body fluids in the refrigerator. This may lead to considerable errors in the determination of total LDH activity and of the relative percentages of the 5 isoenzymes in those sera in which the slowest fractions are increased. In Table I this is shown for the cord blood mentioned above.

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