TOTAL LDH AND LDH ISOENZYME DISTRIBUTION IN THE SERUM OF NORMAL CHILDREN

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SUMMARY

Total LDH activity and LDH isoenzyme distribution were determined in sera of 111 normal children from 4 to 13 years old and compared to a control group of adult sera. It was found that in children the level of total LDH activity and the isoenzyme distribution did not differ significantly from that in adults.

When the children were divided into age groups of one year, such differences could not be detected either. No sex-related differences were found.

The relativity of the normal LDH isoenzyme distribution pattern is discussed.

Many data are available on the total LDH activity and the LDH isoenzyme distribution in sera of normal adults. There is, however, little known of the total LDH activity in the serum of normal children. There is considerable disagreement about the normal level in children[1-4] and, as far as we know, the LDH isoenzyme distribution of normal children has never been studied extensively. In this paper we report the results of an investigation of total LDH activity and the LDH isoenzyme distribution in 111 healthy school-children from 4 to 13 years. The children were divided in groups of 10 to 13 of the same age. Mean value, standard deviation and range of the total LDH activity and the LDH isoenzyme fractions were calculated for each group and compared with the data obtained from a control group of normal adults. Also the occurrence of sex-related differences was investigated. The total LDH activity in serum was determined by the Boehringer spectrophotometric NAD reduction method. The LDH isoenzymes were separated by means of agar gel electrophoresis[5], visualized by the nitroblue tetrazolium reduction method[6,6a] and estimated by scanning.

MATERIAL

Blood from 111 fasting children was collected by venepuncture at school. The samples collected in glass tubes were allowed to clot at room temperature. Two hours after collection, all blood samples were centrifuged at very low speed for 5 min. The serum was roughly decanted from the blood clot and recentrifuged at ± 3500 rev./min during 5 min. The clear serum was removed from the residual erythrocytes.

With a few exceptions, the estimations of the LDH activity and the analyses of isoenzymes were accomplished within 6 h.

METHODS

Total LDH activity

Reagents: Biochemica test combination TG-9-1 15977 (Boehringer, Mannheim)

(1) Buffer–pyruvate: 0.05 *M* phosphate buffer pH 7.5. Pyruvate concentration: $3.1 \cdot 10^{-4}$ *M*.

(2) Aqueous NADH solution: 0.009 *M* NADH.

(3) $K_2Cr_2O_7$: 0.005% solution in water.

To a mixture of 3 ml buffer–pyruvate (1), preincubated at 25° in a heating block (Wissenschaftlich-Technische Werkstätten G.m.b.H. N.V. Oortmersen) 0.05 ml NADH solution (2) and 0.1 ml serum were added. During the next 4 min the decrease of the extinction at 340 nm was measured every minute. (Zeiss PQM II spectrophotometer with automatic 100% adjustment). A 0.005% $K_2Cr_2O_7$ solution was used as a blank.

Calculation: LDH activity in mU/ml = $5053 \times \Delta E$/min.

LDH isoenzymes

Reagents:

(1) Buffer for electrophoresis: barbital–barbituric acid buffer pH 8.36. 6.13 g sodium diethylbarbiturate (E. Merck A.G., Darmstadt) and 2.12 g barbituric acid (Merck) were dissolved in 1 l of distilled water. The pH was controlled before final adjustment of the volume.

(2) Agar: Special Agar Noble (Difco Laboratories, Detroit, Mich. U.S.A.). 0.9% agar solution in barbital–barbituric acid buffer pH 8.36.

(3) Staining solution I: Dissolve 1.2 ml sodium lactate 60% (Onderlinge Pharmaceutische Groothandel), 1.8690 g disodium hydrophosphate Na_2HPO_4 2 aq. (Merck), 0.2722 g potassium dihydrophosphate KH_2PO_4 0 aq. (Merck), 50 mg sodium cyanide p.a. (Merck), 25 mg nitroblue tetrazolium (N.B.T.) (Sigma Chemicals Co., St. Louis, Mo.) in 90 ml distilled water. The solution is filtered. Stable at room temperature for at least one month.

(4) Staining solution II: 10 mg phenazine methosulphate (P.M.S.) (Nutritional Biochem. Co., Cleveland, Ohio) in 10 ml distilled water. In a dark-coloured flask at room temperature the solution is stable for one month.

(5) Incubation bath: Add 8 mg nicotinamide adenine dinucleotide (NAD) to a mixture of 7.2 ml staining solution I and 0.2 ml staining solution II. For each incubation a freshly prepared solution has to be used.

(6) Fixing solution: Ethylalcohol–acetic acid–water (150:10:40 v/v).

Electrophoresis was performed on microscopic slides in a Wieme apparatus (Vitatron). In all experiments 8–9 μl of serum was applied; the run lasted 30 min at 10 mA/cm (power supply Vitatron type GVA/GT). After separation the slides were incubated at 37° for 2 h, then rinsed with water and left for half an hour in the fixing bath. They were then dried at 37° between moist filter paper. For scanning at 578 nm the Vitatron scanning apparatus was used, consisting of an Universal Fotometer

UFD 100, equipped with an optical basis unit UFD 200, a transport unit AAD 100 and an integrating recorder LRP 100.

The isoenzyme fractions were calculated according to the formula

$$\frac{\text{peak area}}{\Sigma \text{ peak areas}} \times 100\% = \% \text{ activity}$$

Reproducibility

Two sera were analysed in 7-fold and 8-fold respectively. The following mean values and standard deviations were found:

Experiment I:

$n = 7$ total LDH: 219 ± 8.2 I.U.

LDH_1	LDH_2	LDH_3	LDH_4	LDH_5
49 ± 2.0	31 ± 2.1	13 ± 1.9	4 ± 0.9	3 ± 1.4

Experiment II:

$n = 8$ total LDH: 150 ± 7.9 I.U.

LDH_1	LDH_2	LDH_3	LDH_4	LDH_5
54 ± 3.1	33 ± 1.5	8 ± 1.6	2 ± 1.2	2 ± 1.9

The standard deviations are calculated according to the formula

$$\text{S.D.} = \sqrt{\frac{\Sigma_1^i (x_i - \bar{x})^2}{n-1}}.$$

RESULTS

In Table I, the total LDH values for each group of children and for the adults are given. Also calculations for all children together were performed.

TABLE I

MEAN TOTAL LDH ACTIVITY, STANDARD DEVIATIONS AND RANGES IN CHILDREN AND ADULTS

Age (years)	*n*	*Total LDH: I.U. mean value*	*S.D.*	*Range*
4–5	13	148	26	113–187
5–6	10	150	17	124–175
6–7	14	135	33	85–241
7–8	11	133	33	78–186
8–9	13	144	23	108–182
9–10	13	134	27	96–177
10–11	12	127	29	96–172
11–12	14	129	23	91–167
12–13	11	133	16	106–152
All children 4–13 years	111	137	26	78–241
Adults	12	148	36	101–207

The isoenzyme fractions were estimated in duplicate. From these duplicates individual means were calculated. These individual values in each group were averaged. The results are given in Table II, together with their standard deviations and ranges.

TABLE II

LDH ISOENZYME DISTRIBUTION IN CHILDREN AND IN ADULTS

Age (years)	*n*	*LDH_1*	*LDH_2*	*LDH_3*	*LDH_4*	*LDH_5*
4–5	13	46 ± 3.9 51.6–40.5	32 ± 3.0 36.6–25.8	13 ± 2.8 17.5–8.9	5 ± 1.3 6.5–2.5	3 ± 1.5 6.5–1.4
5–6	10	48 ± 6.5 56.2–37.7	32 ± 4.2 39.6–24.1	13 ± 3.8 18.2–7.4	5 ± 2.1 7.9–1.4	3 ± 1.9 6.3–0.0
6–7	14	49 ± 5.1 63.1–40.2	32 ± 4.0 38.2–26.2	12 ± 2.8 16.9–7.9	4 ± 1.5 6.6–1.1	2 ± 1.1 4.1–0.0
7–8	11	48 ± 3.7 60.4–42.6	33 ± 3.0 37.2–27.5	13 ± 3.4 17.3–6.9	4 ± 1.2 5.7–2.2	2 ± 1.3 4.4–0.0
8–9	13	49 ± 4.7 56.1–43.9	33 ± 2.1 37.6–29.3	13 ± 2.9 15.9–8.1	3 ± 1.2 5.3–1.2	2 ± 1.3 4.0–0.8
9–10	13	51 ± 6.5 60.6–41.8	32 ± 2.9 36.4–26.8	12 ± 4.3 18.2–6.1	3 ± 1.2 5.3–1.7	1 ± 0.7 2.8–0.0
10–11	12	48 ± 7.9 61.3–35.9	34 ± 4.5 39.8–26.4	13 ± 3.3 18.5–9.1	3 ± 1.5 6.3–1.1	1 ± 1.0 2.8–0.0
11–12	13	48 ± 5.7 57.1–40.8	34 ± 3.9 40.9–26.5	14 ± 3.4 18.6–8.4	3 ± 1.8 7.0–1.0	2 ± 1.0 3.0–0.0
12–13	11	44 ± 4.1 51.3–39.4	32 ± 3.0 37.5–26.9	16 ± 2.0 18.6–11.7	5 ± 1.3 7.1–3.1	3 ± 0.9 4.4–1.3
All children 4–13 years	110*	48 ± 5.7 41.8–63.1	33 ± 3.4 24.1–40.9	13 ± 3.1 6.1–18.6	4 ± 1.5 1.0–7.9	2 ± 1.3 0–6.5
Adults	24	45 ± 4.8 37.0–53.4	35 ± 3.9 26.9–42.1	15 ± 2.4 8.6–19.3	4 ± 1.2 1.6–5.6	2 ± 1.0 0.5–3.7

* 1 failed.

TABLE III

MEAN VALUES AND VARIABILITY OF TOTAL LDH ACTIVITY AND ISOENZYME DISTRIBUTION IN BOYS, GIRLS AND ALL CHILDREN TOGETHER

	Boys (n = 58)	*Girls (n = 53)*	*Boys and girls (n = 111)*
Total LDH act. (I.U.)	141 ± 29 75–241	132 ± 26 78–183	137 ± 26 75–241
Fractions (% of total)	*n* = 58	*n* = 52*	*n* = 110
LDH_1	50 ± 5.6 41.8–61.3	46 ± 5.9 37.7–63.1	48 ± 5.7 41.8–63.1
LDH_2	33 ± 3.4 24.1–39.8	33 ± 3.5 25.8–40.9	33 ± 3.4 24.1–40.9
LDH_3	13 ± 3.2 6.1–18.6	14 ± 3.0 7.9–18.5	13 ± 3.1 6.1–18.6
LDH_4	4 ± 1.6 1.0–7.9	4 ± 1.4 1.4–6.5	4 ± 1.5 1.0–7.9
LDH_5	2 ± 1.3 0–4.5	2 ± 1.4 0–6.5	2 ± 1.3 0–6.5

* 1 failed.

The entire group of children, from 4–13 years, was examined for differences of total LDH activity and isoenzyme distribution according to sex. In Table III, the mean values and variability of total LDH activity and isoenzyme distribution in boys and girls and all children together are listed. Figs. 1, 2 and 3 show the corresponding frequency distribution graphs.

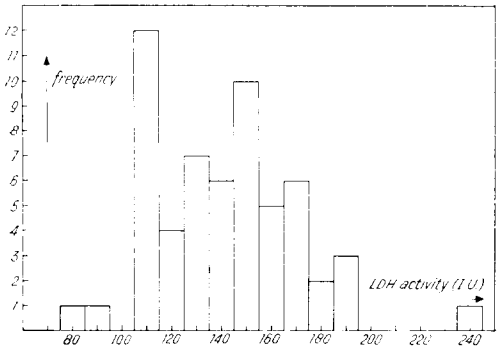

Fig. 1. Frequency distribution of serum LDH in boys (58) aged 4–13 years.

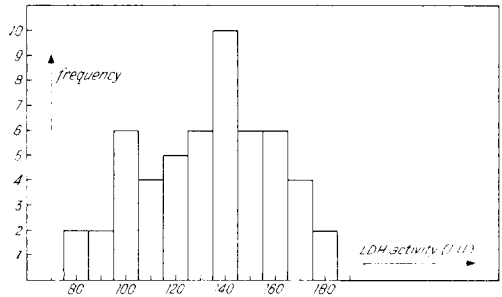

Fig. 2. Frequency distribution of serum LDH in girls (53) aged 4–13 years.

DISCUSSION

Our experimental data do not indicate a significant difference between the serum LDH activity of adults and children, nor obvious differences between the groups mutually. The highest LDH value was found in the group of 5–6 years (150 ± 17 I.U.) and the lowest in the group of 10–11 years (127 ± 29 I.U.). However, there was not a gradual decrease of total LDH activity with increasing age from 4 to 13 years. Only one child had a LDH value (241 I.U.) which was higher than the mean value + 2 × S.D. (201 I.U.). The values for total LDH in adults are in accordance with those given in recent publications[7–9]. This cannot be said from values in children in so far as the techniques used can be compared. Christiansson *et al.*[1] proposed a normal range of 130 to 425 I.U. (converted from Wroblewsky units). Sitzman[2] of 100 to 250 I.U., Bodansky *et al.*[3] gave a mean value of 277.8 ± 57.6 I.U. for children of 5–10 years (converted from Bodansky units) and Stave[4] 113 ± 60.4 I.U. for children of 4–8 days, 50.4 ± 14.6 I.U. for children of 2–3 months, 47.9 ± 12.0 I.U. for 4–12 months, 41.0 ± 13.5 I.U. for 2–6 years and 43.1 ± 14.9 I.U. for children of 7–15 years (converted from Bücher units).

We could not establish any sex-related difference in the serum LDH activity of boys and girls aged 4–13 years. From the frequency distribution diagram (Figs. 1, 2 and 3) it can be seen that the distributions are not symmetrical. For boys as well as for girls the curves seem to be bimodal. Because of the small number of determinations, this phenomenon cannot be validly interpreted.

From our experiments on serum LDH isoenzyme patterns it can be concluded that in children between 4–13 years, no age dependency and no sex-related differences occur. A distribution very similar to that of normal adults was found. It will be necessary to comment on the isoenzyme distribution as found by us, since it is different from the distribution patterns given in the literature[10–15]. LDH_1 was higher and LDH_2 lower than is usually found. However, also in the literature there is little agreement about the fractional values of LDH_1 and LDH_2. We were unable to trace the cause of the inversion of the ratio occurring both in children and adults. It could not be attributed to haemolysis, which was absent to a visible degree. There was no absorption of NBTH by albumin which might contribute to an increase of LDH_1. A

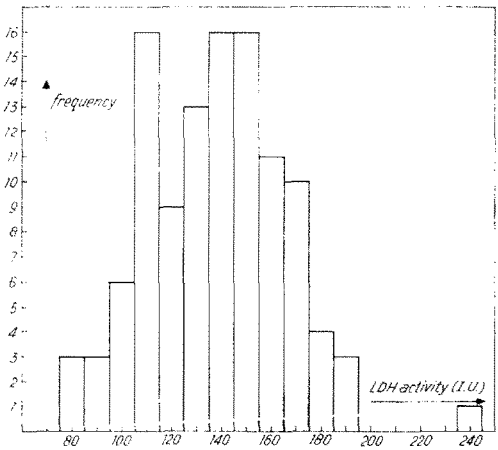

Fig. 3. Frequency distribution of serum LDH in a total of 111 boys and girls aged 4–13 years.

systematic fault in the integration of the peak areas could be excluded. By three different methods of integration the same values were obtained. Washing of the agar with 50% alcohol[16] gave only a slight decrease in the LDH_1 fraction. With two other charges of agar or other brands of NBT the same results were obtained. No effect was seen from aging of the NBT solution for two weeks at 4°. Lowering the temperature of the agar plates during electrophoresis from 15° to 8° resulted only in a slight decrease of the LDH_1/LDH_2 ratio in adults but in children a small increase was observed. Shortening of the incubation time had no effect. It can be concluded that the ratio LDH_1/LDH_2 is determined by experimental factors not yet known. Methods which are apparently identical may yield different results. Biological interpretation of this ratio, as is done by Cohen *et al.*[17], may be risky. They suggested that the ratio for males was less than one, whereas in females it was higher than one. The pattern given for women corresponds remarkably well with the patterns found in our laboratory, for both adults and children. The hypothesis of Cohen *et al.*[17] that the difference might be due to the influence of sex hormones on the combination of the LDH subunits, has to be investigated more thoroughly.

Further investigations are required in order to get acquainted with the experimental factors which determine the distribution pattern of LDH isoenzymes. Also, a better insight into the origin of LDH in normal serum might then be obtained.

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