TOTAL ACTIVITY AND ISOENZYME SPECTRUM OF LACTATE DEHYDROGENASE IN THE NORMAL LENS OF THE HUMAN EYE AND IN SENILE CATARACT

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The human lens was shown to contain three lactate dehydrogenase (LDH) fractions. Total LDH activity in the cortex of the lens is more than four times higher than in the nucleus. As age increases (from 15 to 89 years) LDH activity decreases and its isoenzyme composition is modified. With the development of senile cataract in the lens, changes are observed in the relative proportions of the different molecular forms of LDH on account of an increase in the H-subunits. Particularly marked changes in the complement of molecular forms of LDH take place in the nucleus of the lens affected by cataract.

It is considered that the development of senile cataract is closely connected with and is the result of aging process [9]. According to one theory of the pathogenesis of senile cataract, its development is based on a disturbance of carbohydrate metabolism in the aging organism [13, 15].

The isoenzyme spectrum of lactate dehydrogenase (LDH), the enzyme which catalyzes the final reaction of glycolysis (pyruvic acid $\rightleftharpoons$ lactic acid), is an indicator of the type of metabolism in the tissues [6, 11]. The LDH isoenzyme spectrum can be regarded as a delicate molecular expression of tissue differentiation; each organ and tissue has its own characteristic complement of isoenzymes, which is determined by differences in the functional roles of the H- and M-subunits with their different enzymic properties [5, 12, 14].

In order to detect any disturbances in metabolism during aging of the lens and cataract formation, the total LDH activity and activity of its isoenzymes were determined in the lenses of persons of different ages. In parallel experiments the isoenzyme spectrum of LDH in the lens and blood serum was studied in patients with senile cataract.

TABLE 1. LDH and its Isoenzymes in Human Transparent Lenses at Different Ages (M ± m)

Age of persons from which lenses were taken (in years)	Number of lenses	Total LDH activity*	Activity of isoenzymes (in percent)		
			LDH_3 (H_2M_2)	LDH_4 (H_1M_3)	LDH_5 (M_4)
From 15 to 45	20	250,0±7,6	17,6±0,4	36,1±0,4	45,8±1,0
From 46 to 69	22	208,0±5,0	20,0±0,4	38,8±0,4	41,1±0,4
From 70 to 89	18	149,0±5,5	20,5±0,5	43,0±1,0	36,2±0,7

*Total LDH activity here and in Table 2 is expressed in Wroblewski's units (activity of enzyme in 1 ml serum causing a change in extinction of NAD by 0.001 in 1 min at 340 nm).

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TABLE 2. Total Activity of LDH and Its Isoenzymes in the Human Lens in Senile Cataract (M ± m)

State of lenses	Age of persons whose lenses were taken (in years)	Number of lenses	Whole lens				Cortex of lens				Nucleus of lens			
			total LDH activity	activity of isoenzymes			total LDH activity	activity of isoenzymes			total LDH activity	activity of isoenzymes		
				LDH_3	LDH_4	LDH_5		LDH_3	LDH_4	LDH_5		LDH_3	LDH_4	LDH_5
Normal	60—75	15	203,0±5,0	20,2±0,4	41,3±0,4	38,4±0,5	430,0±2,0	21,4±0,3	40,7±0,2	37,8±1,0	106,5±5,0	30,4±0,4	32,3±0,4	37,2±0,6
Affected by cataract	60—75	30	163,0±8,0	12,6±0,4	42,9±0,9	44,7±1,2	381,0±5,0	11,0±0,3	40,9±0,3	48,1±0,6	99,4±4,2	5,8±0,6	36,1±0,5	58,1±0,5
P			<0,005	<0,001	>0,1	<0,001	<0,001	<0,001	>0,1	<0,001	<0,2	<0,001	<0,001	<0,001

EXPERIMENTAL METHOD

Transparent human lenses were studied not later than 12 h after death; lenses extracted for senile cataract were studied at similar times. Altogether, 60 macroscopically transparent lenses and 30 lenses affected by cataract were investigated. Blood was taken from the patient's finger.

The lenses were carefully washed in physiological saline. By means of an ophthalmic scalpel, the cortex of the lens was separated under a hand lens from the nucleus with its denser consistency. The tissue for testing was homogenized in cold medinal-veronal buffer (pH 8.6) in a Potter's glass homogenizer (1 ml buffer to 100 mg fresh weight of tissue). The homogenate was centrifuged for 15 min at 9000 rpm. The total LDH activity in the supernatant was investigated spectrophotometrically, using pyruvic acid as substrate [10]. Electrophoresis on agar by the method of Yurkov and Alatyrtsev [4] was used to determine the LDH isoenzymes. The stained LDH fractions were measured on a photoelectric colorimeter by means of a special attachment [3].

EXPERIMENTAL RESULTS AND DISCUSSION

The total LDH activity in the lens (Table 1) is evidence of the low rate of glycolysis in it. LDH in the lens consists of 3 isoenzymes of muscular type (H_2M_2, H_1M_3, and M_4) with LDH_5 (M_4) as the predominant fraction, indicating a marked level of anaerobic metabolism. Differential investigation of the various zones of the lens (Table 2) showed that total LDH activity in the nucleus is less than one-quarter that in the cortex. The distribution of the isoenzymes confirmed the more marked anaerobic metabolism of the lens nucleus than in the cortex: fraction H_2M_2 was lower and H_1M_3 higher in the cortex than in the nucleus. The level of glycolytic processes in the nucleus of the transparent lens is thus much lower than in its cortex.

It was also shown that with an increase in age there is a successive decrease in total LDH activity. The following changes take place in the isoenzyme spectrum of LDH: activity of fractions H_2M_2 and H_1M_3 is increased and activity of fraction M_4 is reduced. Fraction H_1M_3 (LDH_4) evidently bears the main responsibility for the change in glucose metabolism in the lens with increasing age.

A study of LDH activity and its isoenzyme spectrum in human lenses affected by cataract showed that in senile cataract there is a decrease in total LDH activity and a change in the complement of its molecular forms: an increase in LDH_5 (M_4) activity and a decrease in LDH_3 (H_2M_2) activity (Table 2). Particularly marked deviations were observed when the nucleus of the lens with cataract was investigated; in this case the activity of fraction H_2M_2 was reduced compared with normal by more than five times, while activity of fractions H_1M_3 and M_4 was considerably increased.

These changes are similar to those taking place in the transparent lens during aging (in both cases there is a characteristic decrease in total LDH activity), but in senile cataract a much more market change in glucose metabolism toward the anaerobic pathway of oxidation of glucose in the lens takes place predominantly in its nuclear zone.

No deviations from normal were found in the spectrum of LDH isoenzymes in the blood serum of patients with senile cataract, evidently because of the low activity of this enzyme in the tissues of the eye by contrast with other tissues of the body.

TABLE 3. Relative Content of M- and H-subunits of LDH in Normal Human Lens and in Lens Affected by Cataract (M ± m)

State of lenses	Whole lens			Cortex of lens			Nucleus of lens		
	total M	total H	M:H	total M	total H	M:H	total M	total H	M:H
Normal	79,3	20,3	3,9	76,6	23,9	3,3	79,1	20,9	3,1
Affected by cataract . .	83,9	17,0	4,9	87,1	11,9	7,3	84,2	15,7	5,3

Calculation of the relative proportions of H- and M-subunits in each of the isoenzymes of the lens, and calculating from these results the sum of the H- and M-subunits, showed a marked predominance of M- subunits in the transparent human lens and marked change in this ratio toward M-subunits in the lens affected by cataract (Table 3).

These changes in the relative content of LDH isoenzymes in the lens affected by cataract can be attributed either to an increase in activity of the M-subunits or a change in the rate of synthesis of M- and H-subunits in favor of the M-subunits.

LDH_5 (M_4) is known to be an allosteric isoenzyme, and its activity increases with a change in pH toward the acid side [3, 8]; synthesis of H-subunits is intensified with a decrease in the oxygen pressure in the surrounding medium [8], or on addition of inhibitors of respiratory catalysts [7]. An excess of substrate has a powerful inhibitory action on LDH [6].

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