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Department of Ophthalmology
(Heads: P. Brændstrup, S. E. Lorentzen, M. S. Norn and K. Nørskov)
and Department of Clinical Chemistry (Head: C. Brun),
Kommunehospitalet, Copenhagen

CONCENTRATIONS OF SOME RIBONUCLEOTIDES, L-LACTATE, AND PYRUVATE IN HUMAN SENILE CATARACTOUS LENSES WITH SPECIAL REFERENCE TO ANTERIOR CAPSULAR/SUBCAPSULAR OPACITY

BY

A. BRUUN LAURSEN

The concentrations of some ribonucleoside tri- and diphosphates, adenosine-5′-monophosphate, L-lactate and pyruvate were determined in human senile cataractous lenses removed during cataract operations. Pyruvate concentrations were found to be negligible (median = 56 μmol/kg lens wet weight) in 15 human senile cataractous lenses.

On the basis of correlations between the biomicroscopic appearances of the senile cataractous lenses (N = 80) and the concentrations and ratios of the metabolites in question, the following classification was found to be justified:

1. Immature cataractous lenses without anterior capsular/subcapsular opacity: high levels of ribonucleoside triphosphates (RTP), high sums of RTP, ribonucleoside diphosphates (RDP), and adenosine-5′-monophosphate (AMP) as well as high levels of L-lactate and high ratios of L-lactate in the lens/L-lactate in the aqueous.
2. Immature cataractous lenses with anterior capsular/subcapsular opacity: intermediate levels of RTP, intermediate values for the sums of RTP, RDP, and AMP, high L-lactate levels, and intermediate values of the ratios of L-lactate in the lens/L-lactate in the aqueous.
3. Totally opaque lenses, which all had extensive anterior capsular/sub-

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capsular opacity: low values for the concentrations of lens RTP, for the sums of RTP, RDP and AMP, and for lens L-lactate. Low ratios of L-lactate in the lens/L-lactate in the aqueous.

Key words: cataracts, senile – opacity, anterior capsular/subcapsular – ribonucleotides – L-lactate – pyruvate.

De Wecker (1886) made a survey of histopathological findings in "cataracte capsulaire" in human senile cataract. This condition was stated to be associated with wrinkles of the lens capsule as well as degenerative changes and vacuolation in the epithelial layer. Hess (1905) and Vogt (1914) described vacuoles at or just beneath the level of the anterior capsule of the lens on biomicroscopical examinations of human senile cataracts. Since the literature seems to contain no information about the concentrations of metabolites in lenses with this particular type of opacity, the present study paid special attention to a possible correlation between the biomicroscopical appearance of the anterior surface of the senile cataractous lenses and the concentrations of certain metabolites: ribonucleoside triphosphates (RTP – see "Methods"), ribonucleoside diphosphates (RDP – see "Methods"), and adenosine-5′-monophosphate (AMP) as well as pyruvate and L-lactate. L-lactate was also determined in the aqueous humour so that the concentration ratio of L-lactate in the lens/L-lactate in the aqueous could be calculated.

Material

The material of this study comprises 80 patients aged 55–93 years with senile cataracts. The patients were fasted for about 10 h before the samples were taken. Patients with additional eye disease, diabetes mellitus, or fasting blood sugar concentration $>$ 6.1 mmol/l were excluded. Moreover patients treated with drugs of the digitalis group, as well as patients treated with local or universal corticosteroid during the period of cataract development were excluded. Lenses extracted extracapsularly and lenses treated with the proteolytic enzyme α-chymotrypsin during the operative procedure were also excluded. Mydriasis for the purposes of biomicroscopical cataract classification and peroperative dilatation was produced by means of 0.5 % cyclopentolate. The patients had retrobulbar anaesthesia (1.5–2.0 ml of 1 % carbocaine, i. e. mepivacaini chloridum NFN, without adrenaline or noradrenaline). The cataracts are classified in the legend, Table III. The extension and density of the cataract

were evaluated by slit lamp examination and ophthalmoscopy: grade 1 lenses comprised immature cataractous lenses with slight extension of the opacities and a high degree of transparency; grade 2 lenses comprised more heavily affected immature cataractous lenses; grade 3 lenses were totally opaque.

Methods

Aqueous humour was aspirated through a keratotomy just before the anterior chamber was opened. The chamber was emptied of aqueous humour. The aqueous was immediately heated to boiling point and then frozen on dry ice (Bruun Laursen & Lorentzen 1975). The lens was cryo-extracted and, while still attached to the tip of the cryo-pencil, rinsed with a few drops of 0.9 % saline. The lens was cautiously wiped with fine gauze and then dropped into liquid nitrogen (−190°C), where it remained until homogenization took place. The frozen lens was crushed in a steel block precooled in liquid nitrogen. The frozen lens powder was carefully mixed and, by means of a steel spatula precooled in liquid nitrogen, transferred to a 5 ml Potter-Elvehjem homogenizer containing 1.00 ml of perchloric acid (0.6 mol/l) precooled at 4°C. Homogenization at 100 r.p.m. took place in ice bath for 2 min. The solution was placed in the refrigerator (4°C) for 30 min and then mixed. Centrifugation was performed at 3000 r.p.m. for 10 min. The supernatant was removed and titrated with a solution containing KOH (3.36 mol/l) – triethanolamine (TEA – 0.33 mol/l) to pH ca. 7. $KClO_4$ precipitated at 0°C and was removed by centrifugation.

The RTP, RDP, AMP, pyruvate, and L-lactate concentrations of the lenses were determined by enzymatic-spectrophotometrical procedures according to Biochemica Boehringer with certain modifications. Readings of the absorbances of the assay systems were taken before and after the additions of enzymes. Changes in absorbance were recorded at 340 nm in Helma 103 glass cuvettes with 10 mm light paths at room temperature by means of a Beckman DB spectrophotometer. Aqua redestillata sterilisata was used. We added equivalent amounts of the same homogenate to the blank cuvette (to which no enzyme was added) and the assay cuvette. This was done in order to avoid a protracted, unspecific rise in absorbance, a phenomenon which was very frequently observed in the assay systems, if water was added to the blank cuvette instead of homogenate. This unspecific rise in absorbance may be due to photoactive pigments in the lenses (Cremer-Bartels 1962). Corrections were made for possible unspecific changes in absorbance due to possible contaminations of the reagents with unwanted enzyme substrates. This was done by recording the absorbance at 340 nm in an assay system where neutralized perchloric acid substituted lens homogenate.

In RDP-AMP determinations we had to add EDTA (91 mmol/l assay mixture) in order to prevent coarse flocculation after the addition of lens homogenate. This flocculation was probably due to precipitation of Mg^{2+} of the assay mixture as phosphates. Furthermore, we added ATP (adenosine-5′-triphosphate – 41 μmol/l assay mixture) in order to ensure that all AMP was determined, since the myokinase activated conversion of AMP to ADP (adenosine-5′-diphosphate) requires ATP. Detailed descriptions of the procedures can be obtained on application to the author. All determinations were duplicated.

The accuracies of the enzymatic-spectrophotometrical procedures appear in Table I. Table II gives information on the reproducibilities of the methods (surgical procedures, homogenization + deproteinization, and enzymatic-spectrophotometrical procedures), as well as on interindividual variations.

According to Biochemica Boehringer the ribonucleoside triphosphate determinations include adenosine-5′-, guanosine-5′-, uridine-5′-, and inosine-5′- triphosphates. According to Biochemica Boehringer and Lowry & Passonneau (1972) the ribonucleoside diphosphates determined are: adenosine-5′-diphosphate and, at essentially lower rates, guanosine-5′-, uridine-5′-, inosine-5′-, and cytidine-5′-diphosphates. AMP determination is not said to be unspecific.

Accuracy and reproducibility for L-lactate determinations in aqueous humour were investigated by Bruun Laursen & Lorentzen (1975) and by Bruun Laursen (1975). Buffer pH 8.5 was used for the determinations of aqueous humour and lens L-lactate concentrations.

The figures were treated statistically by means of non-parametric procedures: medians, 25 % and 75 % percentiles, and ranges (numerical differences between the highest and lowest observations). Comparisons between different groups were carried out by means of the Wilcoxon test for two samples (the Mann-Whitney test), and age-concentration correlations were tested by means of the Spearman rank correlation coefficient. These procedures have been described by e. g. Juul Therkelsen (1968) and Andersen (1972).

Table I.

Recovery experiments in pooled homogenates of human senile cataractous lenses. Individual lens homogenates were pooled. One half of a pool was enriched with a solution containing substrate. An equivalent amount of water was added to the other half of the same pool. The difference in substrate concentration between these two pools was considered to be the recovery. The concentrations are given per kg lens homogenate.

Substrate	N	Added	Recovery median	Range
ATP	9	200 μmol	172 μmol	10 μmol
	11	100 μmol	94 μmol	6 μmol
	11	50 μmol	46 μmol	15 μmol
ADP	14	33 μmol	26 μmol	16 μmol
AMP	14	17 μmol	16 μmol	8 μmol
L-lactate	4	1.5 mmol	1.4 mmol	0
	4	0.75 mmol	0.7 mmol	0
Pyruvate	10	33 μmol	32 μmol	5 μmol

ATP, ADP and AMP: adenosine-5′-tri-, di- and monophosphates, respectively.

Table II.

Variations of concentrations and ratios of some metabolites in eyes with clear lenses from 7 fasting 4–6-month-old pigs. The lenses were removed under general anaesthesia. The time which elapsed from the opening of the anterior chamber to the freezing of the lenses in liquid nitrogen varied from 5 to 12 min. Concentrations are given per kg lens wet weight. The L-lactate concentrations of the aqueous humour were calculated per kg aqueous.

	Parameters						
	RTP	RDP	AMP	RTP + RDP + AMP	$\frac{\text{RTP + 1/2 RDP}}{\text{RTP + RDP + AMP}}$	L-lactate	$\frac{\text{L-lactate lens}}{\text{L-lactate aq.}}$
	μmol/kg					mmol/kg	
N =	12	12	12	12	12	12	5
Median:	2488	600	107	3254	0.87	8.0	1.0
25 % percentile:	2328	422	90	3061	0.85	7.7	
75 % percentile:	2678	717	134	3444	0.91	8.5	
Range:	720	625	102	1004	0.12	1.5	0.8

RTP = ribonucleoside triphosphate, RDP = ribonucleoside diphosphate, AMP = adenosine-5′-monophosphate. See "Methods". Aq. = aqueous humour.

Results

In Table III the lenses are listed in groups in accordance with the biomicroscopical observations. For each group the results of measurements of metabolite concentrations are given. All lens metabolite concentrations refer to lens wet weight.

It appears that the highest ribonucleoside triphosphate concentrations occurred in the groups of immature cataractous lenses without anterior capsular/subcapsular opacity. This is clearly seen in group A, Fig. 1 (median = 1200 μmol/kg lens, range = 1395, N = 33). Significantly lower RTP concentrations ($P < 0.01$) were found in immature cataractous lenses with anterior capsular/subcapsular opacity (group B, Fig. 1: median = 493 μmol/kg lens, range = 966, N = 21). Totally opaque lenses contained still lower ($P < 0.01$) RTP levels (group C, Fig. 1: median = 293 μmol/kg lens, range = 541, N = 26). As for the total amounts of RTP + RDP + AMP a similar gradient was found. The imma-

Table III.

Some metabolite concentrations per kg lens wet weight in 80 human senile cataractous lenses.

Cataract groups according to biomicroscopic findings: 1: posterior subcapsular (posterior subcapsular tufaceous opacity), 2: posterior subcapsular + cortical (veil- or spokelike anterior or posterior cortical opacities not affecting the lens capsule), 3: posterior subcapsular + nuclear (a: nuclear opacities causing blurred insight to the posterior lens capsule, b: disciform opacity on transillumination of the dilated pupil), 4: posterior subcapsular + anterior capsular/subcapsular opacity (whitish dots or irregular greyish coat apparently located in or just beneath the anterior lens capsule – see Fig. 2), 5: posterior subcapsular + cortical + nuclear, 6: totally opaque lenses (uniformly grey or brownish lenses with extensive anterior capsular/subcapsular opacity), 7: nuclear, 8: cortical, 9: immature cortical + nuclear, 10: nuclear + anterior capsular/subcapsular, 11: immature cataracts with anterior capsular/subcapsular + posterior subcapsular + nuclear + cortical opacities, 12: anterior capsular/subcapsular + posterior subcapsular + cortical, 13: anterior capsular/subcapsular + cortical + nuclear.

Abbreviations: see Table II.

	Cataract group	Age years	RTP	RDP	AMP	RTP + RDP + AMP	$\frac{\text{RTP + 1/2 RDP}}{\text{RTP + RDP + AMP}}$	L-lactate in lens	$\frac{\text{L-lactate lens}}{\text{L-lactate aqueous}}$
			μmol/kg					mmol/kg	
	1	N =	7	7	7	7	7	7	6
Median:		73	952	259	36	1172	0.85	8.1	1.7
25 % percentile:			618	154	9	915	0.80	7.0	1.3
75 % percentile:			1287	548	46	1814	0.89	11.5	2.8
Range:		14	1075	563	126	1075	0.23	7.1	1.7
	2	N =	4	3	3	3	3	4	3
Median:		80	932	183	97	1181	0.85	9.9	2.3
25 % percentile:			754					7.2	
75 % percentile:			1271					12.3	
Range:		10	686	208	136	557	0.11	5.5	0.8

	3	N =	1	1	1	1	1	1	1
		65	1560	278	98	1936	0.88	8.4	1.6
	4	N =	7	7	7	7	7	7	7
Median:		70	491	218	20	730	0.82	6.7	1.3
25 % percentile:			254	152	14	497	0.80	5.4	1.1
75 % percentile:			678	293	44	961	0.84	7.0	1.5
Range:		33	623	177	70	784	0.21	8.6	1.7
	5	N =	2	1	1	1	1	2	1
Median:		82	1128	405	75	1593	0.83	7.3	1.4
Range:		9	29					2.0	
	6	N =	26	26	26	26	26	26	25
Median:		75	293	64	3	359	0.89	5.1	1.0
25 % percentile:			261	45	0	320	0.86	4.5	0.8
75 % percentile:			379	79	17	454	0.93	5.9	1.1
Range:		31	541	132	39	662	0.24	8.5	1.6
	7	N =	5	5	5	5	5	5	5
Median:		77	1237	352	126	1718	0.82	5.9	1.8
25 % percentile:			1139	333	63	1535	0.79	5.3	1.2
75 % percentile:			1299	428	199	1924	0.85	8.7	2.1
Range:		26	254	129	244	415	0.12	4.7	1.3
	8	N =	10	10	10	10	10	10	9
Median:		75	1385	528	85	2025	0.84	7.1	1.5
25 % percentile:			1056	193	14	1582	0.79	6.2	1.3
75 % percentile:			1854	637	167	2339	0.90	8.1	1.7
Range:		24	1359	764	189	1994	0.16	4.2	0.7

(cont.)

Table III (cont.).

	Cataract group	Age years	RTP	RDP	AMP	RTP+ RDP+ AMP	RTP + 1/2 RDP / RTP + RDP + AMP	L-lactate in lens	L-lactate lens / L-lactate aqueous
			μmol/kg					mmol/kg	
	9	N =	4	4	4	4	4	4	3
Median:		81	1186	402	87	1674	0.83	7.1	1.3
25 % percentile:			991	307	85	1385	0.82	5.5	
75 % percentile:			1498	477	132	2106	0.83	8.4	
Range:		6	627	213	62	900	0.01	3.5	0.4
	10	N =	2	2	2	2	2	2	2
Median:		70	500	117	22	638	0.86	6.1	1.3
Range:		4	466	65	25	506	0.11	7.8	1.5
	11	N =	5	4	4	4	4	5	4
Median:		68	624	226	30	876	0.84	4.8	0.9
25 % percentile:			438	78	7	442	0.80	4.1	0.8
75 % percentile:			1008	646	48	1752	0.88	8.2	1.1
Range:		18	954	689	44	1687	0.10	6.7	0.4
	12	N =	6	6	6	6	6	6	6
Median:		71	490	177	0	734	0.85	7.2	1.5
25 % percentile:			453	117	0	586	0.78	6.7	1.2
75 % percentile:			724	322	61	968	0.90	9.2	1.8
Range:		27	364	330	109	655	0.14	3.9	1.0
	13	N =	1	1	1	1	1	1	1
		77	467	54	41	562	0.88	4.3	1.0

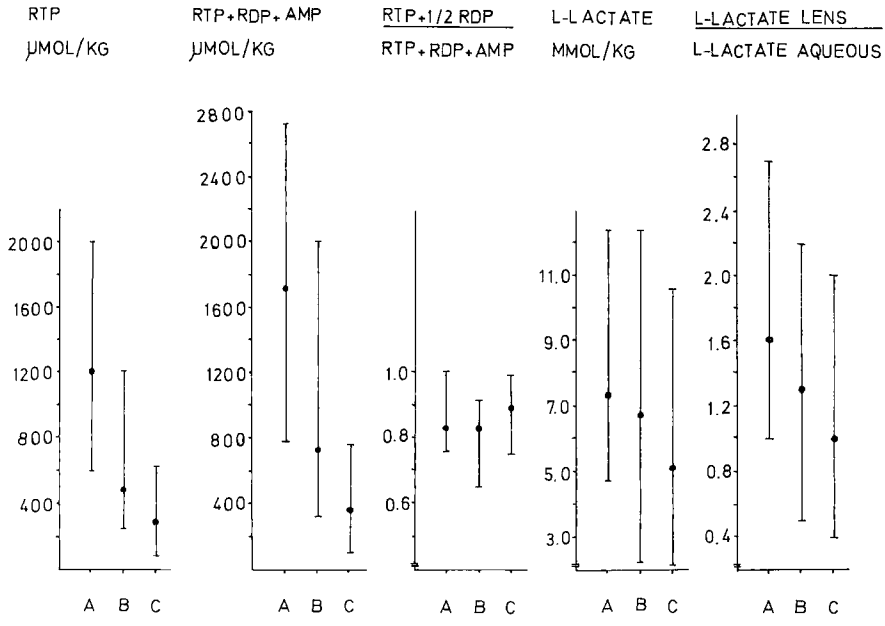

Fig. 1.

Distribution of some ribonucleotides, L-lactate, and the ratios of L-lactate in lens/L-lactate in aqueous in 3 groups of human senile cataractous lenses.

A: Immature senile cataractous lenses without anterior capsular/subcapsular opacity (33 lenses).

B: Immature cataractous lenses with anterior capsular/subcapsular opacity. See Fig. 2. (21 lenses).

C: Totally opaque lenses (26 lenses).

The concentrations refer to lens wet weight. The L-lactate concentrations of the aqueous humour were expressed per kg aqueous. The black dots represent the medians and the vertical lines represent the ranges.

RTP = ribonucleotide triphosphate. RDP = ribonucleotide diphosphate AMP = adenosine-5′-monophosphate. See "Methods". The ratio of RTP + 1/2 RDP/RTP + RDP + AMP represents the energy charge of the lens.

ture cataractous lenses without anterior capsular/subcapsular opacity had a median value of 1718 μmol/kg lens for 31 lenses. The range was 1944 (group A, Fig. 1). The immature cataractous lenses with anterior capsular/subcapsular opacity held lower ($P < 0.01$) total amounts (group B, Fig. 1: median = 734 μmol/kg lens, range = 1687, N = 20), while still lower values occurred ($P < 0.01$) in the group of totally opaque lenses (group C, Fig. 1: median = 359 μmol/kg lens, range = 662, N = 26).

The following observation indicates that the concentrations of the ribonucleotides are lower when anterior capsular/subcapsular opacity appears than when an isolated opacity of the posterior surface of the lens occurs: 7 lenses with isolated posterior subcapsular cataract had higher values for RTP (median = 952 μmol/kg lens) and for the total amounts of RTP + RDP + AMP (median = 1172 μmol/kg lens) than had 7 immature cataractous lenses with both posterior subcapsular and anterior capsular/subcapsular opacity (medians = 491 and 730 μmol/kg lens, respectively, $0.05 > P > 0.02$ in both cases.

When lenses of the same degree of immaturity were compared it was found that the ribonucleotide levels were correlated to the biomicroscopical state of the anterior surface of the lens: 26 lenses without anterior capsular/subcapsular opacity, grade 1 (see "Material") had a significantly higher ($P < 0.01$) median RTP value (1237 μmol/kg lens) than had 7 immature cataractous lenses with anterior capsular/subcapsular opacity, grade 1 (median = 624 μmol/kg lens). As for the total amounts of RTP + RDP + AMP, 24 immature grade 1 cataractous lenses without anterior capsular/subcapsular opacity (median = 1790 μmol/kg

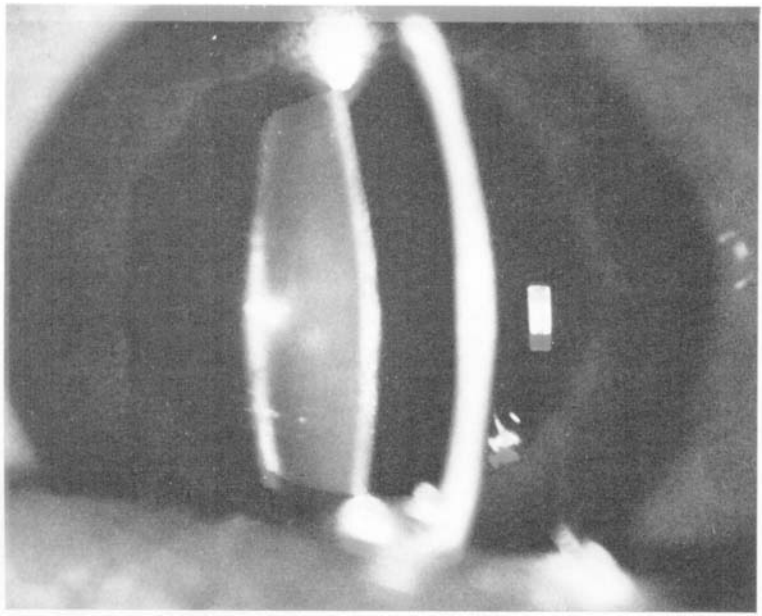

Fig 2.
Anterior capsular/subcapsular lens opacity (slit lamp photograph). The anterior surface of the lens appears to be wrinkled, uneven, and studded with white dots.

lens) had higher ($0.02 > P > 0.01$) values than had 7 immature grade 1 cataractous lenses with this opacity (median = 961 μmol/kg). 79 % of the lenses without anterior capsular/subcapsular opacity were grade 1, while only 33 % of the lenses with this opacity were grade 1.

As for the concentration ratio of RTP + 1/2 RDP/RTP + RDP + AMP, which represents the energy charge of the lens, no significant difference was found between immature cataractous lenses without (group A, Fig. 1: median = 0.83, range = 0.24, N = 31) and with (group B, Fig. 1: median = 0.83, range = 0.26, N = 20) anterior capsular/subcapsular opacity. No decrease in this ratio was found in totally opaque lenses (group C, Fig. 1: median = 0.89, range = 0.24, N = 26) as compared with immature cataractous lenses with anterior capsular/subcapsular opacity. On the contrary, the ratios of totally opaque lenses were significantly higher ($P < 0.01$). This may, however, be due to chance significance, since the difference between the medians of these two groups was very small.

As for L-lactate no significant difference was found between the concentrations of immature cataractous lenses without (group A, Fig. 1: median = 7.3 mmol/kg lens, range = 7.7, N = 33) and with (group B, Fig. 1: median = 6.7 mmol/kg lens, range = 10.2, N = 21) anterior capsular/subcapsular opacity. Most groups, including the aggregate groups of immature cataractous lenses with and without anterior capsular/subcapsular opacity, held significantly more L-lactate than did the group of totally opaque lenses (group C, Fig. 1: median = 5.1 mmol/kg lens, range = 8.5, N = 26). Only the group of pure nuclear cataract (median = 5.9 mmol/kg lens) and the group comprising immature cataractous lenses with anterior capsular/subcapsular opacity + posterior subcapsular + cortical + nuclear opacities (median = 4.8 mmol/kg lens) did not differ significantly from the group of totally opaque lenses as far as L-lactate concentrations were concerned.

The ratios of L-lactate in the lens/L-lactate in the aqueous were found to be higher ($0.02 > P > 0.01$) in eyes with immature cataractous lenses without anterior capsular/subcapsular opacity (group A, Fig. 1: median = 1.6, range = 1.9, N = 28) than in eyes with immature cataractous lenses with anterior capsular/subcapsular opacity (group B, Fig. 1: median = 1.3, range = 1.9, N = 20). Still lower values ($0.05 > P > 0.02$) for this ratio were found in eyes with totally opaque lenses (group C. Fig. 1: median = 1.0, range = 1.6, N = 25).

Lenses with pure cortical cataract did not differ significantly from lenses with pure posterior subcapsular or nuclear cataract in any of the five parameters depicted graphically in Fig. 1.

Immature cataractous lenses without anterior capsular/subcapsular opacity were tested for possible correlations to age of the five parameters in question. No such correlation was found.

In the present study the median pyruvate concentration of 15 human immature and totally opaque senile cataractous lenses was 56 μmol/kg lens. The 25 % and 75 % percentiles were 26 and 87 μmol/kg, respectively. The range was 148 μmol/kg lens. However, the decreases in absorbance recorded were so small that these figures cannot be considered reliable.

No normal human lenses were available.

Discussion

These experiments point to a correlation between the ribonucleotide levels and the biomicroscopical condition of the anterior surface in human senile cataractous lenses. This observation does not appear to have been reported before in the literature. Lenses with anterior capsular/subcapsular opacity in this study occupied an intermediate position between immature cataractous lenses without this type of opacity (higher ribonucleotide concentrations) and totally opaque lenses (lower ribonucleotide concentrations). However, the energy charges (ratios RTP + 1/2 RDP/RTP + RDP + AMP) of the lenses were no lower in immature cataractous lenses with anterior capsular/subcapsular opacity and in totally opaque lenses than in immature cataractous lenses without anterior capsular/subcapsular opacity.

We were not able to locate the site(s) of the anterior capsular/subcapsular opacity precisely. However, it does seem to be located close to the epithelium of the lens (Fig. 2, de Wecker 1886) which in clear lenses forms a single layer of cells beneath the anterior lens capsule. ATP consuming cation transport of the lens is assumed to take place chiefly in the epithelium (Kinsey & Reddy 1965; Harris & Becker 1965). It is noteworthy that totally opaque lenses, which have extensive anterior capsular/subcapsular opacity and low concentrations of ribonucleotides, have been found to have higher water and sodium contents, but lower potassium concentrations than have clear lenses and pure early cortical cataractous lenses (Maraini & Mangili 1973). In the present study immature cataractous lenses with anterior capsular/subcapsular opacity belonged mainly to the more opaque immature cataractous lenses. This may be due to a disturbance of the normal cation and water balance of these lenses. Anterior capsular/subcapsular opacity has not been seen in clear lenses of healthy eyes (Hess 1905; Vogt 1914, the author of the present study).

As far as the pure forms of nuclear, cortical, posterior subcapsular cataracts, and totally opaque lenses are concerned the ribonucleotide concentrations of the present study are comparable with those found for the same groups by Maraini et al. (1967) for RTP and Friedburg (1972) for RTP and RDP.

Most of the ATP production of the lens is supposed to be derived from glycolysis. Hockwin & Korte (1968) estimated that glycolysis accounted for 94 % of rabbit lens glucose consumption. Hockwin et al. (1971) estimated that 21 % of calf lens ATP was derived from the citric acid cycle. Since the adenine nucleotides have been found to make up about two thirds of the ribonucleotides of human senile cataractous lenses (Maraini et al. 1969 – concentrations referred to lens dry weight), and since ATP was found by the same authors to constitute two thirds of the ribonucleoside triphosphates, it would be interesting to compare the ribonucleotide concentrations with the glycolytic activity of the cataractous lenses *in vivo*. The ratio of lactate/pyruvate was found by Hohorst et al. (1961) to represent the oxidation/reduction state of the cytoplasmatic nicotinamide dinucleotide in liver cells *in vivo*. This ratio has been used as a parameter for glycolysis in the cornea (Reim & Lichte 1965; Reim et al. 1968) and in the aqueous humour (Schütte et al. 1972). However, in the present study, this ratio could not be determined precisely, since the pyruvate concentrations of the lenses were found to be so low that they could not be determined with sufficient accuracy. The figures obtained indicate, however, that the lens L-lactate steady state concentrations are, on an average, 100–200 times higher than those of pyruvate. This finding indicates a high rate of glycolysis in the cataractous lenses. Wu & Racker (1959) estimated the glycolytic activity of Ehrlich ascites tumour cells, which also have a high rate of glycolysis, by means of one measurement of the lactate concentrations of the cells.

In the present study the L-lactate concentrations were statistically identical in immature cataractous lenses with and without anterior capsular/subcapsular opacity, although there was a highly significant difference in the ribonucleotide concentrations of these groups. However, in totally opaque lenses low ribonucleotide levels and low L-lactate levels did occur simultaneously.

Murata & Okazawa (1972) found higher L-lactate mean values per kg lens wet weight than we did: 7.9 mmol/kg in 5 totally opaque, 17.6 mmol/kg in 4 nuclear cataractous lenses. As far as pyruvate is concerned, these authors also found higher concentrations than we did (mean values ranging from 647 to 1114 μmol/kg). As far as can be determined these authors gave no details about the accuracies of their methods (Biochemica Boehringer) in their laboratory (written in Japanese). No further information has been found in the literature concerning pyruvate and lactate concentrations in human senile cataractous lenses *in vivo*.

The ratio of L-lactate in the lens/L-lactate in the aqueous may give an impression of the balance between the lactate concentration in the water phase of the lens and the lactate concentration of the aqueous humour. The average water percentages of the cataractous lenses in question probably range from

66 % to 77 %, and the upper limit seems to be 88 % (Maraini & Mangili 1973). Therefore, if the L-lactate concentrations of the lens water and the aqueous humour were identical, an average ratio between 0.66 and 0.77 would be expected, and the maximal value for the ratio would be 0.88. The average value of the present material for the ratio was 1.0 in the group of lenses with the lowest ratios, viz. the group of totally opaque lenses (N = 25). In 3 cases the ratio was lower than 0.77. These findings indicate that even in totally opaque lenses there is a production of L-lactate, since the surplus of lactate in the lens water cannot be derived by diffusion from the aqueous humour.

The variations within the individual groups of cataract are large in this material and in the few comparable materials in the literature, as far as the parameters in question are concerned. This is probably not due to technical errors only (see Tables I and II). One possible explanation might be an omission to quantify the extent of the opacification within the individual lenses. E. g., one immature cataractous lens might have 10 % of its anterior surface affected by anterior capsular/subcapsular opacity, while another lens had 90 % of its anterior surface affected. Both were classified as immature cataractous lenses with anterior capsular/subcapsular opacity.

On the basis of the information presented in this study it seems justifiable to distinguish between 3 types of human senile cataracts. This distinction is founded on a correlation between biomicroscopical and biochemical findings:

1. Immature cataractous lenses without anterior capsular/subcapsular opacity: high levels of lens RTP, RTP + RDP + AMP, and L-lactate. High ratios of L-lactate in the lens/L-lactate in the aqueous.

2. Immature cataractous lenses with anterior capsular/subcapsular opacity: intermediate lens RTP, RTP + RDP + AMP. High L-lactate concentrations. Intermediate concentration ratios of L-lactate in the lens/L-lactate in the aqueous.

3. Totally opaque lenses, which all had extensive anterior capsular/subcapsular opacity: low values for lens RTP, RTP + RDP + AMP, L-lactate, and for the ratios of L-lactate in the lens/L-lactate in the aqueous. Possibly high ratios of RTP + 1/2 RDP/RTP + RDP + AMP (energy charge).

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Author's address:

A. Bruun Laursen,
Department of Ophthalmology,
Rigshospitalet, Blegdamsvej,
2100 Copenhagen Ø,
Denmark.