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LIPID COMPOSITION AND ($Na^+ + K^+$)-ATPase ACTIVITY IN RAT LENS DURING TRIPARANOL-INDUCED CATARACT FORMATION

GEORGE R. MIZUNO, CLIFFORD J. CHAPMAN, JACQUES R. CHIPAULT and DOUGLAS R. PFEIFFER

Hormel Institute, University of Minnesota, 801 16th Avenue N.E., Austin, MN 55912 (U.S.A.)

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The development of triparanol cataracts in rats is accompanied by the loss of lens ($Na^+ + K^+$)-ATPase activity and by alterations in the lens content and composition of phospholipids, sterols and phospholipid acyl groups. The lipid changes occur along the same time course as the loss of ($Na^+ + K^+$)-ATPase activity. Triparanol feeding produces a decrease in lens phospholipid content. The percentage contents of phosphatidylcholine and phosphatidylserine decrease while the content of sphingomyelin substantially increases. The amounts of oleic acid in lens phospholipids decrease while stearic and palmitic acids increase; however, these changes are relatively small. Sterol content is also decreased while the percentage content of desmosterol increases markedly. Feeding of the cataractogenic agents galactose and diazacholesterol also alters the lens lipid compositions and ($Na^+ + K^+$)-ATPase activity. A loss of phosphatidylserine is the only change in lipid properties which always accompanies a loss of the enzyme activity. The possible relationships between the lens content of phosphatidylserine, ($Na^+ + K^+$)-ATPase activity and the mechanism of triparanol-induced cataract formation are discussed.

Introduction

The importance of maintaining normal osmotic balance to the maintenance of transparency in lens tissue is widely recognized. During cataract formation of most types, the lens undergoes an uptake of Na^+ and loss of K^+ (see Ref. 1 for review). The total cation movements constitute a net increase in monovalent cation content which results in water uptake and swelling of the lens. The accompanying changes in refractive index contribute to opacification and the overall process probably contributes to subsequent membrane disruption and other degradative changes as the cataractous process proceeds.

The changes in monovalent cation content and composition may result from increased membrane permeability, decreased ($Na^+ + K^+$)-ATPase activity or a combination of both. ($Na^+ + K^+$)-ATPase is known to be depressed in galactose-induced cataracts [1–4] and in human senile cataracts [5]. It has been proposed that a failure of this enzyme may be the primary defect in certain animal hereditary cataract models [1,6].

The role of ($Na^+ + K^+$)-ATPase inhibition in the mechanism of cataract formation induced by triparanol, 4-chloro-α-(4-(2(diethylamino)ethoxy)phenyl)-α-(4-methylphenyl)benzeneethanol, is uncertain. In this type of cataract, the lens accumulates Na^+ and loses some K^+, effects which tend to reverse during spontaneous clearing following withdrawal of the drug [7–9]. While these effects are consistent with a critical role for this activity in the triparanol cataract, cation flux studies have indicated that an increase in the permeability of lens membranes to cations or inhibition of another Na^+ transporter may largely explain the altered distributions [8]. Furthermore, an enzyme staining technique failed to show any differences in the ($Na^+ + K^+$)-ATPase activities of normal, triparanol cataract and clearing lenses [10].

In this communication we report that ($Na^+ + K^+$)-ATPase activity is almost absent from triparanol cataractous lenses. This enzyme is known to require lipid

for activity and to display considerable specificity in the structural requirements for lipids to reactivate delipidated preparations (see Refs. 11–15 for review). Previous studies from this laboratory have established that significant lipid composition changes occur in triparanol cataract [16]. Since these changes might influence the $(Na^+ + K^+)$-ATPase as well as affect membrane permeability, we have extended our previous studies to other lipid parameters and compared the time courses of cataract formation, loss of $(Na^+ + K^+)$-ATPase and changes in lens lipid content and compositions. Changes in $(Na^+ + K^+)$-ATPase and lipid parameters in lenses made cataractous by other techniques have also been investigated to provide further insight into possible causal relationships. The results of those studies are presented here.

Experimental

Treatment of experimental animals. Triparanol cataracts were produced by feeding a pelletized diet of rat chow containing 0.075% triparanol. Weanling Wistar rats weighing approx. 50 g were maintained on this diet for 68 days and then returned to normal rat chow [8,17]. Galactose cataracts were produced by feeding a ground 50% galactose, 50% rat chow diet for an appropriate period of time (cf. Ref. 18) as described in the table legends below. These rats weighed approx. 100 g at the beginning of the feeding period. Diazacholesterol was administered to weanling rats by feeding a ground lab chow diet containing 0.067% diazacholesterol. 15 g of this mixture was provided per day, thereby restricting the daily drug intake to 10 mg [19]. The rats weighed approx. 50 g initially and were maintained on the diet for 42 weeks. Control animals were maintained on normal rat chow for the appropriate period.

Animals were sacrificed by diethyl ether administration and the lenses from seven or eight animals were removed and pooled to provide samples of adequate size. Capsules were immediately removed by dissection. Following wet weight determination, the lenses were homogenized in three volumes of deionized water at 4°C using a motor driven Potter-Elvehjem apparatus. Initial homogenization was carried out at a slow speed (approx. 150 rev./min) after which the pestle was stopped and adhering lens tissue removed with a spatula and returned to the homogenate. The speed was approximately doubled and the process was repeated. After a third cycle of homogenization at approx. 6000 rev./min, tissue no longer adhered to the pestle and aliquots of the homogenate were lyophilized and stored at −20°C until analysis.

Normal human lenses were obtained from autopsy material whereas cataractous lenses were from surgery patients. Four to six hours normally passed between the time of death or surgical removal of the lenses and the subsequent steps of sample preparation. The lenses were not decapsulated due to fragility; however, the procedures were otherwise the same as for the rat lenses.

$(Na^+ + K^+)$-ATPase activity. Lens $(Na^+ + K^+)$-ATPase was assayed essentially as described by Bonting et al. [3] with appropriate volume reductions to increase the assay's sensitivity. Weighed aliquots of the lyophilized homogenate were reconstituted with deionized water to give a 10% suspension (approximately a 4-fold dilution of the original water content). This suspension was further diluted with an equal volume of the assay solution [3] and the suspension incubated at 37°C for 1 h. We verified that the reactions were linear over the time course of the routine single point determinations. The $(Na^+ + K^+)$-ATPase activity was obtained as the ouabain-sensitive fraction of the total ATPase activity. The values presented are the mean of three determinations from the pooled lens samples. The triplicate determinations agreed with each other within ±5% of the mean value. The assayed activities of different homogenates from normal animals showed a comparable variation. To investigate the direct effects of triparanol on this enzyme the drug was added to the reconstituted homogenate in negligible volumes of ethanol. The suspensions were then sonicated for 2 min at 10°C and the resulting preparation assayed as described above. Preliminary studies established that the control enzyme activity was not diminished by this procedure and that the extent of inhibition produced by triparanol was maximal after 2 min sonication.

Lipid extraction. Water reconstituted suspensions of lyophilized homogenate were extracted with chloroform/methanol (1 : 1, v/v). Approx. 6 ml of solvent was employed per lens equivalent. The residue obtained by centrifugation was reextracted twice with the same solvent mixture and subsequently with chloroform/methanol (2 : 1, v/v). Solvent was removed

from the pooled extracts on a rotary evaporator and the extract subsequently partitioned by the technique of Folch et al. [20]. The remaining small amounts of proteolipid was dissociated by three repeated extractions with 4 ml of chloroform of the insoluble residue suspended in 1 ml of water [21].

Thin-layer chromatography. Lipids were separated by two-dimensional chromatography on Silica gel HR 60, essentially as described by Anderson et al. [22]. The second solvent mixture was changed to chloroform/methanol/acetic acid/water (135 : 60 : 18 : 3, v/v) as this provided improved resolution. Lipid fractions were located by spraying the plates with a 0.001% aqueous solution of rhodamine 6G, exposing to ammonia vapor and visualizing under ultraviolet light.

Phospholipids were identified by their R_F values and quantitated by phosphorus analysis [23] following acid digestion as described by Broekhuyse [24]. The values presented have been corrected for reagent and silica gel phosphorus blanks and are the mean of two or three determinations. The mol percentage of lipid phosphorus variation between the repetitive determinations was normally less than ±1.

Phospholipid acyl group composition. After separation of phospholipid classes by thin-layer chromatography and drying [25], phospholipid acyl groups were converted to the methyl esters by transesterification essentially as described by Rouser et al. [26]. Methyl heneicosanoate was added prior to drying and carried through the procedure to serve as an internal standard. The methyl esters were recovered by repeated extraction into hexane, the extract neutralized and the methyl esters determined by gas-liquid chromatography. A 6 feet × 1/8 inch column of 10% EGGS-X was employed and separations were conducted isothermally at 170°C. Peaks were identified by their retention times, either by direct comparison to known compounds or by the equivalent chain length technique when the required standard was not available. Quantitation was conducted graphically or by employing a microcomputer based integrating device. Care was taken throughout all procedures to maintain a nitrogen atmosphere so as to minimize the oxidative degradation of polyunsaturated fatty acids.

Other procedures. Lens sterol contents and composition were determined as described previously [16]. Dry weight determinations were carried out by drying aliquots of lens homogenates to constant weight over KOH in vacuo. Protein determinations were conducted by the technique of Lowry et al. as modified by Hess and Lewin [27]. Bovine serum albumin (Sigma fraction V) was utilized for protein standards. Glassware was routinely cleaned with 50% HCl. Solvents were redistilled shortly before use. The materials and reagents are available from commercial sources.

Results

The loss of ($Na^+ + K^+$)-ATPase in triparanol cataract

The effects of triparanol feeding on the incidence of cataracts and ATPase activities in lens tissue is shown in Fig. 1. Animals which received the drug during an initial period showed a high incidence of cataract development and an accompanying loss of ($Na^+ + K^+$)-ATPase activity compared to the animals fed a normal diet (Fig. 1A). The ($Na^+ + K^+$)-ATPase data were obtained for pooled samples of lenses, chosen from the experimental group at random, some of which were cataractous and some of which were clear. The activities are thus the mean for all animals at the time indicated. Other determinations (not shown) on cataractous lenses only, gave values substantially lower than the mean, indicating that formation of this type of cataract is accompanied by a nearly complete loss of this enzyme activity. In contrast, the total activity of other ATPase was only partially inhibited by triparanol feeding (Fig. 1B), suggesting that loss of the ($Na^+ + K^+$)-ATPase activity was not the result of nonspecific protein denaturation.

We investigated the possibility that loss of ($Na^+ + K^+$)-ATPase was due to direct inhibition by triparanol accumulating in the lenses. The content of the drug was not determined directly; however, with the solvent systems employed, its presence in significant quantities would have been noted as an extra component on the two-dimensional TLC plates. By that criterion, triparanol accumulation in lens was substantially less than 5 mol% with respect to the content of phospholipid. Fig. 2 demonstrates that triparanol at levels as high as 40 mol%, when incorporated by sonication into the lipids of the enzyme preparation, produced only a modest inhibition of the ($Na^+ + K^+$)-ATPase or of the other activities.

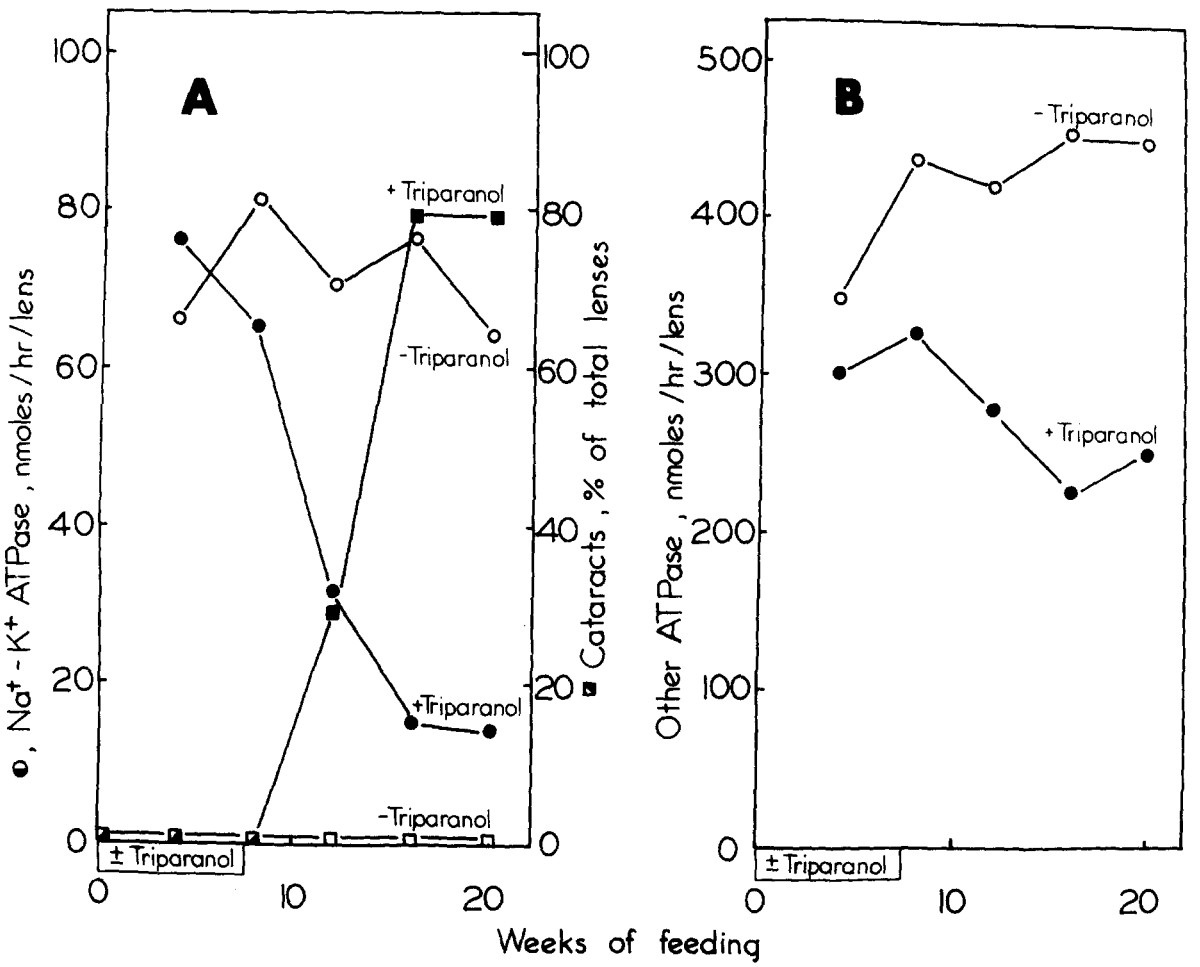

Fig. 1. The effect of triparanol feeding on ATPase activities of rat lens. Animals were fed a triparanol-containing diet or a normal diet for 68 days as described in Materials and Methods. Following this period all animals were fed normal chow. At each time point lenses from 7 or 8 animals chosen at random were pooled, homogenized and assayed as described in Materials and Methods. The incidence of cataracts at each time point represents that particular pool of lenses. Panel A: ■———■, the incidence of cataracts in triparanol-fed animals; □———□, the incidence of cataracts in normal animals; ●———●, $(Na^+ + K^+)$-ATPase activity in lens from triparanol fed animals; ○———○, $(Na^+ + K^+)$-ATPase activity in lens from normal animals. Panel B: ●———●, total non $(Na^+ + K^+)$-ATPase activity in lens from triparanol-fed animals; ○———○, total non $(Na^+ + K^+)$-ATPase activity in lens from normal animals.

The effects of triparanol on the lipid properties of lens

Since triparanol is classically an inhibitor of the conversion of desmosterol to cholesterol [28,29], and since cholesterol can activate delipidated $(Na^+ + K^+)$-ATPase [30,31], we examined the time course of changes in lens sterol content and composition induced by feeding the drug. The results are shown in Fig. 3. Triparanol produced a minor depression in the total sterol content which returned to the control levels after the animals were returned to the normal diet (Fig. 3A). In the normal animals, nearly all of the sterol present was cholesterol. As expected, triparanol feeding depressed the percentage cholesterol content and increased the level of desmosterol. Smaller amounts of earlier cholesterol precursors also accumulated, accounting for the difference between the decline in the percentage content of cholesterol and the increase in desmosterol content [16]. Like the effects on total sterol levels, the changes in composition tended to revert to control values after the drug was withdrawn (Fig. 3B).

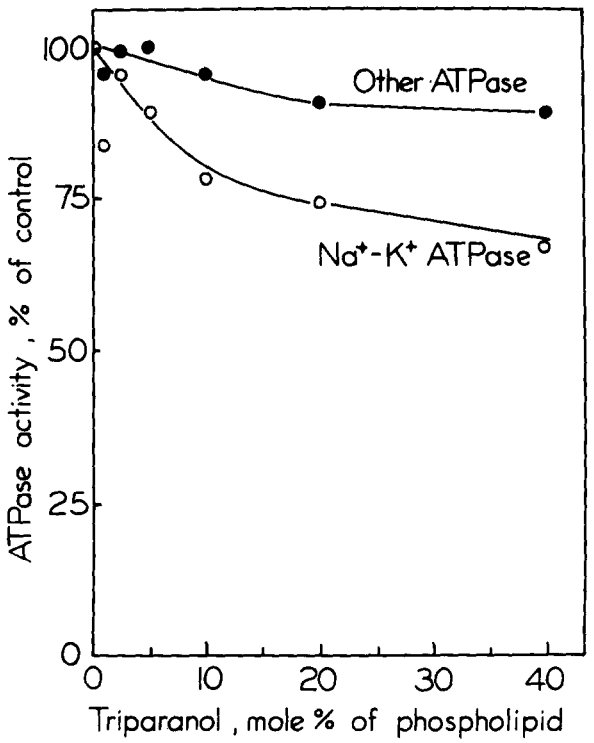

Fig. 2. Direct inhibition by triparanol of ATPase activities in rat lens. Determinations were conducted with material from normal lens as described in Materials and Methods. o———o, $(Na^+ + K^+)$-ATPase activity; •———•, total non $(Na^+ + K^+)$-ATPase activities.

The effects of cataractogenic agents on lens phospholipid composition and structure is of particular interest in view of the apparent requirement of $(Na^+ + K^+)$-ATPase for acidic phospholipid (see Refs. 11–15 for review) and of the alterations in membrane permeability which accompany cataract formation. As with the sterol content, the total phospholipid content is depressed by triparanol treatment but returns to normal after the drug is withdrawn, as shown in Fig. 4A. We have included in this panel data on the water content of triparanol and normal lens to allow the conversion of the data presented to other units.

Substantial phospholipid composition changes produced by triparanol feeding can be seen by comparing panels B and C in Fig. 4. Normal lens displays age-related alterations in phospholipid composition during the time of these experiments, as seen in panel B. There is normally a gradual loss of phosphatidylcholine and phosphatidylethanolamine, whereas phosphatidylserine and sphingomyelin gradually increase in terms of percentage composition (see also Ref. 32). Triparanol feeding eliminates the decrease in phosphatidylethanolamine while substantially accelerating the loss of phosphatidylcholine and accumulation of sphingomyelin (Fig. 4C). The acidic phospholipid phosphatidylserine declines in triparanol lenses rather than accumulating as in the normal tissue. The levels of two other minor acidic phospholipid components, phosphatidylinositol and phosphatidic acid, are not much affected by aging or by triparanol.

The alterations in phospholipid acyl group composition produced by triparanol are less dramatic than those in phospholipid composition, as seen in Table I.

TABLE I

CHANGES IN RAT LENS PHOSPHOLIPID ACYL GROUP COMPOSITION PRODUCED BY TRIPARANOL FEEDING

The abbreviations PC, PE, PS, Sph and PI are defined in the legend to Fig. 4. Determinations were conducted as described in Materials and Methods. The values presented are in percent of the total acyl groups in the indicated phospholipid class. There were no detectable changes (n.d.) in the PC acyl group composition. C, control level; T, triparanol-treated level.

Phospholipid class and acyl group	Percentage					
	9 weeks		23 weeks		46 weeks	
	C	T	C	T	C	T
PC	n.d.		n.d.		n.d.	
PE						
18 : 1	76.0	66.0	80.1	73.0	81.2	74.2
20 : 4	2.1	5.8	2.2	4.5	2.4	6.6
22 : 4	0.3	1.8	0.7	1.7	0.9	1.4
22 : 6	5.3	9.3	3.2	5.0	3.0	4.1
PS						
18 : 0	14.2	16.3	13.6	15.1	16.9	18.7
18 : 1	59.4	57.2	64.7	61.9	67.6	60.2
22 : 0	2.4	3.3	1.2	1.6	0.9	1.9
Sph						
16 : 0	52.0	66.0	55.4	64.8	53.2	58.9
22 : 0	2.7	1.6	1.9	1.5	2.5	1.8
22 : 1	3.7	3.2	2.2	2.9	2.3	3.1
24 : 0	2.0	1.4	3.2	1.7	3.9	2.4
24 : 1	20.0	17.7	22.3	18.5	27.5	24.0
PI						
18 : 0	9.8	12.8	7.6	14.2	9.0	12.9
18 : 1	37.7	37.2	39.7	32.0	41.5	27.7
20 : 3	5.6	3.4	11.4	3.0	11.8	8.9

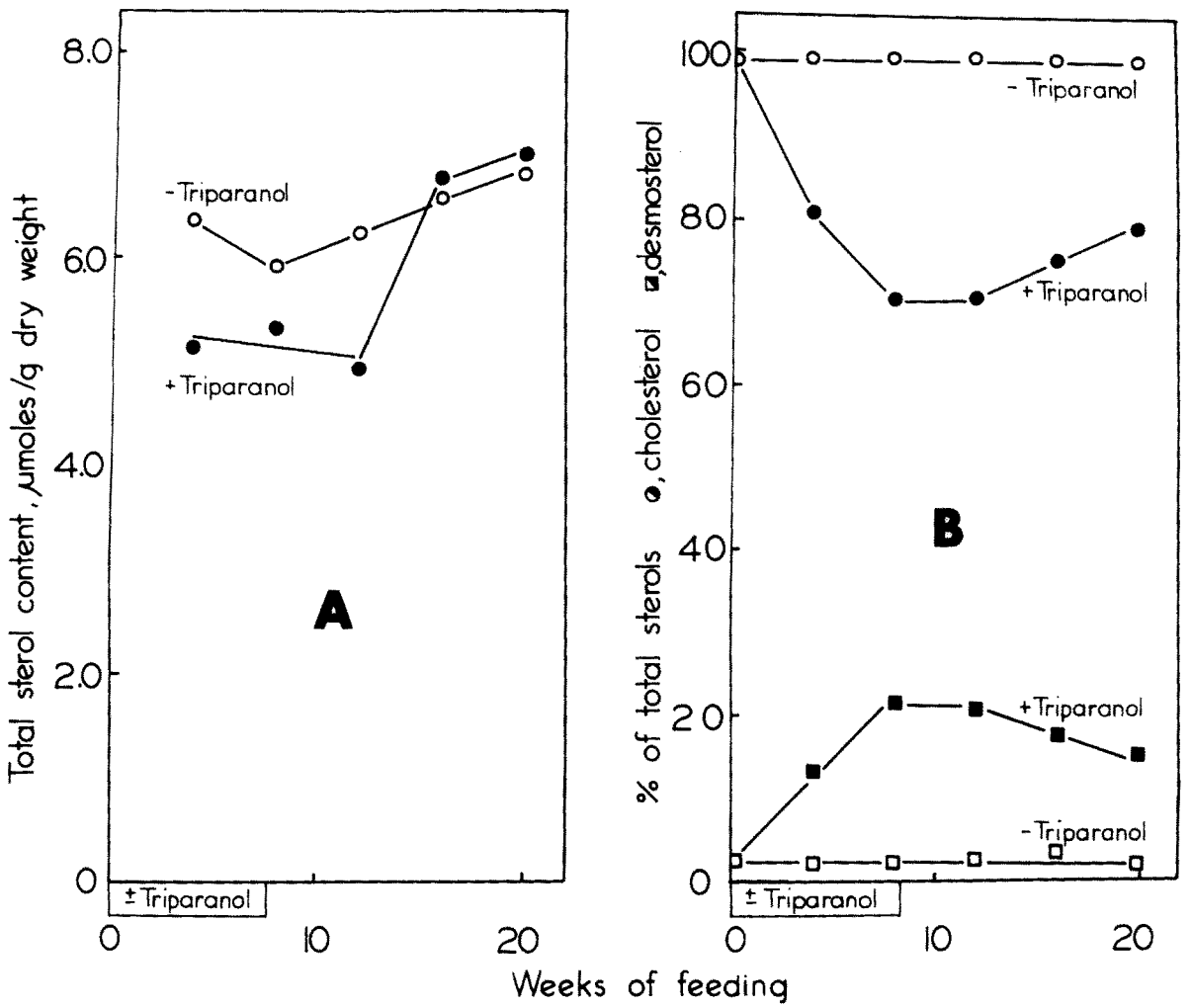

Fig. 3. The effect of triparanol feeding on lens sterol content and composition. Aliquots of the same pooled homogenates described in the legend to Fig. 1 were assayed for sterol content and composition as described in Materials and Methods. Panel A: ●——● and ○——○, the total sterol content in lens from triparanol-fed and normal animals, respectively. Panel B: ●——● and ○——○, the percentage of total sterols which was cholesterol in lens from triparanol-fed and normal animals, respectively. ■——■ and □——□, the percentage of total sterols which was desmosterol in lens from triparanol-fed and normal animals, respectively.

We have only included data of those fatty acids where the differences between normal and experimental values were clearly outside of the range of experimental error. The most substantial effects were seen in the amounts of oleic acid in phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol, which were decreased compared to normal animals of the same age, and the levels of palmitic and stearic acids in phosphatidylinositol and sphingomyelin, respectively, which were increased. Some of the alterations tended to revert slowly after the drug was withdrawn (e.g. 16 : 0 in sphingomeylin), whereas others were maintained or became more divergent (e.g. 18 : 1 in phosphatidylinositol) with no clear pattern emerging. It is interesting to note that phosphatidylethanolamine, phosphatidylserine and sphingomyelin contained 80% or more of saturated plus monounsaturated fatty acids. The same was true for phospha-

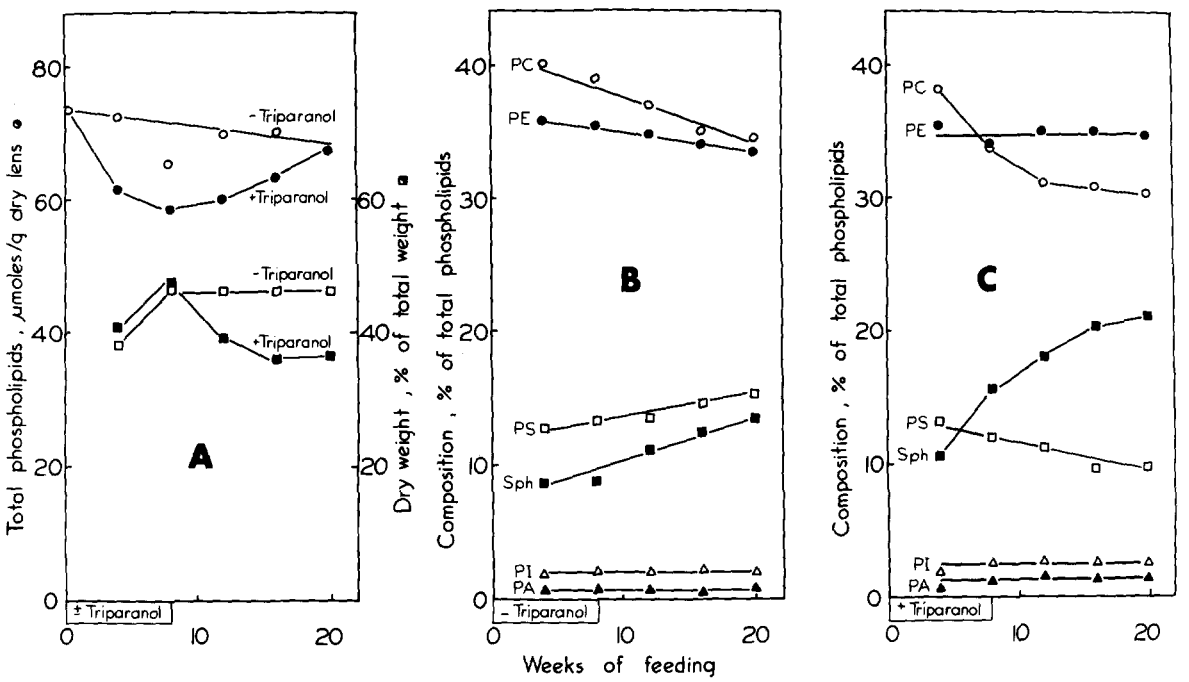

Fig. 4. The effect of triparanol feeding on lens phospholipid content and composition. Aliquots of the same pooled homogenates described in the legend to Fig. 1 were assayed for phospholipid content and composition as described in Materials and Methods. Panel A: ●——● and ○——○, total phospholipid content in lens from triparanol-fed and normal animals, respectively. ■——■ and □——□, dry weight percentage of wet weight (see Materials and Methods) of lens from triparanol-fed and normal animals, respectively. Panel B: the phospholipid composition of normal lens. Panel C: the phospholipid composition of lens from triparanol-fed animals. The individual components in Panels B and C are identified in the figure: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; Sph, sphingomyelin; PI, phosphatidylinositol; PA, phosphatidic acid.

tidylcholine (data not shown). Thus, lens membranes containing these phospholipids are unusually devoid of polyunsaturated fatty acids, which suggests a lack of fluidity (Mizuno, G.R., unpublished results).

The relationships between ($Na^+ + K^+$)-ATPase and lipid composition in other cataracts

Potentially, any of the changes in lens lipid properties of triparanol cataracts could be contributing to the inhibition of the ($Na^+ + K^+$)-ATPase. To gain further insight into these interrelationships, similar studies were carried out on lenses from animals treated with other cataractogenic agents and on human lenses which had developed senile cataracts. The data in Table II confirm that as with triparanol, there is a loss of ($Na^+ + K^+$)-ATPase from lenses made cataractous by galactose feeding; however, the progression of phospholipid changes are rather different.

Total phospholipid and phosphatidylcholine increase instead of decrease, whereas the increase in sphingomyelin and the changes in sterol properties produced by triparanol are not seen. Only the decline in phosphatidylserine is the same with both agents. The cataractogenic agent diazacholesterol was sufficiently toxic to prevent maintenance of animals on it for long enough to produce cataracts. However, its precataractous effects are significant. The ($Na^+ + K^+$)-ATPase is slightly activated compared to control values, as is seen in other tissues of animals receiving this drug [33]. The content of phosphatidylserine was essentially the same as in precataractous lenses from the galactose and triparanol fed animals; however, phosphatidylcholine and cholesterol were markedly depressed whereas sphingomyelin and desmosterol were markedly elevated. Together these results show a positive correlation (see Discussion) between changes in lens ($Na^+ + K^+$)-ATPase activity

TABLE II

THE EFFECT OF CATARACTOGENIC AGENTS ON $(Na^+ + K^+)$-ATPase AND LIPID COMPOSITION IN LENS

The abbreviations PC, PE, Sph, PS, PI and PA are defined in the legend to Fig. 4, LPE and LPC refer to 1-acyl lyso derivatives of phosphatidylethanolamine and phosphatidylcholine, respectively. Rat lenses were obtained from animals fed experimental diets containing the cataractogenic agent indicated. The designation 'clear' indicates that the diet had not yet resulted in cataract formation (20 and 266 days feeding for galactose and diazacholesterol, respectively). The designations 'IM' and 'M' for galactose refer to immature and mature cataracts, respectively, as judged by the degree of opacification and the size of the involved area. The immature cataracts were from animals which had been fed the galactose diet for 20 days. For mature cataracts, galactose was fed for 51 days followed by 34 days on normal chow. Galactose-fed animals were 1 year old and hence would have a higher control value for PS content (approx. 18%) than the weanling animals given the other agents. Each sample was composed of the pooled lenses from eight animals. All determinations were carried out as described in Materials and Methods.

Lens type	$(Na^+ + K^+)$-ATPase *	Phospholipid content and composition **									Sterol content and composition ***			
		Total	PC	PE	Sph	PS	PI	PA	LPE	LPC	Total	Cholesterol	Desmosterol	Other
Control	74.0	3.09	35.8	33.2	11.7	14.5	2.1	0.9	1.1	0.7	2.57	97.4	2.6	–
Triparanol														
Clear	39.0	2.50	27.5	34.7	20.7	11.9	2.8	1.6	0.8	–	2.37	70.9	23.8	5.3
Cataract	7.4	1.43	29.7	34.8	21.6	9.3	2.8	–	1.8	–	2.14	76.8	18.7	4.4
Galactose														
Clear	20.5	1.78	36.4	34.5	8.6	16.5	2.7	1.3	–	–	1.86	96.1	3.9	–
IM cataract	14.7	2.12	43.2	35.4	8.4	9.6	2.6	0.5	0.3	–	2.22	95.9	4.1	–
M cataract	5.4	2.53	41.1	35.5	10.3	8.0	2.7	1.4	0.5	0.4	3.57	98.2	1.8	–
Diazacholesterol														
Clear	93.0	3.29	22.0	32.9	29.0	12.2	1.5	1.5	1.0	–	3.29	32.9	65.4	1.8

* nmol P_i/h per lens.

** Total content in μmol/g wet weight, composition in percentage of total.

*** Total content in μmol/g wet weight, composition in percentage of total.

and phosphatidylserine content but no consistent pattern between the activity of this enzyme and the level of other lens lipids.

The relationship between phospholipid composition, ATPase activities and related parameters in human senile cataractous lenses are shown in Table III. Consistent with an earlier report [5], these lenses retain approx. 50% of $(Na^+ + K^+)$-ATPase activity compared to normal tissue of a similar age. These lenses did not show an elevated water content indicating that this remaining activity was adequate to maintain normal cation content and that failure of osmotic control is not the primary cause of this cataract. Also confirming earlier work [21], no significant alterations in phospholipid content were apparent. The normal lenses did show a high variation in sterol content and composition, which may represent normal variation since the patients were not known to be taking medication at the time of death.

Discussion

The data presented here demonstrate that development of the triparanol cataract is accompanied by loss of the $(Na^+ + K^+)$-ATPase activity in the cortex plus nuclear fraction of the lens. The failure of earlier studies to reveal this may be attributed to the less direct and sensitive approaches that were employed in those studies [8,10]. The loss of this activity is probably an early and pivotal step in the production of this experimental opacity since the net uptake of Na^+, with subsequent water accumulation (reversible with clearing), appears to be the immediate cause of swelling and loss of transparency [8,9]. The primary defect in the hereditary Nakano mouse cataract is also believed to be the loss of $(Na^+ + K^+)$-ATPase activity which is interesting since both abnormalities first appear as a nuclear opacification [6,9]. Most investigators agree that the epithelial layer $(Na^+ + K^+)$-

TABLE III

ATPase ACTIVITIES, LIPID COMPOSITION AND RELATED PROPERTIES IN HUMAN, NORMAL AND CATARACTOUS LENS

The abbreviations PC, PE, Sph, PS, PI, PA, LPE and LPC are defined in the legends to Fig. 4 and Table II. All determinations were carried out as described in Materials and Methods. ATPase activities are in nmol P_i/h per lens. Phospholipid and sterol contents are in μmol/g dry lens. Normal human lenses were obtained from autopsy material. Cataractous lenses were obtained at the time of their surgical removal. Each sample was composed of two lenses. With normal samples, both lenses were from the same individual. With cataractous samples, the two lenses were from different individuals. Data presented are the mean of three samples followed by the range observed between the lowest and highest values.

Parameter	Normal lens		Cataractous lens	
	Mean	Range	Mean	Range
Average age of lens (yr)	81	68–92	66	60–69
Average wet weight (mg)	250	230–260	210	200–220
Dry weight (%)	31.8	28.7–35.4	30.9	30.2–32.0
$(Na^+ + K^+)$-ATPase	361	308–413	180	157–224
Other ATPases	320	316–325	245	203–294
Phospholipid content	24.6	21.6–26.6	23.9	20.1–28.6
Phospholipid composition (%)				
PC	3.3	2.6–3.8	3.4	2.9–3.7
PE	10.6	9.7–11.1	9.0	8.1–9.7
Sph	61.4	56.2–64.5	63.3	61.5–66.4
PS	7.1	5.9–7.7	7.2	5.7–8.4
PI	4.8	4.2–5.2	4.2	3.5–4.9
PA	0.5	0.5–0.5	0.3	0.2–0.4
LPE	11.5	10.9–12.1	11.7	11.1–12.4
LPC	0.9	0.8–1.0	0.9	0.8–1.0
Sterol content	34.4	24.8–49.3	42.0	38.7–45.3
Sterol composition (%)				
Cholesterol	45.1	11.4–98.9	99.2	98.9–99.5
Desmosterol	54.9	1.1–88.6	0.8	0.5–1.1

ATPase is primarily responsible for maintenance of normal cation distribution in lens; however, the fiber cell activity may also be important (see Refs. 34 and 35 for reviews). The epithelial fraction of the activity was not investigated in this study whereas the data from the Nakano mouse cataract represents total lens activity. Further studies on the time course of the loss of this activity in individual lens fractions during the development of these two types of cataracts could help to clarify the relative importance of fiber cell $(Na^+ + K^+)$-ATPase in the maintenance of lens transparency.

If loss of $(Na^+ + K^+)$-ATPase is responsible for the development of triparanol cataract then it is not likely to be caused by denaturation secondary to general cell and membrane disruption. These are relatively late developments in the cataractogenic process whereas changes in lens cation content occur early, while cataract formation is still reversible. Loss of the $(Na^+ + K^+)$-dependent activity is much more rapid than the other ATPases which also argues against the importance of nonspecific denaturation. In view of the data in Fig. 2, it also appears unlikely that triparanol is a direct inhibitor of $(Na^+ + K^+)$-ATPase, although potentially an inhibitory metabolic product of the compound could be formed in vivo and be contributing to loss of the activity.

There remain several possible explanations for the loss of the activity. The enzyme is sulfhydryl dependent (see Ref. 36 for review) and since an oxidation and loss of both protein and nonprotein sulfhydryl groups occurs in this cataract [37], changes in the redox state of the tissue may be a contributing factor. Recent studies indicate that the lens enzyme, like that from other tissues, is inhibited by Ca^{2+} [38]. The nominal concentration of this cation increases to 5 mM in fully developed triparanol cataracts [9]. After the 8-fold dilution involved in the assay procedure, the final nominal concentration is in the range which significantly inhibits $(Na^+ + K^+)$-ATPase from other tissues (cf. Ref. 39). However this crude enzyme preparation still contains the full complement of lens proteins, phospholipids and metabolites which can potentially bind Ca^{2+}. The Ca^{2+} activity is therefore likely to be substantially lower than the nominal concentration making it difficult to evaluate the role of this inhibitor in the loss of activity.

The data presented here demonstrate substantial changes in lens lipid composition in this cataract which may also be contributing to the loss of $(Na^+ + K^+)$-ATPase. There are substantial data demonstrating the importance of lipid for the maintenance of this activity although the degree of specificity for the lipid required remains controversial. Early studies indicated high specificity for phosphatidylserine or for acidic phospholipid (cf. Ref. 40). Thus, it is of particular interest that changes in lens content of phosphatidylserine are most closely correlated to changes in $(Na^+ + K^+)$-ATPase activity. If it is assumed that a linear relationship between $(Na^+ + K^+)$-ATPase activity and phosphatidyserine content should exist, then a regression analysis between these parameters using the data from Fig. 1, panel A and Fig. 4, panel C yields a positive correlation coefficient of 0.96. This value remains positive but is reduced to 0.59 when the data from triparanol and galactose fed animals in Table II are considered together and is further reduced to 0.21 when the values from the diazacholesterol fed animals are also included. Diazacholesterol in particular is known to have other effects on membrane properties which alter the activity of $(Na^+ + K^+)$-ATPase [19,33]. Including these data in the regression analysis is therefore of doubtful value. The lipid alterations produced by diazacholesterol feeding remain of interest however since they tend to rule out a loss of phosphatidylcholine or the accumulation of sphingomyelin and desmosterol as factors contributing to the loss of enzyme activity resulting from the other cataractogenic agents.

Although there is a good correlation between $(Na^+ + K^+)$-ATPase activity and phosphatidylserine content in the triparanol-fed animals, the value of this analysis remains uncertain. The relationship between enzyme activity and acidic phospholipid content might not be linear. More recent work has indicated that the phosphatidylserine requirement is actually a requirement for negative membrane surface charge and may be more significant for the reactivation of delipidated enzyme than for isolated enzyme associated with boundary lipid (Ref. 40 and references therein). Studies employing enzymatic modification of phospholipid associated with $(Na^+ + K^+)$-ATPase indicate that the acidic phospholipid must be severely depleted before a loss of activity becomes apparent [40,41]. Regional depletion of phosphatidylserine in lens is a possibility since the high cholesterol and

saturated fatty acid contents should result in low fluidity and the potential existence of microdomains in the membrane. These findings emphasize that the relationship between activity and phosphatidylserine content could be complex.

The alterations in lens lipid composition and $(Na^+ + K^+)$-ATPase activity occurring in triparanol cataracts may also occur in cataracts induced by other agents such as chloroquine, chlorphentermine and iprindole (cf. Refs. 42 and 43). All of these compounds belong to a large group of amphiphilic pharmacological agents which share the ability to interfere with lysosome function and polar lipid degradation in many types of tissues (see Refs. 44 and 45 for review). These common properties suggest a common mechanism of cataract formation. Regardless of whether or not the lipid changes associated with development of the triparanol and other cataracts contribute to loss of $(Na^+ + K^+)$-ATPase, the alterations demonstrate that the highly differentiated fiber cells still retain considerable ability to metabolize lipid and alter their membrane lipid composition. The accelerated accumulation of sphingomyelin and loss of phosphatidylcholine produced by triparanol and diazacholesterol feeding may represent an acceleration of a process normally occurring with aging as this reciprocal alteration of composition normally proceeds as the lens grows older [32].

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