Reversal of triparanol-induced cataracts in the rat

II. Exchange of $^{22}\text{Na}$, $^{42}\text{K}$, and $^{86}\text{Rb}$ in cataractous and clearing lenses

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The cataract which develops in the rat fed triparanol is accompanied by a marked increase in sodium and a more modest decrease in the potassium. Sodium is excreted as the lens clears and potassium returns to normal. Initially triparanol causes an increase in permeability to cations. This appears before the lens becomes cataractous. Permeability returns to normal as the lens clears. In the cataractous lens the cation pumps are accelerated. The data are compatible with the view the there are two sodium pumps, one which is ouabain insensitive and excretes sodium plus chloride from the lens, and another which is ouabain sensitive and is involved in exchange of equivalent units of sodium and potassium. The cataract develops when the ouabain-insensitive pump is either overwhelmed by the increased permeability or ceases to function.

Key words: rat, lens, triparanol, cataract, cation transport, permeability

In a previous paper we reported that cataracts produced in rats by the ingestion of a cholesterol-reducing drug, triparanol (MER-29), were reversible in some animals. Gross and biomicroscopic examinations of the lenses of animals during the period of drug ingestion and for several months after removal of the drug from the diet revealed that the lenses passed from transparency through a stage of dense opacity involving only the nucleus or both the nucleus and the cortex and, finally, over a period of some months, some of the opacities became less dense until grossly the lens was transparent and when examined with the slit lamp only a very few vacuoles remained.

As is true of most lenses, the development of the cataract was associated with a marked hydration. There was a concomitant increase (as much as tenfold) in the sodium content of the lens. However, the potassium content decreased only slightly in contrast to cataractous lenses produced by other techniques, (e.g., galactose feeding). As the triparanol cataracts cleared, the sodium and water were excreted from the lens while the potassium content remained fairly constant. It was our conclusion that the in-
flux of water and subsequent excretion was due to the changes in total base which was reflected by the sodium movement probably accompanied by chloride.

Since under most experimental circumstances potassium is lost when sodium is gained, the finding of an exception to the rule should help to further clarify knowledge of the factors involved in control of cation content of the lens. This paper is concerned with exchange of labeled cations in cataractous and clearing lenses.

Methods and materials

Weanling Wistar rats weighing 30 to 50 grams were fed a diet containing 0.05 or 0.075 per cent triparanol for 67 to 69 days. The rat chow containing triparanol was prepared by General Biochemicals. Rats were returned to the basic chow without the drug after the 67 to 69 days. The 67 to 69 day time period previously had been found to be optimum for producing a high degree of cataractogenesis followed by a considerable degree of clearing in a large number of the lenses within six to eight months following removal of the drug from the diet.* A comparable number of control rats fed the same chow without triparanol were followed throughout the experiment. This was necessary, as we had previously found that the potassium content decreased with age. For the purpose of this paper, lenses will be divided into three groups: (1) controls; (2) cataractous, those with a dense nuclear opacity and with either a clear or a hazy peripheral zone; (3) clearing, those which were previously grossly cataractous but which showed a marked degree of clearing.

This included grossly clear ones, ones with a suggestion of haze throughout, and those with a hazy nucleus. Slit lamp changes consisting largely of opacities along the posterior sutures were often seen some weeks prior to development of the gross opacity. Like the gross opacity of the galactose cataract, that of the triparanol-fed animal appeared suddenly, usually overnight. The clearing process, on the other hand, was slow, occurring over a period of weeks or months.

It should be emphasized that there was considerable variability in the time at which cataracts developed and when they cleared. Thus many of the cataracts developed after the experimental group was returned to a diet free of triparanol. This is in contradistinction to the mature galactose cataracts which, if not present, do not go on to develop after the animal is returned to a normal diet.

The radioisotope under study was incorporated into the modified* Tyrode's solution we have normally used for in vitro lens culture. The concentration of tracer was so small that further changes in the media were unnecessary. In uptake studies using $^{45}K$, $^{22}Na$, and $^{40}Rb$, lenses were incubated at $37^\circ$ C. in 2 ml. of the appropriate medium for the desired period of time, rinsed with three drops of nonlabeled media or water, rolled on gauze pads to remove excess moisture, weighed into tared tubes, and counted in a Packard Tri-Carb gamma spectrometer. Lenses were then dried to constant weight, reweighed, charred with the aid of a drop of concentrated sulfuric acid,ashed in the muffle furnace, and sodium and potassium determined on the ash with an IL flame photometer. Aliquots of the bathing media were also counted at the end of the experiment. Where indicated, varying concentrations of ouabain were sometimes added to the media bathing one of a pair of lenses.

The efflux studies were done in the manner described by Becker and Cotlier. Lenses were loaded with the isotope by incubating for one hour at $37^\circ$ C. in 2 ml. of media containing the isotope under consideration. They were then carefully removed, rinsed with three drops of nonlabeled media, and incubated in 2 ml. of nonlabeled media for an additional hour. Where appropriate, ouabain was added. Aliquots of media, 0.1 ml., were removed at 15, 30, and 45 minutes and counted, and a 1 ml. sample was counted at the end of 60 minutes. At the termination of the incubation, lenses were weighed into tared tubes and counted. The total isotope taken up was assumed to be the sum of the counts remaining in the lens at the end of the experiment plus the counts recovered from the media.

Where possible lens-media specific activity ratios are reported instead of the conventional counts per milliliter lens H$_2$O-counts per milliliter media. This is done because experimental cataractous lenses were hydrated, dry weights were decreased, while total wet weights and actual sizes of the cataractous lenses were often greater than the normal control lens of the same age. Cataractous lenses had a marked increase in sodium content with only a slight decrease in potassium content so that the conventional lens-media ratio presented a misconception of the data.

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*It should be pointed out that a similar diet fed to young rabbits did not produce cataracts even after a more prolonged feeding. Unlike rats, the rabbits continued to thrive in the same manner as did the controls.

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*Specific activity ratio = \( \frac{\text{Cts.} / \text{ml lens H}_2\text{O}}{\text{Cts.} / \text{ml media}} \)
Results

Accumulation of $^{22}$Na. In Fig. 1 sodium and potassium content of fresh control lenses of comparable age is given for comparison, as well as the cation content and $^{22}$Na uptake of control, cataractous, and previously cataractous but clearing lenses of triparanol-fed rats. Although the controls for the clearing lenses were older by several months than those for cataractous lenses they behaved similarly and hence were grouped together. With the use of sodium and potassium content of fresh lenses as a reference, it appeared that the manipulation and incubation had no effect on the cation content of control lenses. Sodium content of triparanol-induced cataractous lenses was significantly higher and the potassium content significantly lower than those in the controls. In our original studies the potassium content of cataractous lenses was slightly decreased but not to this extent. Therefore, one must consider that further sampling might have revealed lower potassium content or that cataractous lenses were more susceptible to potassium loss with manipulation and incubation.

The lens-media specific activity ratios of cataractous lenses were less than that of controls. Since the sodium content of the cataractous lenses was five times that of the controls, it follows that the exchange of sodium ions across the lens barriers was considerably more rapid in the cataractous than in the control lens. The $^{22}$Na lens-media ratio per unit water was higher in cataractous lenses than in controls as would be expected because of the increased sodium content. A rather surprising finding was that the specific activity ratio of the clearing lenses had reached (even exceeded) unity after one hour of incubation and no further change occurred in it or in the lens-media ratio after three hours' incubation. The sodium content was significantly greater in these lenses than in control lenses but it was definitely approaching normal levels.

Accumulation of $^{42}$K. Cation content and $^{42}$K uptake studies are given in Fig. 2. As was noted with the previous data, sodium and potassium content of clearing lenses was similar to those of controls although not yet normal, while the sodium content of cataractous lenses was markedly increased and that of potassium was decreased. The $^{42}$K specific activity ratio was
similar in control and clearing lenses but was higher in cataractous lenses. This was the reverse as noted with $^{22}\text{Na}$ uptake studies and suggests an increased potassium transport. The lens-media ratio per unit water was lowest in cataractous lenses. This results from two factors: first, there was a definite decrease in potassium content, and, second, the calculation was based on wet weights and the cataractous lens was markedly hydrated.

**Effect of ouabain.** The effect of $1 \times 10^{-4}$M ouabain on cation content and $^{22}\text{Na}$ uptake of cataractous and clearing rat lenses is presented in Fig. 3. This concentration of ouabain had the predictable effect of increasing the ratio of specific activities of control lenses by interfering with the sodium pump. By contrast, the ouabain-sensitive sodium pump appeared to be eliminated or markedly reduced in the cataractous or clearing lenses where ouabain had essentially no effect on the specific activity ratios. This is probably illusory as is noted in the discussion. No effect of ouabain would be expected in the clearing lens, since in media without ouabain the lens-media ratio of specific activities had already exceeded unity in one hour. In the cataractous lenses it seems likely that the flux of sodium was so rapid that the portion which was ouabain sensitive was within experimental error.

This was confirmed by studying the effect of $5 \times 10^{-6}$M ouabain on the efflux of $^{22}\text{Na}$ from isotope loaded lenses (Fig. 4). The higher uptake of $^{22}\text{Na}$ in the cataractous and clearing lenses attests to the greater permeability of the cataractous and clearing lenses to sodium. This concentration of ouabain had a significant effect on the efflux of $^{22}\text{Na}$ from the cataractous and clearing lenses.

Ouabain had a pronounced effect on the uptake of $^{42}\text{K}$ as judged by measurements of the specific activity (Fig. 5). Indeed it appeared to have a more profound effect on potassium transport in the cataractous than in the control lenses. This is in keeping with the conclusion that the active transport of potassium was actually stimulated in the cataractous lenses. The clearing lenses behaved in a manner similar to that of the controls.
Effect of $1 \times 10^{-4}$M ouabain on $^{22}$Na lens-media specific activity ratio in rat lenses. Each bar for the control and clearing lenses represents an average of nine or more lenses. In the cataractous group, an average of four lenses comprised the one-hour data and average of six lenses made up the three-hour data.

Fig. 3. Effect of $5 \times 10^{-4}$M ouabain on $^{22}$Na efflux in rat lenses. With one exception the data given are based on the average of eight or more lenses. The exception, effect of ouabain on clearing lenses, is based on four lenses.

**Accumulation of $^{86}$Rb.** The uptake of $^{86}$Rb by the control, cataractous, and clearing lenses is shown in Fig. 6. Since specific activities could not be measured, the data presented are ratios of counts per milliliter of lens water to counts per milliliter of medium. The uptake of $^{42}$K is shown in similar units for comparison. Generally, the uptake of the two isotopes was similar in the control and clearing lenses, although different in magnitude for $^{42}$K and $^{86}$Rb. The ratios of the two isotopes in the cataractous lens were less for both $^{42}$K and $^{86}$Rb. This may reflect the increased water content or the decreased potassium. In an attempt to determine which was the variant, we refrigerated rat lenses for 18 hours (Table 1). To our surprise they did not behave like rabbit lenses which simply exchange sodium for potassium and do not hydrate. The lens-media ratio for the refrigerated lenses was considerably lower. The data suggest but do not prove that the potassium level is the determining factor. This is under further study at present.

In any event, the smaller uptake of $^{86}$Rb by cataractous lenses was not due to decreased active transport. Studies of $^{86}$Rb efflux demonstrated an increased permeability to cations (Fig. 7). When uptake was inhibited by ouabain, 10.8 per cent of the total counts in the control lens ef-
Fig. 5. Effect of $5 \times 10^{-4}$M ouabain on $^{42}$K lens-media specific activity ratio. Data for effect of $5 \times 10^{-4}$M ouabain on cataractous and clearing lenses were based on an average of four lenses; the remainder of the data represents averages of seven or more lenses per group.

Fig. 6. Cation content and $^{42}$K and $^{86}$Rb lens-media ratios per milliliter water in rat lenses. $^{42}$K data based on averages of seven or more lenses. With one exception, data for $^{86}$Rb are based on averages of six or more lenses. The exception, the one-hour value for clearing lenses was based on four lenses.

fluxed in one hour in contrast to 46.7 per cent effluxed in the cataractous lens. Adding this efflux to the total counts taken up in one hour, it can be calculated that the active transport of $^{86}$Rb was stimulated by 38 per cent. A similar estimate from the specific activity ratios of $^{42}$K suggests the potassium transport to be increased by 33 per cent with the use of the one-hour figure and 47 per cent with the three-hour value.

The clearing lenses and their controls were several months older than cataractous lenses and their controls. They took up less label. Ouabain had little effect on the run out as compared to younger lenses. This
was in contrast to the $^{42}$K uptake by clearing lenses which was inhibited by ouabain. The reason for this discrepancy is not clear.

The above studies have indicated that a major feature of the triparanol cataract is an increased permeability to cations. In order to ascertain when this increase appeared, $^{86}$Rb efflux studies with ouabain on control lenses and on lenses from animals on triparanol for varying periods of time were done (Fig. 8). The percentage of $^{86}$Rb effluxed from the control lenses was remarkably consistent for all intervals sampled. By the second week the one-hour rubidium efflux of lenses from triparanol-fed rats was double that of the controls and by five weeks (at about the time first changes were noted by slit lamp in some of the lenses) it was five times greater than that of the controls. After the triparanol was removed from the diet and the lens changes had progressed to mature cataracts, the $^{86}$Rb efflux remained at essentially this level. Some eight months later, when lenses which had previously been grossly cataractous and were then quite clear, the efflux returned to essentially the same value as the control lenses. This is convincing evidence that the increased permeability was reversible.

**Discussion**

The results here presented are compatible with the following explanation of the observed marked increase in sodium and
relatively small loss of potassium in the triparanol cataracts. As triparanol is fed the lens becomes increasingly permeable to cations. This is initially compensated for by an increased active transport of both potassium and sodium. A similar increase in active transport of potassium and rubidium was observed by Kinoshita and associates in the lens cultures in media containing galactose. Cotlier and co-workers observed an increased active transport of rubidium into swollen lenses bathed in hypotonic media. At some stage just prior to the development of the cataract, the sodium pump is overwhelmed and sodium and probably chloride increase markedly. Conversely, the increased active transport of potassium compensates fairly well so that a relatively small decrease in potassium is noted. As cation permeability returns to normal sodium is extruded and the lens clears.

Whittembury and Proverbio present convincing evidence for two sodium pumps, one which is capable of excreting sodium together with chloride and which is sensitive to ethacrynic acid but not to ouabain. This pump controls the cell volume. The second pump which is ouabain sensitive involves an exchange of sodium for potassium. In the cataract which develops following triparanol feeding, there is a marked increase in sodium and presumably chloride. This may be caused by a failure of the ouabain-insensitive pump or may simply mean that the pump is overwhelmed. As permeability returns toward normal, sodium plus chloride are excreted and lens water is reduced.

Our failure to observe an ouabain-sensitive sodium exchange in the cataractous lenses may simply be due to the fact that the turnover of sodium is so rapid that the potassium-linked exchange is not observed. Sodium efflux data do show a ouabain effect. It seems likely than that the ouabain-sensitive linked potassium and sodium exchange does not fail.

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REFERENCES


