Table III

Exp. No.	Pupil diameter Experimental/Control	Pre-Drug Gas (Minutes)	Dose (mg.)	Pupil diameter experimental/						
				0	5	10	15	20	25	30
17	1.00	55	1.0			1.04				1.08
18	1.03	150	1.8	1.00						1.00
19	1.05	120	2.1		1.12		1.06			1.00
20	1.00	30	2.1	1.00		1.00		1.00		1.07
21	1.00	60	2.1		1.06	1.06		1.10		1.00
22	1.00	60	2.1		1.07	1.05		1.16		1.07

*Goggles removed.

the relative anaerobiasis of lid closure or gas goggles increased the permeability of the cornea to eucatropine.

Administration of atropine systemically and the use of nitrogen goggles avoided both the problem of corneal permeability alterations and of possible drug trapping in the conjunctival sac. The drug must have reached both irises at the same time and in the same amount. Any difference in pupil size of the two eyes must be attributed to difference in reactivity of the drug. The anaeorobic eye developed the larger increase of lactate content of the aqueous from inhibition of aerobic glycolysis in the cornea.[2] Increased lactate should decrease pH of the aqueous. Lowered pH should suppress ionization of atropine, and the unionized form, being more fat soluble, should penetrate cell membranes more readily and thus be more active.[3]

The human rather than the rabbit or cat was chosen as experimental subject because of the approximately equal bilateral distribution of pupillomotor nerve impulses, a distribution not found in many lower animals. Thus, for these experiments it was not necessary to control illumination or accommodation, for all pupillomotor impulses from these stimuli are equally distributed to the two eyes in primates.

The finding that lid closure enhances the mydriatic action of atropinic drugs may find use in therapeutics when maximum mydriatic effect is desired, as in breaking posterior synechias. It is suggested that in such cases the eye be bandaged between applications of atropine.

It is noted in passing that slight, transient slowing of the pulse rate was found in some subjects after systemic atropine while acceleration of the pulse was not found. This is contrary to the statement that atropine in comparable dose increases the pulse rate.[4]

Mydriatic effect of atropinic drugs is enhanced by lid closure and by nitrogen goggles. The enhancement is noted both with topical and with systemic administration of the drug. The enhancement is attributed to acidification of the aqueous resulting from corneal anaerobiasis.

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Cyanate effects on the lens in vitro. JIN H. KINOSHITA AND LORENZO O. MEROLA.

Recently, it has been shown that when red cells from patients with sickle cell disease were incubated with cyanate, the cells were inhibited from sickling upon deoxygenation.[1, 2] The cyanate inhibition was irreversible. Since cyanate can carbamylate other proteins besides hemoglobin the possibility exists that cyanate may adversely affect the proper functioning of a cell. However, this does not appear significant since it was shown that a number of enzymes of the glycolytic pathway are not affected to any extent by cyanate.[2] In addition, the effect of cyanate did not alter the levels of the nucleotides.[2] Cerami[2] states that animals can be given daily doses of cyanate without adverse effect. Since no specific effects on the lens have been mentioned in these studies,

control time after drug (minutes)

35	40	45	50	55	60	65	70	75	80	85	90	95	100
		1.04					1.01				1.07		
		1.07			1.00								
		1.12			1.15	°	°	1.06°					
1.03	1.03	1.11	1.14	1.07	1.10	1.14		1.00	1.00		1.00	1.00	1.02
	1.02				1.00	1.00	1.00		1.00				1.00
	1.07		1.14	1.15	1.07			1.15	1.15	1.25	1.12°		

we thought it would be worthwhile to study the effects of cyanate on the isolated lens.

Rabbit lenses weighing approximately 170 mg. obtained from animals weighing about 1.5 pounds were used in these experiments. The organ culture procedures, description of the medium, glutathione determination, and the rubidium uptake experiments are given in previous reports.[3-5]

In the isotope experiments, aliquots of medium and of the trichloroacetic acid filtrates of the lenses were taken for counting radioactivity.[3-5] The results were calculated as the L/M ratio, that is the concentration of ^{86}Rb in the lens compared to that of the medium.[6] Potassium cyanate purchased from Baker and Adams was used in these experiments.

It was obvious that the lens were adversely affected when incubated in 10 mM. cyanate for an overnight period. At this level of cyanate, the lens turned cloudy and appeared swollen. Analyses revealed that large increases in water and in electrolytes occurred. The effects at lower levels of cyanate were therefore studied. The minimal concentration that caused definite visible lens changes after 24 hours of incubation was at 2 mM. At this level, a ring of opacities around the periphery was found (Fig. 1). At 5 mM., almost a complete opacity occurred, except for the clear central region and the suture line (Fig. 1). Only a faint ring of opacity was observed at 1 mM. and no change in appearance was found at 0.5 mM. of cyanate. It does appear that the cyanate has some drastic effects on the incubated lens at millimolar levels.

As first shown by Becker,[6] the ability of the lens to accumulate rubidium (Rb) is a good index of the effectiveness of the cation pump mechanism. This method was used to further study the cyanate effects on the lens. The lens was incubated in cyanate for a 24-hour or a 48-hour period, after which ^{86}Rb ion was added to the medium and the lens was allowed to accumulate Rb during an additional 3-hour period. The control lenses incubated for 3 hours in medium containing ^{86}Rb ion were able to concentrate this cation so that the (L/M ratio was 4.0 ± 0.2 (S.D.). As shown in Fig. 2, the lenses

incubated in cyanate show a significant decrease in the ability of the lens to concentrate rubidium. After 24 hours in cyanate at 2 to 5 mM., a 25 to 35 per cent decrease in L/M ratio was observed. Prolonging the exposure to 48 hours further accentuated the cyanate effects. The accumulation of rubidium was reduced even at 0.5 mM. cyanate and there was a progressive decrease in the ^{86}Rb recovered in the lens with increasing levels of cyanate. At 5 mM. over a 50 per cent decrease in accumulation of Rb was observed. The effect of cyanate on the lens glutathione content was also determined. After 24 hours of exposure to cyanate at 1 mM. there was a slight lowering of 15 per cent of lens glutathione that gradually dropped to 25 per cent at 5 mM. of cyanate.

Cerami and Manning[1] reported that 80 per cent of the red blood cells (S/S) could be inhibited from sickling by potassium cyanate in concentrations 10 to 100 mM. In solution, hemoglobin S undergoes gel formation after deoxygenation. Cerami and Manning[1] found that 1 mM. of cyanate prevented the gelling of a hemoglobin-S solution. Thus, as might be expected, the cyanate effect can be demonstrated at much lower concentrations when hemoglobin is in solution than in cells. Using potassium cyanate from the same source we found that 10 mM. cyanate has a very drastic effect on the isolated lens in culture leading to complete opacification. Visible lens changes can also be observed at 2 and 5 mM. cyanate (Fig. 1). Our preliminary results show that some phase of the cation concentrating mechanism is affected at the millimolar level of cyanate. The cation concentrating mechanism is important in maintaining proper electrolyte balance which in turn, governs the normal state of hydration in the lens.[7] Any change that occurs in making this mechanism less effective could lead to localized accumulation of fluid which could increase light scattering and cause opacities. The cyanate-induced loss in transparency in the incubated lens could be related to this phenomenon. An alternative explanation for the opacification is the direct interaction of cyanate with the lens proteins.
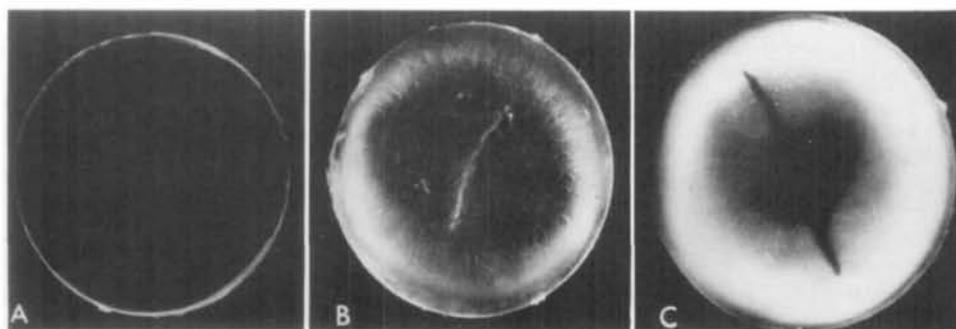
Fig. 1. The effect of cyanate on the lens. The result of incubation for 24 hours in the presence of 2 or 5 mM. cyanate. *A*. Lens in control medium. *B*. In 2 mM. cyanate. *C*. In 5 mM. cyanate.
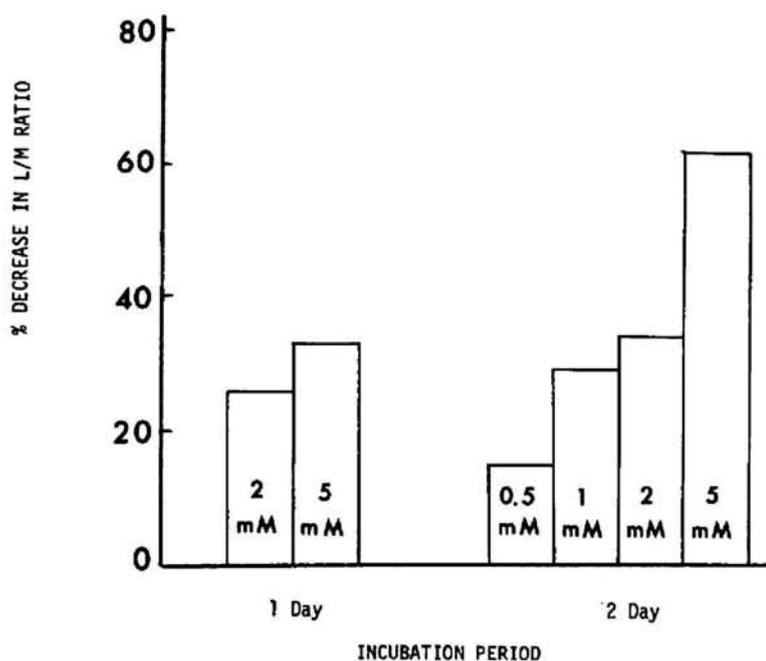

Fig. 2. The effect of cyanate on lens transport of cations. The incubations are for a 24- or 48-hour period. The concentrations are designated. The results are expressed as the per cent decrease in the L/M ratios of ^{86}Rb of the lens exposed to cyanate compared to the control lens. The differences noted are all statistically significant.

The cyanate effect on hemoglobin has been shown to be the result of the carbamylation of certain amino groups.[8] Cyanate has been known to interact with sulfhydryl groups even more rapidly than with amino groups.[8] However, the -SH groups in hemoglobin are probably masked sufficiently to render them unreactive since labeled cyanate was incorporated primarily into amino groups of valine.[1]

In our laboratory[4, 5] we found that diamide, a specific glutathione oxidant, and azoester, an oxidant of -SH groups, were effective in decreasing the accumulation of ^{86}Rb in the lens. The observed membrane changes suggested that certain -SH groups are present in the membranes which are susceptible to oxidation when glutathione levels decrease precipitously. These membrane -SH groups are involved in the uptake process (Na-K ATPase) as well as in determining the degree of cation permeability. One of the roles of glutathione appears to maintain these crucial -SH groups in the reduced form. The cyanate effect on Rb uptake is similar to the effects of -SH oxidants. It is quite possible that cyanate

reacts with these -SH groups. In this case, there would be an alkylation of the -SH groups rather than undergoing oxidation. The result would have similar adverse effects. These possibilities will have to be further explored.

A report by Glader and Conrad[9] indicates that a possible side effect of cyanate is the irreversible inactivation of glucose-6-phosphate dehydrogenase (G-6-PD). The cyanate inhibition of this enzyme led to a 50 per cent lowering of red cell glutathione. These experiments were patterned after Cerami and Manning's study in which the cyanate concentrations used were 10 to 50 mM. These high levels of cyanate were too deleterious for the lens, but at 5 mM. cyanate a 25 per cent decrease in lens GSH was observed. Whether this is due to inhibition of G-6-PD or to alkylation of GSH is uncertain.

Potassium cyanate, an antisickling agent, was shown to have a deleterious effect on the lens. Upon incubation in the presence of cyanate at millimolar levels, the lens develops opacities. It was shown that the lens cation transport mechanism is interfered with at these concentrations as evidenced by the loss in the ability to concentrate rubidium ion. It is significant that to demonstrate the antisickling effects on the affected red cells by cyanate a much higher level of 10 to 100 mM was used.

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