Distribution of 5,5 dimethyl-2,4-oxazolidinedione (DMO) in intraocular and cerebrospinal fluids of rabbits. III. Effect of ammonium chloride and probenecid

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The effect of ammonium chloride (systemic acidosis) and probenecid on the distribution of DMO in intraocular and cerebrospinal fluids has been studied. These drugs increased the level of DMO in intraocular fluids so that DMO was found in excess of plasma levels at plasma levels which normally result in a deficit of DMO in intraocular fluids. Their action seemed to be explained in part by a blocking of the secretion out of DMO at the ciliary process and perhaps an increased diffusion in the posterior segment of the eye. Probenecid may well affect the distribution of DMO in cerebrospinal fluid. It appears that the use of the partition of DMO between compartments as an indication of pH should be substantiated by another method for each set of experimental conditions.

The drug, 5,5 dimethyl-2,4-oxazolidinedione (DMO), has been used by several investigators to study the pH of tissues in vivo. In such instances the drug primarily penetrates by diffusion. Although a recent study may indicate that a transport process is involved,[1] the data can be interpreted on the basis of diffusion and pH partition.[2] Previous studies have shown that the distribution of DMO may be used to estimate the pH of intraocular and cerebrospinal fluids in rabbits.[3] Posterior and anterior aqueous humors are alkaline (DMO in excess), pH of approximately 7.50 to 7.60, and vitreous humor and cerebrospinal fluid are acid (DMO in deficit), pH of approximately 7.20 to 7.30 relative to plasma pH. However, in the case of posterior and anterior aqueous humors of rabbit eyes, the relative concentration of DMO is dependent on the plasma level; at low plasma levels DMO is in deficit and at high plasma levels DMO is in excess[4] of plasma concentration. The level of DMO in cerebrospinal fluid does not appear to be so dependent. Following the administration of a carbonic anhydrase inhibitor, the concentration of DMO increased in intraocular fluids although the plasma level decreased. The increased level of DMO in the vitreous humor was striking. These changes probably relate in part to the inhibited secretory

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process and also to a relative increase in alkalinity of aqueous humors to plasma because of the systemic acidosis which occurs following the administration of such drugs. In addition it has been found that probenecid competes for a secretion out process of organic acids in the ciliary processes.[11] It seemed worthwhile to explore the effects of systemic acidosis (ammonium chloride) and of probenecid on the distribution of DMO in intraocular fluids.

Methods

The general experimental protocol, methods of obtaining samples, and analysis of DMO were as previously discussed.[3, 4] Probenecid does not interfere with analysis of DMO. DMO was used as a 5 per cent solution neutralized to approximately pH 7 and administered subcutaneously at a dose of 50 mg. per kilogram of DMO and 9.2 mg. per kilogram of the parent compound trimethadione.

DMO was given at the start of the experiment and allowed to approach steady state in intraocular fluids. At 4 hours the control samples were taken and the drugs administered. The experimental samples were taken at 8 hours. Ammonium chloride was used as a 3 per cent aqueous solution and was administered intraperitoneally in doses of 90 to 300 mg. per kilogram with a second dose, one half the initial amount, given 2 hours later. Probenecid was just solubilized at a concentration of 100 mg. per milliliter with a mole equivalent of sodium (hydroxide) and was administered intraperitoneally at a dose of 200 mg. per kilogram. In the probenecid control animals, probenecid was given simultaneously with DMO at the start of the experiment and a second dose, one half the initial amount, was administered at 4 hours. Cerebrospinal fluid samples were taken from the cisterna magna immediately (1 to 2 minutes) after an intravenous lethal dose of pentobarbital sodium.

Results

The data in Fig. 1, *A* showed that in control animals the plasma level of DMO remained reasonably stable over a 4 hour period from 4 to 8 hours after administration of DMO. This level is attained in 1 to 2 hours.[4] In agreement with previous studies of low concentrations of DMO in plasma, the concentration of DMO in intraocular fluids was not in excess relative to plasma concentrations throughout this time. At 4 hours, the average concentration in posterior aqueous humor of control eyes for the various groups (all except Fig. 1, *B*) ranged from 88 to 100 per cent of the plasma concentration; that of anterior aqueous humor ranged from 80 to 90 per cent; that of vitreous humor ranged from 71 to 81 per cent of the plasma concentration.

In contrast, the animals given ammonium chloride showed increasing concentrations of DMO in intraocular fluids with increasing doses of ammonium chloride although a decreasing plasma level occurred, apparently caused by the acidosis resulting from the administration of ammonium chloride (Fig. 1, *E*, *G*, and *I*). The rise in the concentration of DMO in vitreous humor was very consistent and was apparent after the lowest dose (90 mg. per kilogram) of ammonium chloride (Fig. 1, *C*), whereas at this dose the concentration in posterior and anterior aqueous humor followed closely the decrease in plasma concentration. After administration of 300 mg. per kilogram of ammonium chloride (Fig. 1, *I*) the concentration of DMO in posterior aqueous humor rose to 114 per cent, anterior aqueous humor to 94 per cent, and vitreous humor concentration to 103 per cent of the control (4 hour) plasma concentration although the plasma concentration decreased to 87 per cent of this level.

Probenecid (Fig. 1, *B*) caused a slight loss of DMO from plasma. At 4 hours, these animals had an average of 12.2 mg. DMO per 100 ml. plasma compared to values of 13.3 to 14.7 mg. per 100 ml. plasma for other groups. The concentration in posterior aqueous humor was 114 per cent and anterior aqueous humor was 102 per cent compared to plasma concentration. The concentration of DMO in intraocular fluids continued to increase, although there was a further small decrease in plasma concentration. These results contrast with those of the lower doses of ammonium chloride.

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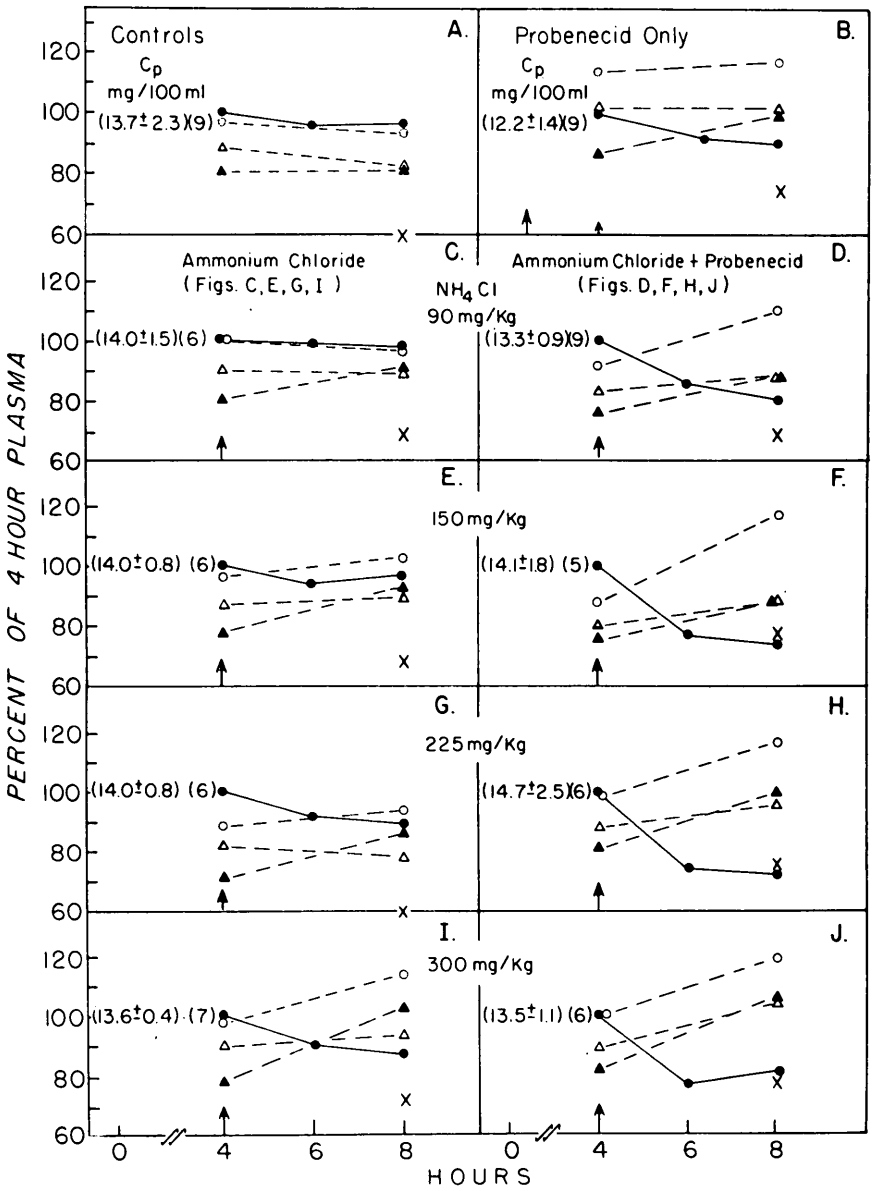

Fig. 1. The effect of ammonium chloride and/or probenecid on the concentration of DMO in intraocular fluids. The values in parentheses are the actual concentrations and the number of animals. Symbols represent plasma (●), C_h (○), C_a (△), C_v (▲), and C_{csf} (x).

The simultaneous administration of ammonium chloride and probenecid appeared additive in effect and caused an increase in concentration of DMO in intraocular fluids although the plasma concentration decreased markedly. After probenecid and 90 mg. per kilogram of ammonium chloride, the plasma concentration of DMO decreased to 86 per cent at 6 hours and to 80 per cent at 8 hours (2 and 4 hours after administration of drugs) compared to the 4 hour predrug level. A maximal change in plasma concentration of DMO was seen after 150 mg. per kilogram of ammonium chloride and probenecid with a decrease at 6 hours to 76 per cent of control values.

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The plasma levels were relatively stable for the following 2 hour period (6 to 8 hours). However, the concentration of DMO in posterior aqueous humor increased to an approximately maximal level of 117 per cent compared to the pretreatment (4 hour) plasma concentration. The concentration of DMO in anterior aqueous humor and vitreous humor showed increasing concentrations with increasing doses of ammonium chloride with probenecid and were 95 and 100 per cent, respectively, after 225 mg. per kilogram, and 103 and 105 per cent, respectively, after 300 mg. per kilogram. The concentrations of DMO in intraocular fluids relative to plasma concentrations at the 8 hour period, of course, were considerably in excess since the plasma level decreased after administration of the drugs. After 300 mg. per kilogram of ammonium chloride with probenecid the excess of DMO in posterior aqueous humor was 147 per cent; in anterior aqueous humor 129 per cent, and in vitreous humor 131 per cent compared to plasma concentration. It was not possible to increase the dose of these drugs, as higher doses were frequently lethal.

Ammonium chloride administration tended to cause an increase in the concentration of DMO in cerebrospinal fluid, but the variability of response was sufficiently great that the change was not significant. The level remained considerably below that of plasma. Probenecid caused a consistent increase in concentration of DMO in cerebrospinal fluid. Ammonium chloride and probenecid were not additive in their effects, as seen in intraocular fluids. However, since the combination of drugs caused a marked decrease in plasma concentration of DMO, the concentration in cerebrospinal fluid was generally equal to the plasma concentration.

Discussion

The hypothesis set forth by Jacobs,[5] elaborated for gastric juice by Shore, Brodie, and Hogben,[6] and more recently discussed by Schanker[7] indicates that weak organic acids are distributed across membranes in accordance with their ionization constant and the pH of the fluid in the compartments. The process is one of diffusion influenced by molecular size, charge, and lipoid solubility. DMO distribution agrees with this hypothesis when used to measure the intracellular pH of muscle[8, 9] and distribution into mitochondria.[10] This concept has the inherent assumption that the distribution of the un-ionized molecule in each compartment is equal.[6] That this may not be true for secretory processes, as in the eye, is discussed for urea and DMO.[4] If diffusion were the primary process involved in the distribution of DMO in intraocular fluids, one would expect to find the concentration of DMO in posterior and anterior aqueous humor in excess of that in plasma. The present data confirm those of previous studies[3, 4] showing that at low plasma concentrations DMO is not in excess in intraocular fluids. However, after the administration of ammonium chloride (systemic acidosis) the DMO level does increase in posterior aqueous humor and become in excess. The predrug plasma level is used for comparison to avoid the problems arising from a falling plasma concentration. Of interest is the fact that systemic acidosis appears to have its initial and more marked effect on the posterior uvea with the level of DMO in vitreous humor increasing after a dose which does not affect the anterior blood aqueous barrier (Fig. 1, *C*). Only with the highest dose of ammonium chloride, 300 mg. per kilogram, (Fig. 1, *I*) does the level in the posterior aqueous humor increase enough to prevent the level in the anterior aqueous humor from decreasing in parallel with plasma changes. In contrast, probenecid administration affects primarily the anterior blood aqueous barrier, and the level of DMO in posterior aqueous humor is in excess at the initial sampling period (Fig. 1, *B*). After the second dose of probenecid the level in vitreous humor also increases. One might speculate that at this dosage probenecid results in a relative systemic

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acidosis approximately equal to that of 90 to 150 mg. per kilogram of ammonium chloride. Although more marked plasma decreases are seen after the combined use of ammonium chloride and probenecid, the level of DMO increases enough to prevent a fall in level of DMO in anterior aqueous humor. The increase in vitreous levels of DMO are approximately the same as after ammonium chloride alone, although these increases occur in the presence of more marked decreases in plasma levels. Thus, the combination of ammonium chloride and probenecid mimics to a considerable degree the effect of carbonic anhydrase inhibitors.[4] The latter data are summarized in Fig. 2. However, the striking increase in vitreous humor levels to equal or exceed those of posterior aqueous humor after carbonic anhydrase inhibition does not occur after ammonium chloride and probenecid administration. These data as well as those of Fig. 1, *A* and *C* indicate that the diffusion of DMO out of the eye is rapid enough to follow changes in plasma level closely. In the studies of carbonic anhydrase inhibitors the excess of DMO in intraocular fluids persists. These phenomena may be explained in part by the secretion out mechanism for organic acids as observed by Forbes and Becker[11] for iodopyracet, which is inhibited by probenecid. As this mechanism becomes saturated, DMO appears in excess in the posterior aqueous humor; when the mechanism is fully saturated or blocked, an excess in anterior aqueous humor is found and the ratio of levels in these fluids to that of plasma approximates those expected on the basis of pH.[3] It appears necessary to conduct kinetic studies under conditions of steady state plasma levels and to determine whether probenecid crosses the blood aqueous barriers to a significant degree. These studies are in progress. The effect of acidosis on the posterior segment of the eye, suspected in previous studies[4] and again observed in the present experiments, remains unexplained. Concentrations of DMO in frozen slices showed that the gradient still existed from ora serrata to the optic disc, and altered permeability of

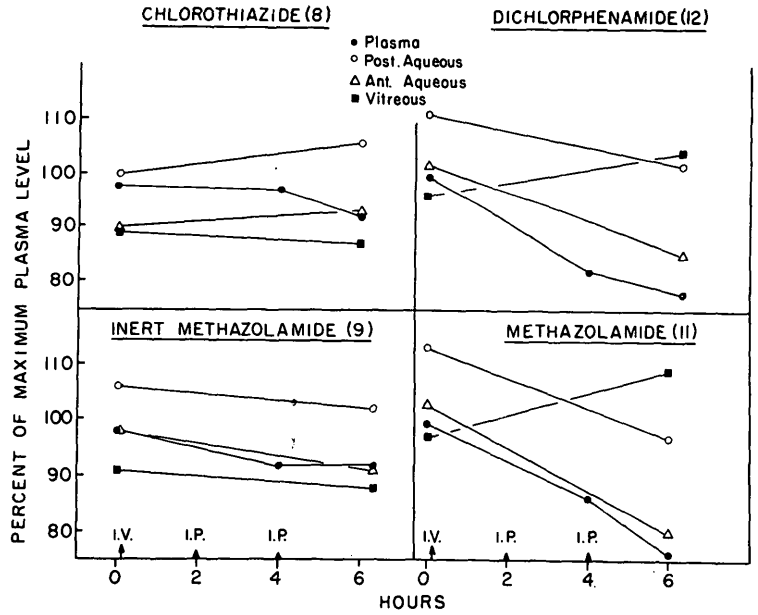

Fig. 2. The effect of carbonic anhydrase inhibitors and control drugs. Since various plasma levels were studied, some 50 to 60 per cent of each group had plasma levels less than needed to result in an excess in C_h (INVEST. OPHTH. 1: 609, 1962).

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retinal vessels does not appear to be a major factor. Probenecid appears to have little effect on this process.

Also inherent in the pH hypothesis of distribution of weakly ionized compounds[6] is that a decrease in the pH of plasma causes an increase in the distribution ratio of compartment concentration (intraocular fluids) to plasma concentration. This effect is presented graphically in Fig. 3. At a pH of 7.55 for posterior aqueous humor, plasma pH of 7.4, the ratio C_h/C_p should equal 1.40. This value is approached after the combined dosage of 90 mg. per kilogram of ammonium chloride and 200 mg. per kilogram of probenecid (1.34), and it is exceeded, reaching 1.58, after 150 mg. per kilogram of ammonium chloride, 1.66 after 225 mg. per kilogram, 1.43 after 300 mg. per kilogram with probenecid. If the plasma pH decreased to pH 7.3, the ratio should be approximately 1.75. Thus the data are compatible with a relative alkalosis of intraocular fluids and approximate values of pH. On the other hand, Langham and Lee[16] found that 2.5 hours after 270 mg. per kilogram of ammonium chloride was administered to rabbits by stomach tube the pH of plasma decreased to 7.22 but that of anterior aqueous humor similarly decreased so that the gradient remained essentially the same as in normal rabbits. The data suggest that blocking agents may be found which would permit the use of DMO distribution for pH studies at low levels of DMO to avoid pH changes which may be caused by the drug itself at high concentrations.[12] However, the dose used in the present studies is approximately one tenth the dose used to cause acidosis and at a level used as an indicator dose.

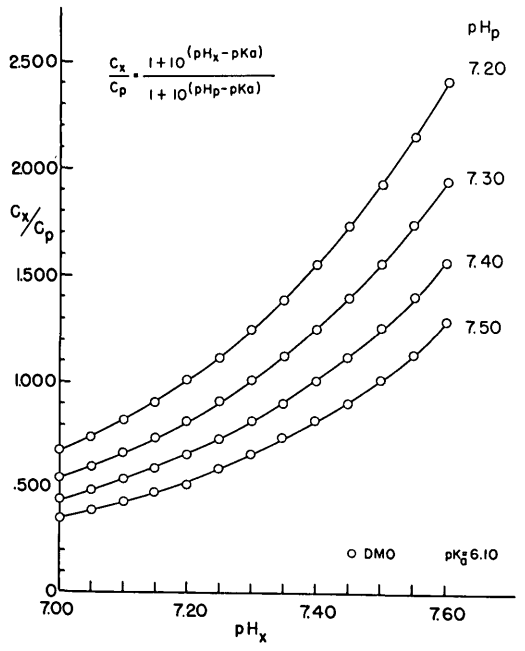

Fig. 3. The calculated ratio Cx/Cp expected at different values of pH of plasma and pH of intraocular fluids.

It is apparent from these studies that the question of whether or not DMO distribution reflects hydrogen ion gradient must be demonstrated by an independent method, such as glass electrode, for each change of conditions. As is well known for studies on intraocular fluids, the plasma concentration must be maintained at a stable level for some 6 hours to allow a new steady state to be reached in the intraocular fluids. In the present studies the postdrug plasma level of DMO was reasonably stable for 2 to 3 hours; this permits the new DMO level in intraocular fluids to approach its final value by some 80 to 90 per cent. Similar considerations may well be necessary for cerebrospinal fluid, which attains maximal level of DMO in 2 to 3 hours and is not affected by plasma levels.[3] Robin[13] and others found that there was a relative alkalosis in cerebrospinal fluid associated with systemic acidosis resulting from hyperventilation. In the present studies no consistent change was seen on a population basis in the DMO level of cerebrospinal fluid associated with systemic acidosis caused by ammonium chloride. However, all animals given probenecid showed higher levels of DMO in cerebrospinal fluid, and the level equaled that of plasma when ammonium chloride at a dosage of 150 mg. per

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kilogram or more, was given with probenecid.

The distribution of bicarbonate has been used to study intracellular pH.[8] Kinsey and Reddy[14] have noted that since CO_2 is more rapidly diffusible across cell membranes than the bicarbonate ion, it is essentially trapped in the more alkaline intraocular fluids of rabbits. As noted previously, this mechanism is inherent when a compound exists in un-ionized and ionized state, such as in the case of DMO. Some unpublished data on bicarbonate concentrations in the eye, before and after administration of a carbonic anhydrase inhibitor, including vitreous humor levels are summarized in Table I for comparison with similar DMO studies. Both acids have a pK of 6.1. There are striking similarities in the ratio Cx/Cp in the saturated systems of both ions and striking differences in the ratio Cv/Cp when secretion is inhibited. In the approximately saturated systems (active secretion for HCO_3 and high Cp for DMO) the ratio Ch/Cp ranges 1.36 to 1.57; Ca/Cp, 1.20 to 1.26; Cv/Cp, 1.00 to 1.08. These values indicate that a difference in pH of posterior and anterior aqueous humors should be present and the pH of vitreous humor should equal or be slightly more alkaline than that of plasma instead of the finding of no difference between the pH of anterior and posterior aqueous humors and a relatively acid, pH 7.2, vitreous humor.[15] Following carbonic anhydrase inhibition, the bicarbonate ratios decrease and that of Cv/Cp shows a deficit. In the less saturated animals given DMO, the ratios are considerably less than in the saturated series, but following carbonic anhydrase inhibition the 2 groups are indistinguishable. Both groups show a large excess in Cv. The conflicting Cx/Cp ratios after inhibition can be explained in part on a non-steady-state change relating to systemic acidosis which would favor a loss of bicarbonate from the eye and may favor an increased rate of diffusion of the un-

Table I. Effect of carbonic anhydrase inhibitor* on bicarbonate concentration in intraocular fluids (mM./Kg. water)

Time (minutes)	*Ch (pH 7.6)*	*Ca (pH 7.6)*	*Cv (pH 7.2)*	*Cp (pH 7.4)*	*(pH 7.2)*
0	36.50	29.20	24.20	23.20	
60	28.70	25.70	21.20	22.40	(21.80)
180	26.20	20.60	16.00	18.90	(18.40)
Cx/Cp—bicarbonate					
0	1.57	1.26	1.04		
180	1.39	1.09	0.85		
300	1.48	1.07	0.85		
Cx/Cp—DMO					
Saturated system†	1.40	1.20	1.00		
Fig. 2‡					
0	1.19	1.08	0.97		
300	1.32	1.16	1.34		
Fig. 2‡ saturated					
0	1.36	1.20	1.08		
300	1.34	1.15	1.33		

*Dichlorphenamide intravenously administered initially at 25 mg. per kilogram followed by 12.5 mg. per kilogram, intraperitoneally at 2 hour intervals. The total Co_2 found by the Van Slyke method has been corrected, with the Henderson-Hasselbach equation, to bicarbonate concentration at assumed pH taken from published data.

†Data taken from Am. J. Ophth. 51: 98, 1961.

‡Data taken from Fig. 2 (DCP) and INVEST. OPHTH. 1: 609, 1962. Fig. 2 includes all data with average Cp of 29.1 and a range of 13 to 53. Thus, approximately 60 per cent of the animals were at unsaturated plasma levels. The "saturated" data are from the same group with Cp above 30 mg. per 100 ml. The average Cp initially was 41.6 mg. per 100 ml. Since the true saturation level is not known, these animals can be considered only as at relatively saturated levels.

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ionized DMO into the eye, a more rapid attainment of saturation levels of the ionized molecule, and subsequent increase in vitreous levels. However, over a period of several hours it would be expected that as a new steady state is approached the ratios would again tend to be similar for bicarbonate and DMO. This does not appear to be the case. Kinetic studies show that the increased Cv/Cp persists for an additional 5 hours. The present studies only partially help to explain this paradox in the vitreous levels of DMO by suggesting an interrelated effect of acidosis, competive inhibition, carbonic anhydrase activity and saturation at the level of the ciliary body, and possibly altered diffusion in the posterior segment of the eye.

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