Distribution of 5,5-dimethyl-2,4-oxazolidinedione (DMO) in intraocular fluids

V. Effect of plasma level, probenecid, and dichlorphenamide on time course

Marguerite A. Constant

The kinetics of penetration of DMO into intraocular fluids at different plasma levels or after the administration of probenecid or dichlorphenamide have been studied. Many similarities were found to other systems which have been studied. The data provided further evidence for the probability that DMO is secreted out of the eye and that this mechanism is inhibited by probenecid and dichlorphenamide.

DMO penetrates most of the tissues studied by a diffusional process.[1, 2] Although DMO should be in excess in the anterior intraocular fluids of the rabbit because the pH of these fluids is alkaline relative to that of plasma, an excess is seen only when the plasma level is high or the animal has been treated with probenecid or dichlorphenamide.[3, 4] It was of interest to conduct time-course studies under these various conditions to obtain further information concerning the mechanisms of the penetration of DMO into intraocular fluids.

Methods

The lowest plasma level (15 mg. per 100 ml.) of DMO was achieved by parenteral administration of 50 mg. per kilogram DMO and 9.2 mg. per kilogram TMO (trimethadione); the second level (25 mg. per 100 ml.) at 100 mg. per kilogram DMO and 13.8 mg. per kilogram TMO; the foregoing given 50 per cent intravenously and 50 per cent intraperitoneally; the third level (40 mg. per 100 ml.) at 175 mg. per kilogram DMO and 13.8 mg. per kilogram TMO, 57 per cent intravenously and 43 per cent intraperitoneally; and the fourth level (54 mg. per 100 ml.) at 250 mg. per kilogram DMO and 13.8 mg. per kilogram TMO, 45 per cent intravenously and 55 per cent intraperitoneally. The two highest levels were maintained by administering additional doses of TMO (intraperitoneally) at 4 and 8 hours and for dichlorphenamide treated animals at 3, 5, and 7 hours. TMO is quantitatively demethylated to DMO.[5] Because the plasma level was falling at the highest level, the 7 hour TMO was increased in a series of animals. Six animals were used per experiment. Samples from the right eye were taken during the early time periods and from the left eye at late time periods to cover the 11 to 12 points for the time course (10 hours). A minimum of 5 such experiments were conducted to obtain data on a minimum of 5 animals per point for each curve with the exception of the animals receiving the highest dosage. Probenecid or dichlorphenamide was administered 30 to 45 minutes before DMO at a dose of 200 mg. per kilogram (probenecid) intraperitoneally with a second

From the Department of Ophthalmology and the Oscar Johnson Institute, Washington University Medical School, St. Louis, Mo.

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dose of 100 mg. per kilogram in 4 hours or 25 mg. per kilogram (dichlorphenamide) intravenously with a repeat dose of 12.5 mg. per kilogram intraperitoneally at 3 and 6 hours. DMO was determined by the method of Waddell and Butler appropriately scaled down to permit analyses on the small samples of aqueous humor.[3]

Results

The data are plotted as the per cent of plasma level in Fig. 1, *A-F*. At a plasma level of 15 mg. per 100 ml. (Fig. 1, *A*) the concentration of DMO in intraocular fluids did not exceed that of the plasma over a 10 hour period. The highest concentration was found in posterior aqueous humor, averaging 98 per cent of plasma from 6 to 10 hours. At a plasma level of 25 mg. per 100 ml. (Fig. 1, *B*) the DMO concentration in posterior aqueous humor exceeded that of plasma at 150 minutes and remained in excess by approximately 10 per cent from 6 to 10 hours. The level in the anterior aqueous humor did not exceed that of plasma. At a plasma level of 40 mg. per 100 ml. (Fig. 1, *C*), both posterior and anterior aqueous humor showed a DMO excess of 15 and 5 per cent, respectively. The excess in posterior aqueous humor was found at 90 to 120 minutes. A similar finding was obtained at a plasma level of 54 mg. per 100 ml. (Fig. 1, *D*) with an excess 20 and 6 per cent for posterior and anterior aqueous, respectively. The vitreous humor concentration of DMO reached 94 to 97 per cent of plasma level in these experiments.

Following probenecid (Fig. 1, *E*) an excess in posterior aqueous humor was found at 90 to 120 minutes and reached approximately 12 per cent excess over the plasma concentration. The level in anterior aqueous humor continued rising and equaled that in plasma. The vitreous humor concentration approximated 94 per cent of that of plasma. As found previously,[4] probenecid lowered the plasma level expected from a given dose of DMO from 15 mg. to 11 mg. per 100 ml. Following the administration of dichlorphenamide, an excess of DMO relative to plasma level (13 mg. per 100 ml.) (Fig. 1, *F*) was found in posterior aqueous humor in 90 to 120 minutes and reached a value of 131 per cent. The level in anterior aqueous humor continued to rise and was in slight excess in some animals at 8 to 10 hours. It was difficult to keep the plasma level stable after 7 hours. The vitreous humor level showed a striking increase, exceeding the plasma level at 3 hours and equaling or exceeding the level in posterior aqueous humor at 5 hours.

Schanker[6] has suggested that a plot of concentration versus log t may be used to estimate the t½ for diffusional processes. The data were plotted in this manner (Fig. 2, *A-F*). These plots assisted in giving an estimate of steady state values (24 to 40 hours). Little difference was seen in the slopes of these lines except for the slope of posterior aqueous humor in the animals receiving probenecid. Values for t½ are given in Tables IA and IB. For the various plasma levels, t½ values for posterior aqueous humor ranged from 6.2 to 9.0 minutes but not in any definite pattern in relation to plasma concentration, that for anterior aqueous humor was 42 to 48 minutes, and that for vitreous humor was 74 to 80 minutes. The half-times for the animals receiving probenecid were less, especially the posterior aqueous humor which gave an average value of 3.5 minutes, that of anterior aqueous humor was 34 minutes, and that of vitreous humor was 62 minutes. These data suggested that probenecid administration increased the diffusion rate. After dichlorphenamide the half-time value for posterior aqueous humor was increased to an average of 11 minutes, and the values for anterior aqueous humor or vitreous humor were indistinguishable from values for untreated animals and were 40 minutes and 80 minutes, respectively.

The foregoing method of analyzing the data did not take into account the fact that the intraocular fluids are a multicompartment system. Consequently, the data were plotted to take into account flow from the posterior aqueous humor as developed by Kinsey and Palm and further elaborated by

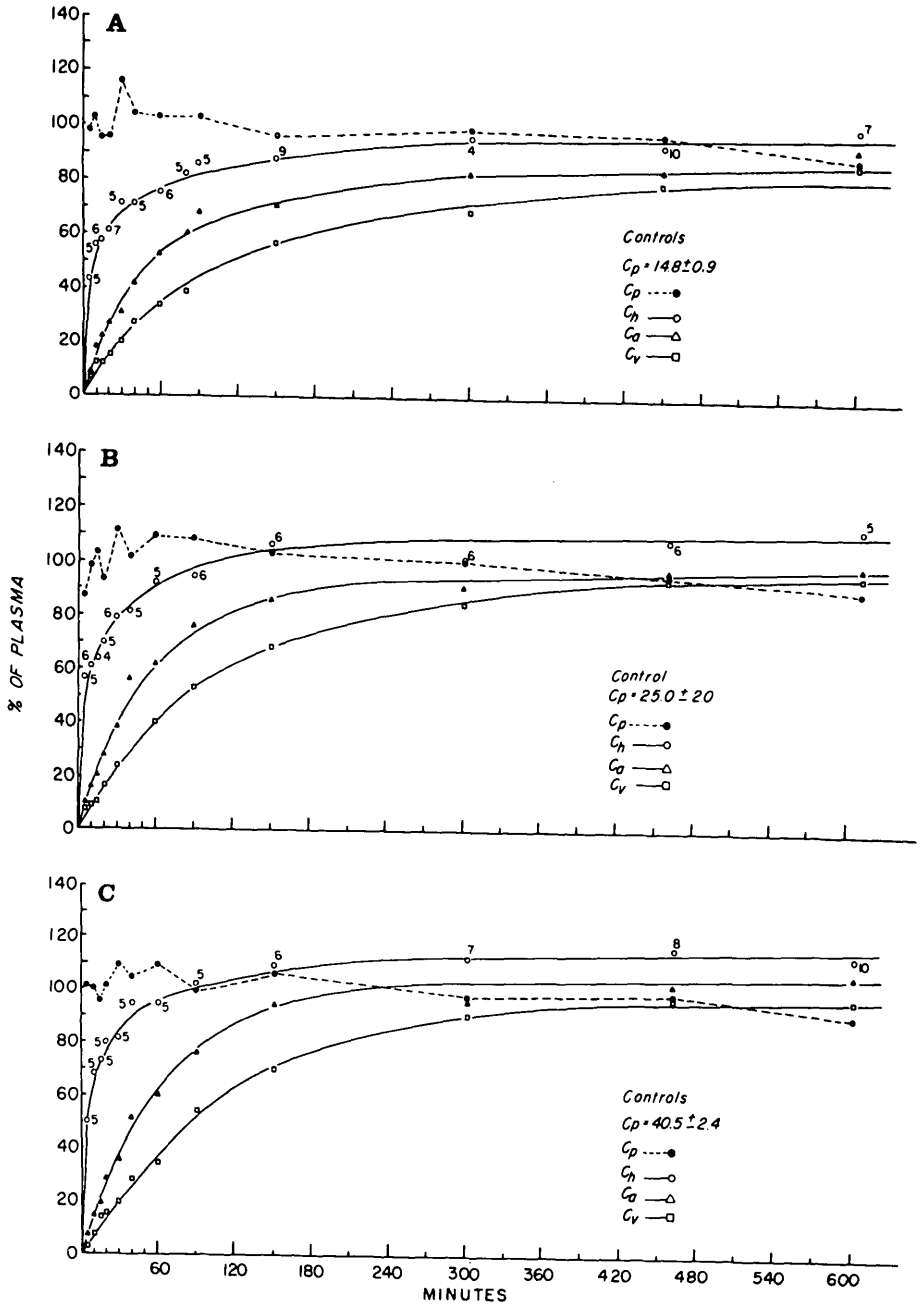

Fig. 1. The concentration of DMO with time in intraocular fluids at different plasma levels of DMO or after probenecid or dichlorphenamide. Data are expressed as the per cent of plasma concentration at the individual time periods. The plasma concentrations were averaged and the plasma curve presents the deviation from the average.

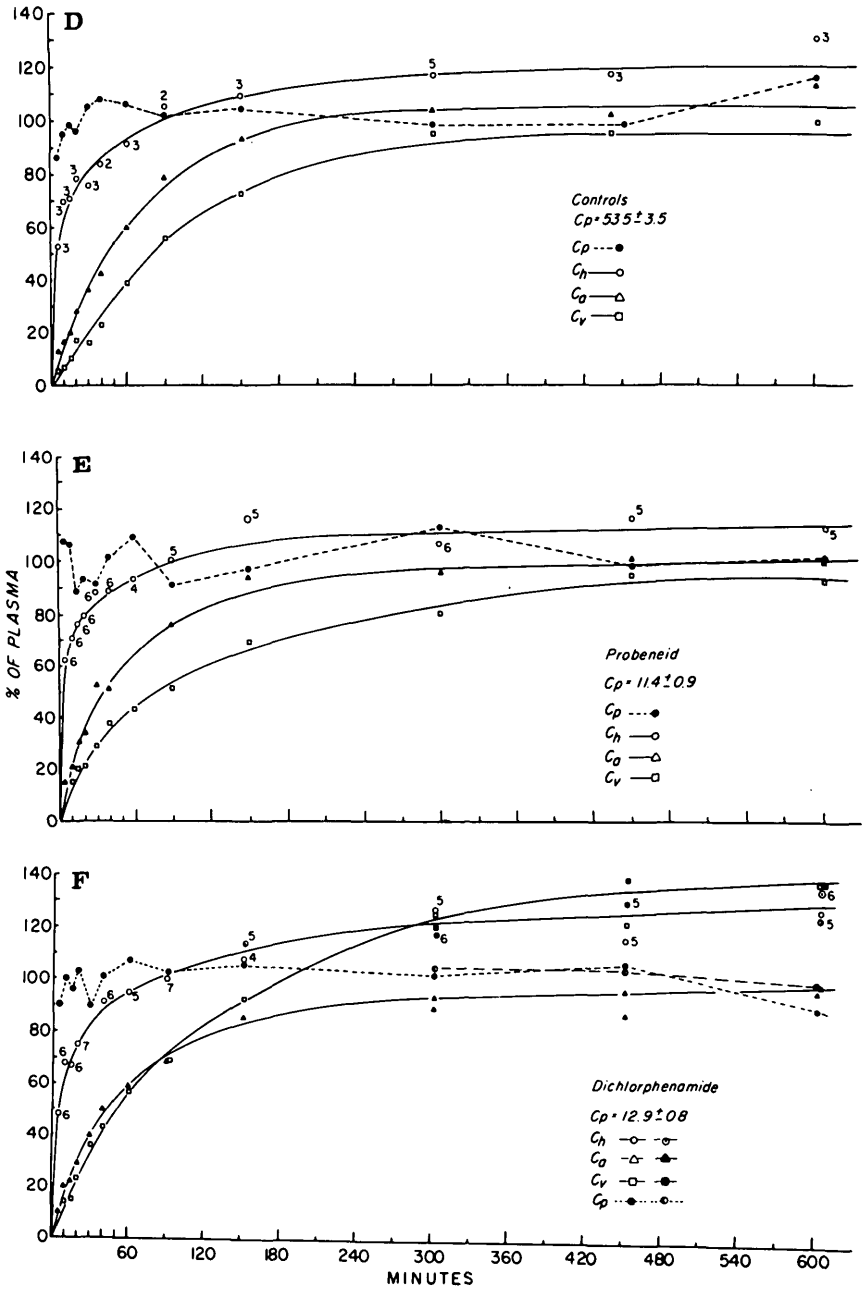

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Table IA. The half-time values (Fig. 2) and various coefficients (Fig. 3) of DMO turnover rates in intraocular fluids

Drug	C_p DMO (mg./ 100 ml.)	Posterior aqueous humor ($t\frac{1}{2}$ minutes)	Anterior aqueous humor ($t\frac{1}{2}$ minutes)	Vitreous humor ($t\frac{1}{2}$ minutes)
—	15	7.8	44	80
—	25	8.5	42	74
—	40	6.2	48	80
—	54	9.0	46	76
Probenecid	11	3.5	34	62
DCP	13	11.0	40	80

Friedenwald and Becker.[7] These plots are presented in Fig. 3, *A-F* and the various rate coefficients* obtained are summarized in Tables IA and IB.

With the exception of animals with the lowest plasma level, the turnover of DMO in the vitreous humor of untreated animals resolved into two log-linear phases. Values for late "environment filling" coefficients (K_{v_3}) were found which ranged from 0.00238 to 0.00266. In these animals values for the initial filling phase (K_{v_2}) increased from 0.00554 to 0.00925 with increasing C_p, and consequently the time at which the late "environment filling" rate was reached decreased from 450 minutes (25 mg. per 100 ml.) to 400 minutes (45 mg. per 100 ml.) to 360 minutes (54 mg. per 100 ml.). The initial phase of transport into the vitreous humor of the untreated animals with the lowest plasma level and of those receiving probenecid showed a short rapid phase; the log-linear portion (K_{v_2}, 1½ to 10 hours) gave a value of 0.00495 and 0.00630, respectively. The entire time course for vitreous humor turnover of DMO of animals receiving dichlorphenamide appeared to be log-linear with a value of 0.00815.

Similar values for late "environment filling" rate was found for anterior aqueous humor (K_{a_3}) for almost all groups, except the untreated animals with the lowest plasma level, with values ranging from 0.00223 to 0.00330. A higher value of 0.00463 was found for those animals. Similarly for the untreated animals (C_p25 to 54 mg. per 100 ml.) values for late environment filling rate for posterior aqueous humor (K_{h_3}) ranged from 0.00231 to 0.00266. The value for animals receiving probenecid was 0.00315, probably not different from the untreated animals. The values for untreated animals with the lowest plasma level (C_p15 mg. per 100 ml.) and the animals receiving dichlorphenamide were 0.00463 and 0.00462, respectively.

The values for K_{h_2} and K_{a_2}, environment filling based on 5 hours, were similar throughout and ranged from 0.00603 to 0.00707 with the exception of untreated animals with the highest plasma levels where values of 0.00795 (K_{h_2}) and 0.00925 (K_{a_2}) and the animals receiving dichlorphenamide, value of 0.00502 (K_{a_2}) were found. Since a small error in plotting could make such a difference, it is felt that these values may not be different from those of the other groups of animals.

A value for K_{h_1} (turnover rate of posterior aqueous humor) of 0.106 was found for the untreated animals with the lowest plasma level. This value was lower by some 40 to 65 per cent in animals of all other groups with the lowest value of 0.0346 found in the probenecid group and the highest value of 0.0628 in the dichlorphenamide group.

A diffusion coefficient ($K_{d.pa}$) value of 0.0183 was found in untreated animals with the lowest plasma level (C_p 15 mg. per 100 ml.). This value was lower in the

*The coefficients, K_1 and K_2, for posterior and anterior aqueous humor were obtained in the same fashion as used by Friedenwald and Becker, assuming data were collected for 5 hours. The coefficient K_2 is derived from the linear tail portion of the curve of a plot of C_∞-C_t versus t (C_∞ or C_t is the concentration in aqueous humors at infinity or a particular time period, respectively) and approximates a reservoir filling coefficient based on 5 hour data. K_1 approximates the turnover rate of the posterior aqueous humor and is obtained by subtracting K_2 (posterior aqueous humor) from the initial portion of the curve. Since the data were collected for 10 hours, it was possible to extend the analyses and obtain a reservoir filling coefficient at later time periods. This coefficient is represented by K_3. Subscripts are used to designate the source of the data ($K_{h_1} = K_1$, K_{h_2}, K_{h_3}; $K_{a_2} \approx K_2 = K_{h_2}$, K_{a_3}; K_{v_1}, K_{v_2}, K_{v_3} are from posterior, anterior, vitreous humors, respectively). The ratio $K_{f.a}/K_{d.pa}$ (flow: diffusion coefficient) was taken from Fig. 2, *A-F*, and equals $C_p - C_a/C_h - C_a$ at steady state.

Table IB

	C_p (mg./100 ml.)					
	15	*25*	*40*	*54*	*Probenacid*	*DCP*
$\frac{K_{f.a}}{K_{d.pa}}$	1	0.14	0.55	0.57	0.37	0.19
K_0	0.0384	0.0314	0.0248	0.0178	0.0315	0.0260
$K_{f.a}$	0.0201	0.0040	0.0087	0.0065	0.0081	0.0041
$K_{d.pa}$	0.0183	0.0274	0.0161	0.0113	0.0221	0.0219
K_{h_1}	0.106	0.0385	0.0495	0.0532	0.0346	0.0628
K_{h_2}	0.00707	0.00665	0.00603	0.00795	0.00603	0.00618
K_{h_3}	0.00463	0.00266	0.00231	0.00247	0.00315	0.00462
K_{a_2}	0.00693	0.00660	0.00603	0.00925	0.00603	0.00502
K_{a_3}	0.00463	0.00243	0.00330	0.00247	0.00223	0.00231
K_{v_1}	—	0	0	0	—	0
K_{v_2}	0.00495	0.00554	0.00867	0.00925	0.00630	0.00815
K_{v_3}	—	0.00266	0.00238	0.00247	—	—

See footnote of text for explanation of symbols.

untreated animals with the highest plasma level, 0.0113 (C_p 54 mg. per 100 ml.). It was little changed in the probenecid group, 0.0221, and the dichlorphenamide group, 0.0219. A flow coefficient value ($K_{f.a}$) of 0.0201 was found for the untreated animals with the lowest plasma level of DMO which agrees well with values found for Na, SCN, and ascorbate.[7, 13] However, this value was lower in other animals, 0.0087 (C_p 40 mg. per 100 ml.); 0.0065 (C_p 54 mg. per 100 ml.); 0.0081 (probenecid); and 0.0041 (dichlorphenamide).

A turnover value for the anterior aqueous humor ($K_o = K_{f.a} + K_{d.pa}$) for DMO of 0.0384 was found for untreated animals with the lowest plasma level. This value was found to be progressively lower with each increase in plasma level of untreated animals to a value of 0.0178 for animals with C_p of 54 mg. per 100 ml. The value was slightly lower in the animals treated with probenecid, 0.0315, and still lower in the animals which received dichlorphenamide, 0.0260.

Discussion

The values for the late "environment filling" coefficients of the vitreous humor (K_{v_3}) of 0.238 to 0.247 per cent per minute are in good agreement with the turnover of Na in the vitreous (0.155 per cent per minute) found by Friedenwald and Becker.[7] With few exceptions similar values for late environment filling coefficients (K_{h_3}, K_{a_3}) were found for the posterior and anterior chambers.

The early environment filling coefficients for posterior and anterior chambers (K_{h_2}, K_{a_2}), based on collection of data for 6 hours were very consistent and showed an average value of 0.00626 (omitting the highest plasma level group). This average value is somewhat higher than found for Na (0.0044)[7] and more similar to that of the anion SCN (0.0057)[7] and probably Cl (which enters the vitreous more rapidly than Na).[8]

With the exception of the untreated animals with lowest plasma level of DMO and the animals receiving probenecid, the turnover of DMO in vitreous humor appears to be a single exponential function. In the exceptional animals the log-linear phase is approached in 60 to 90 minutes. The values for turnover coefficients for vitreous humor (K_{v_2}) increase with increasing plasma concentration from 0.00495 to 0.00925. This may be explained in part by the increasing diffusion gradient and/or the saturation of a mechanism which secretes organic anions out of the eye.[9] Additional evidence that DMO may be transported by this mechanism has been presented previously.[4, 10]

Although the plasma level of the animals

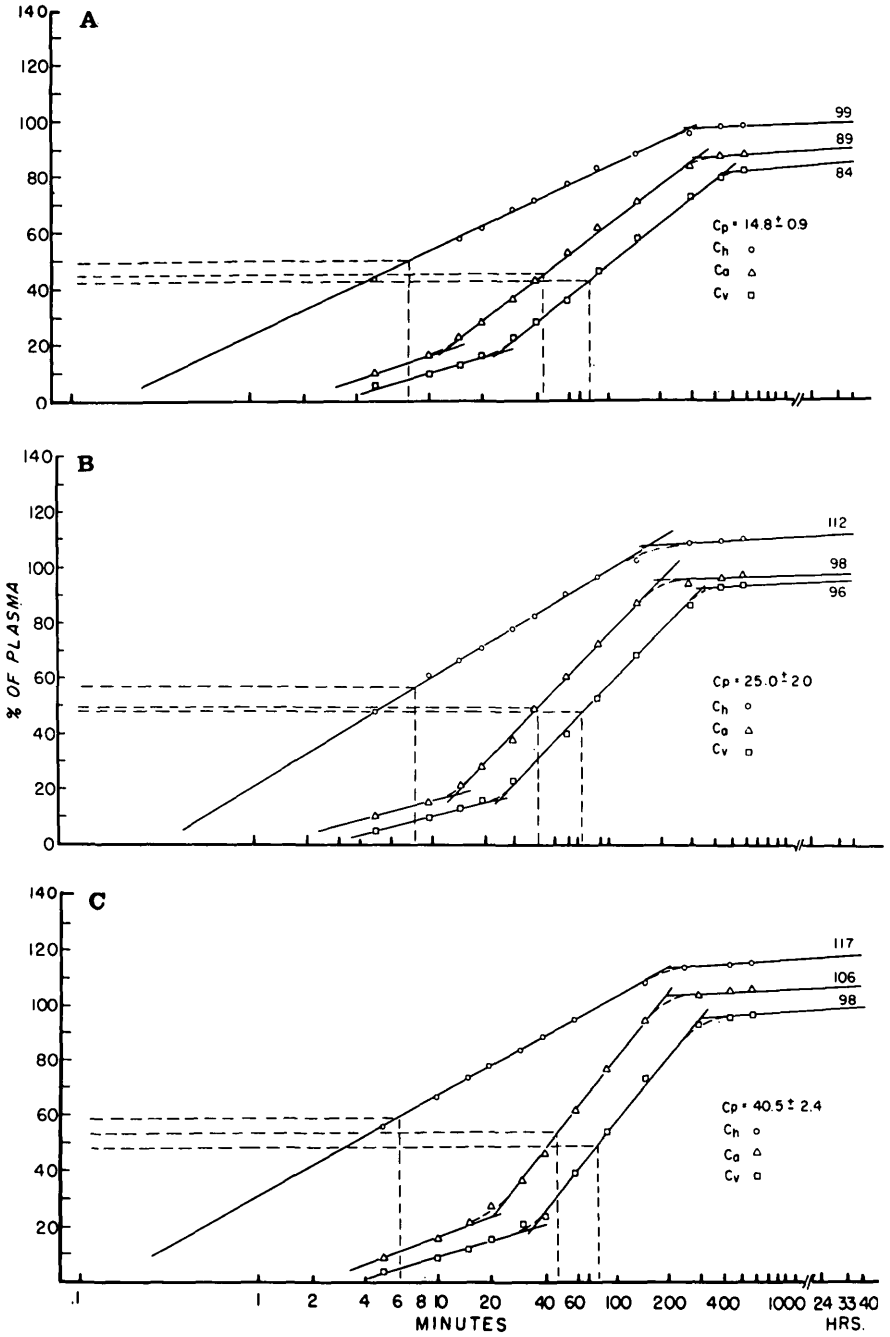
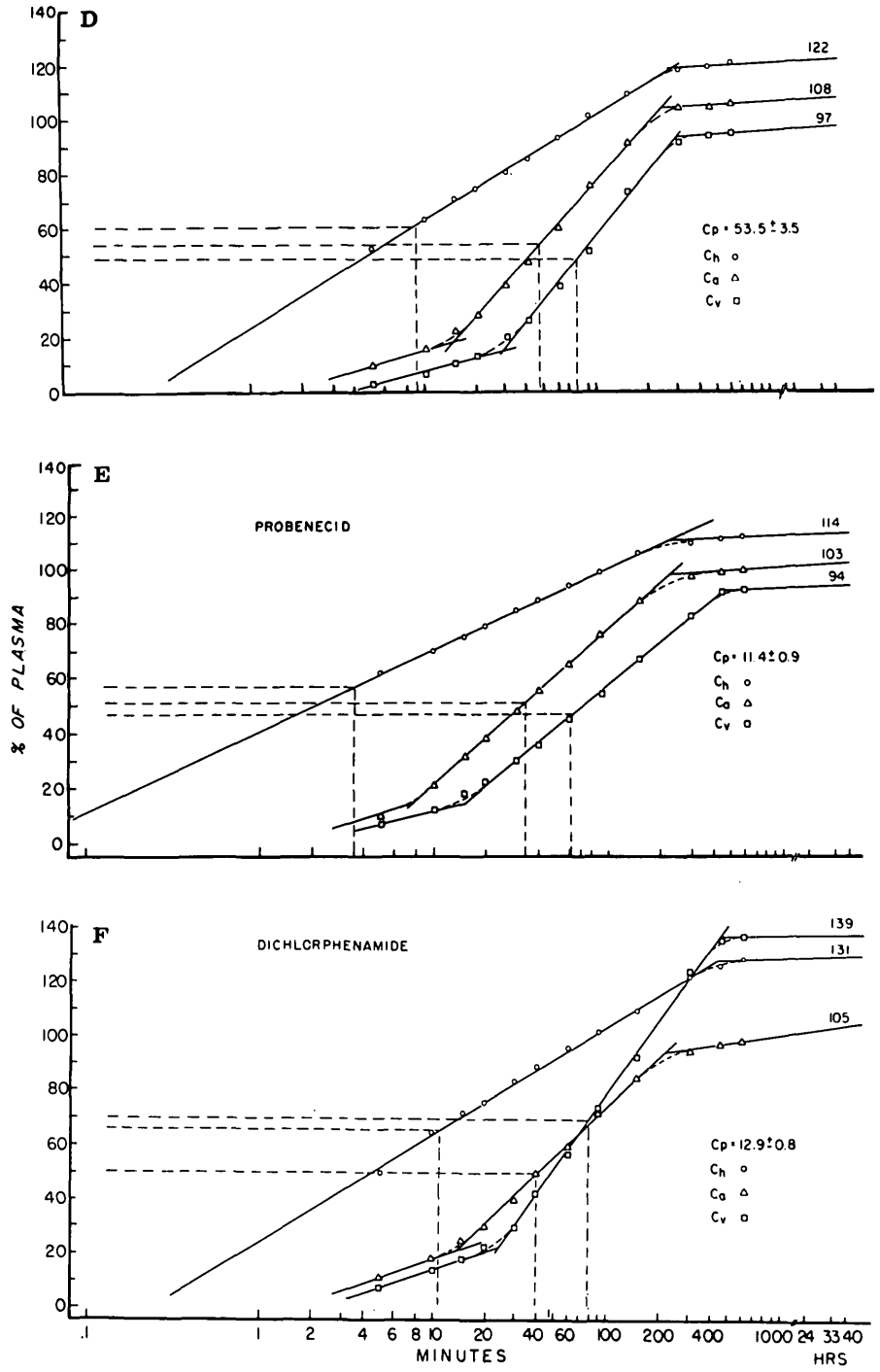


Fig. 2. The concentration of DMO with time in intraocular fluids at different plasma levels of DMO or after probenecid or dichlorphenamide expressed as the per cent of plasma concentration with time as a log function.



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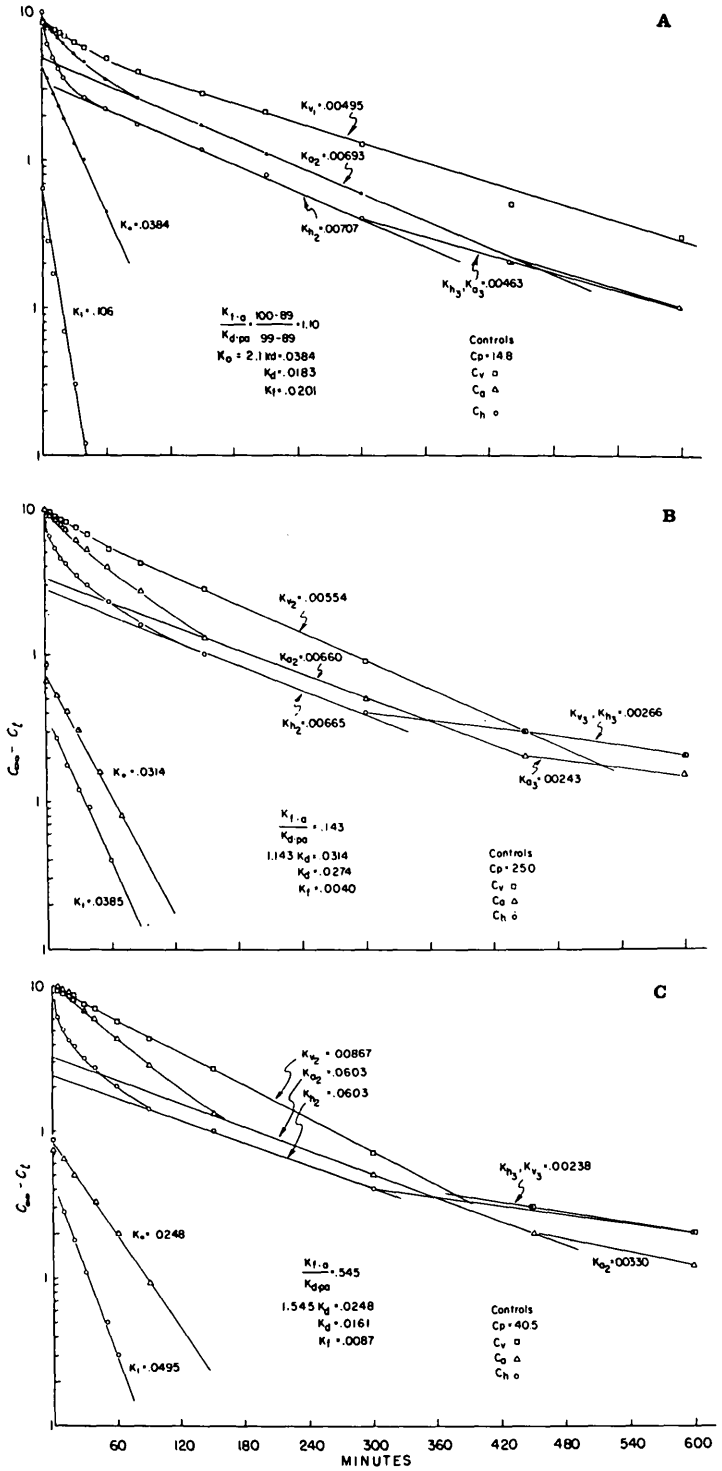
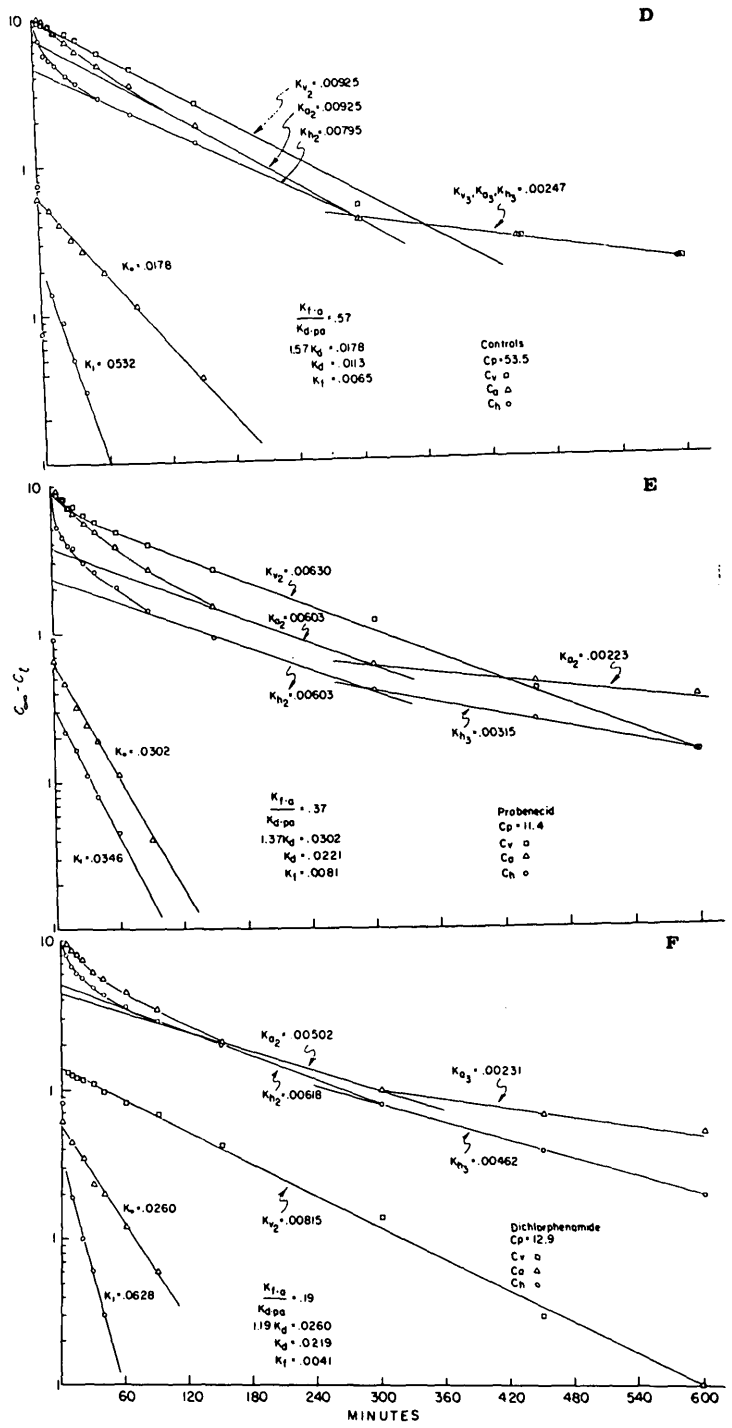


Fig. 3. Graphic exponential analysis of data where the ordinate is $C_\infty - C_t$ and the abscissa is t. C_∞ was taken from Fig. 2.



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receiving dichlorphenamide was only 13 mg. per 100 ml., the K_{v_2} coefficient, which is one of the highest (0.00815), appears to be a single exponential throughout the experimental period and does not show a late environment filling rate as seen with higher levels of DMO in the untreated animals (K_{v_2}, 0.00554 to 0.00925). The vitreous humor concentration reaches levels equal to or exceeding that of posterior aqueous humor (131 per cent of plasma level compared to 115 to 120 per cent in the untreated animals with high plasma levels). It appears that after dichlorphenamide the vitreous humor has a greater capacity to trap DMO. This was explained previously[4] as possibly related to a relative alkalosis of vitreous humor. On the other hand bicarbonate ion concentration (trapped CO_2) markedly decreases in vitreous humor after dichlorphenamide.[4] Another explanation may be more likely. Recently Butler and co-workers[11] have found for rat and dog (but not man) that after the administration of an acidosis-producing dose of DMO for several days there is a compensatory mechanism whereby an equivalent of chloride is lost from plasma with the presence of a foreign anion. The total anion (HCO_3^- + Cl^- + DMO^-) remains the same. The acidosis level of DMO in plasma used was some 8 mEq. per liter (dog) to 14 mEq. per liter (rat). In the present experiments the highest plasma level in untreated animals was 4 mEq. per liter, and in the animals receiving dichlorphenamide it was only 1 mEq. per liter (a tracer dose).[11] The rabbit may be more sensitive as evidenced by the difficulty in maintaining stable plasma levels at higher doses.

In addition to the reduction in bicarbonate in vitreous humor, a review of unpublished data reveals that dichlorphenamide causes a significant decrease in chloride concentration of posterior aqueous humor with little change in plasma concentration. In one series of 5 animals (5 hours after 50 mg. dichlorphenamide intravenously and 10 mg. intraperitoneally in 2 hours) the average values in millimoles per liter, before and after, were C_p, 111.2 to 108.5; C_h, 100.8 to 94.4; and C_a, 102.6 to 100.5. A second series of 11 animals a year later showed C_p, 109.6 to 110.4; C_h, 95.4 to 90.4; and C_a, 102.2 to 99.9. Unfortunately, vitreous humor analyses were not of interest and were not done, but the findings of Kinsey would indicate that the level in vitreous humor would be decreased. Kinsey[8] has shown that chloride enters more rapidly into posterior aqueous humor than into vitreous humor. The present studies show that DMO similarly penetrates more rapidly into posterior aqueous humor than vitreous humor and a similar anterior to posterior gradient is found in frozen slices of vitreous humor even after dichlorphenamide.[3] Such studies do not account for the higher concentration of chloride in vitreous humor compared to posterior aqueous humor at steady state.[12] It appears from the indirect evidence of the present study, and a previous study where probenecid was found also to increase in vitreous humor after dichlorphenamide, that a mechanism may exist in the posterior portion of the eye for maintaining a status quo of total anions. It is known that the osmolarity of posterior aqueous humor tends to remain constant.[16] These findings raise the question of what physiologic anion replaces bicarbonate (and probably chloride) when these anions are reduced in vitreous humor after treatment with dichlorphenamide. A more complete study of acid-base balance of vitreous humor under these conditions seem to merit study.

The value for turnover coefficient for posterior aqueous humor (K_{h_1}) of 10.6 per cent per minute of the untreated animals with the lowest plasma level of DMO is similar to that for ^{24}Na (9 per cent),[7, 8] or SCN (11.5 per cent).[7] This coefficient value is reduced to some 5 per cent in the animals with higher plasma level (saturated system) and is approximately one half of a similar saturated system—ascorbate.[13]

In the untreated animals with the low-

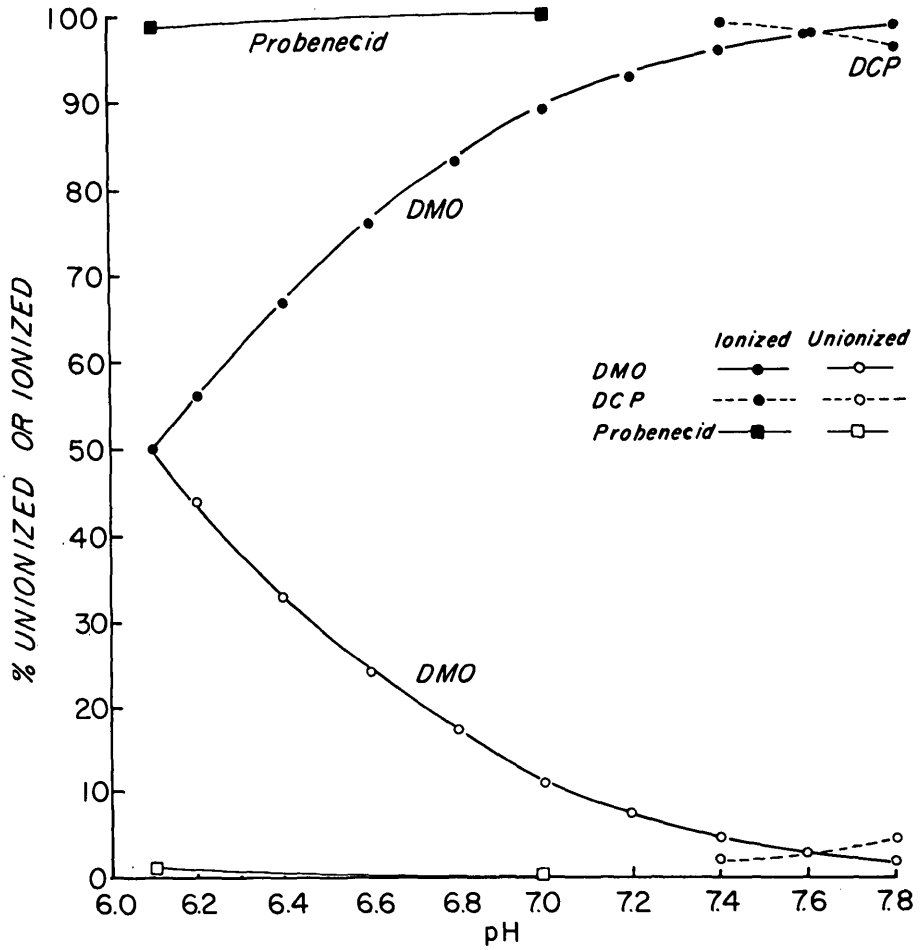

Fig. 4. Graphic presentation of the per cent ionized or unionized DMO (pK 6.1), probenecid (pK 3.4), dichlorphenamide (pK 9.15) at various pH values and calculated by use of the Hendelson-Hasselbalch equation.

est plasma level, DMO appears to enter the anterior aqueous humor approximately equally by diffusion and flow. The flow coefficient ($K_{f.a}$) of 2.01 per cent per minute is comparable to that for ascorbate of 1.73 per cent[13] or ^{24}Na and ^{36}Cl of 1.3 per cent per minute each.[8] This value was reduced to 0.7 to 0.8 per cent per minute in the untreated animals with the higher plasma levels or in animals receiving probenecid, a finding which is compatible with saturation (former group) or competitive inhibition (latter group) of the secretion out mechanism for organic anions. Under these conditions the flow rate is approximately 40 to 50 per cent of the diffusion rate. This is somewhat comparable to ^{36}Cl ($K_{f.a}$, 1.3 per cent; $K_{d.pa}$ 1.8 per cent)[8] but in contrast to the saturated ascorbate system ($K_{f.a}$ 1.73 per cent; $K_{d.pa}$ 0.4 per cent)[13] or the probenecid inhibited iodopyracet system ($K_{f.a}$ 1.5 per cent, $K_{d.pa}$ 0.3 per cent per minute).[15] Another difference between the organic anion system of iodopyracet and that of DMO was observed previously.[4] Iodopyracet penetrates the intraocular fluids negligibly from plasma and probenecid fails to increase its penetration into vitreous or aqueous humors.[9] This difference may be related in part to molecular size.

The value for flow coefficient is reduced

an additional 40 to 50 per cent after the administration of dichlorphenamide. This result is compatible with the usual finding of inhibition of secretion by carbonic anhydrase inhibitors and confirms the observation by Becker[14] that sulfonamides inhibit the secretion out mechanism for organic anions. A similar finding is obtained when the ratios of the flow coefficient: diffusion coefficient at steady state is compared. This ratio decreases from 0.56 (saturated) to 0.37 (probenecid) to 0.19 (dichlorphenamide).

Although there are many similarities between the various turnover coefficients of DMO in intraocular fluids with those of other electrolytes (Na, SCN, or Cl) and behavioral characteristics of organic anions (ascorbate, iodopyracet), such observations can be only qualitative at best. DMO exists in both the unionized and ionized state and there is no reason to assume that the diffusion or transport coefficients of these states are the same. In fact, the successful use of nonionic diffusion to indicate pH is dependent on the (relative) impermeability of the membrane to the ionized molecule. The data in Fig. 4 indicates that probenecid (pK 3.4) or dichlorphenamide (pK 9.15) are essentially entirely ionized but that DMO may vary from 92 to 97 per cent ionized at the probable pH gradients involved. The picture is, of course, further complicated by the participation of DMO in a mechanism which secretes organic anions out of the eye.

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