Phosphate and protein concentrations of intraocular fluids

I. Effect of carbonic anhydrase inhibition in young and old rabbits

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The effects of a carbonic anhydrase inhibitor, dichlorphenamide, on the phosphate and protein concentrations of intraocular fluids have been studied.
The administration of dichlorphenamide caused a marked increase in phosphate concentration of posterior and anterior aqueous humors. The changes in steady-state concentrations were compatible with a decrease in the flow coefficient of approximately 54 per cent.
Simultaneous treatment with probenecid has been found to prolong the inhibitory effect on aqueous secretion of a single dose of dichlorphenamide as measured by tonography and by phosphate concentrations in intraocular fluids.
The protein concentrations in intraocular fluids of the experimental eyes were not significantly different from those of the control eyes.

The possibility seemed worthy of exploration that changes in phosphate concentration in intraocular fluids might be used as an indicator of the partial suppression of aqueous humor secretion by inhibitors of carbonic anhydrase. Such partial suppression of secretion has been shown to alter the concentrations of ascorbate or bicarbonate in aqueous humors, and suitable considerations of aqueous humor dynamics permitted an estimation of the degree of suppression of secretion.[1] Ascorbate and bicarbonate concentrations in the aqueous humor of rabbits were in excess of plasma concentrations. Similar studies on the effect of carbonic anhydrase inhibitors on electrolytes in deficit in aqueous humor compared to plasma apparently have received little attention. The present experiments showed that the repeated administration of the carbonic anhydrase inhibitor, dichlorphenamide, resulted in a markedly increased phosphate concentration in intraocular fluids which was maintained for several hours. Since probenecid has been shown to decrease the renal excretion of a variety of organic acids,[2] including the carbonic anhydrase

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inhibitor acetazolamide, studies also were made to determine whether probenecid prolonged the effect of a single dose of dichlorphenamide.

Methods

In most experiments, immature male albino rabbits (Haskins Rabbitry) weighing 2 to 3 kilograms were used. In studies with older animals, male rabbits (received weighing approximately 2.2 kilograms) kept in our animal quarters approximately 9 months, and does, approximately 2 to 3 years of age when obtained from the rabbitry, were used. Samples of intraocular fluids were obtained from eyes proptosed under topical tetracaine anesthesia. The drugs used were dichlorphenamide (20 mg. per milliliter) and probenecid (100 mg. per milliliter) which were brought into solution with equivalents of sodium hydroxide. Dichlorphenamide was given in doses of 30 mg. per kilogram intravenously initially with repeated doses of 15 mg. per kilogram intraperitoneally every 2 hours, except as noted otherwise. Probenecid was given intraperitoneally in doses of 200 or 100 mg. per kilogram as noted with the summarized data. Only in 8 hour experiments was a repeat dose of probenecid (100 mg. per kilogram) given at 4 hours. Immediately after samples from the control eye were obtained, the drugs were administered and experimental samples were obtained from the opposite eye at the time intervals noted in the figures and tables. Heparinized blood samples were obtained from the heart just before paracenteses. The data for each group of animals were obtained from multiple experiments.

Phosphate was determined by the method of Fiske and Subbarow[3] with volumes appropriately reduced in proportions to permit analyses on 25 μl samples. The protein precipitate from the 25 μl samples was used for protein determinations according to the method of Lowry and associates.[4] Purified bovine albumin was used as the protein standard.

Results

Scattergrams of the phosphate concentration in anterior and posterior aqueous humors of control eyes of 171 young rabbits and 49 older rabbits are presented in Figs. 1 and 2. There appeared to be no correlation between the phosphate concentration in the posterior aqueous humor (C_h) and that in the plasma (C_p) of young rabbits. The standard error of the estimate (S_y) = 0.41 and the regression coefficient (r) = 0.25. The phosphate concentration of posterior aqueous humor of old rabbits

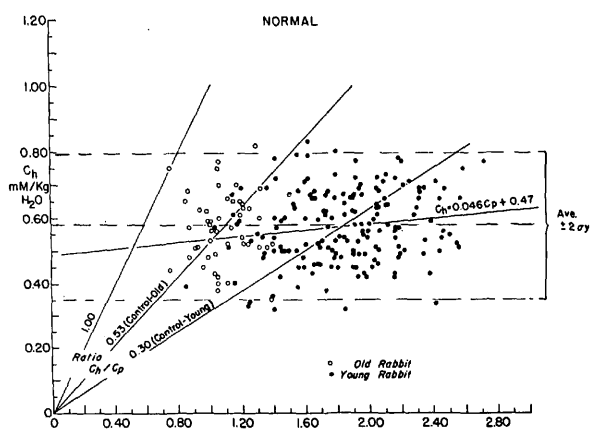

Fig. 1. Scattergram of the concentration of phosphate in the posterior aqueous humor (C_h) in normal young rabbits (filled circles) and old rabbits (open circles) in relationship to plasma concentration. There appears to be no relationship of C_h to C_p (the regression coefficient was found to be 0.25).

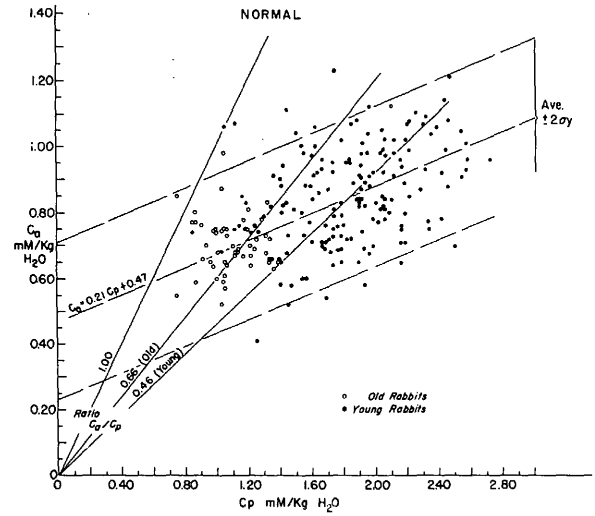

Fig. 2. Scattergram of the concentration of phosphate in the anterior aqueous humor (C_a) in normal young (filled circles) and old (open circles) rabbits in relationship to plasma concentration.

averaged 0.57 mM. per kilogram water and was indistinguishable from average values obtained from young rabbits. The concentration of phosphate in the plasma of old rabbits averaged 1.11 mM. ± 0.08 per kilogram water compared to 1.85 mM. per kilogram water for young rabbits. Of 344 young rabbits studied to date, only 15 per cent had plasma values below 1.45 mM. and 3 per cent below 1.30 mM. A significant correlation between the concentration of phosphate in the anterior aqueous humor (C_a) of rabbits and C_p was found (Fig. 2). The formula expressing this relationship was found to be $C_a = 0.21\ C_p + 0.47$ with $S_y = 0.12$, $r = 0.75$, and $p = < 0.001$.

During the course of these experiments, control data were obtained from the same population groups as were used for the dichlorphenamide studies to determine the similarity of concentrations of phosphate between the left and right eyes, and the effect, if any, of paracentesis of one eye on the fellow eye. These data are summarized in Table I. The agreement between the phosphate concentrations of the intraocular fluids of the two eyes was very good. The effect of paracentesis (removal of samples from posterior and anterior chambers) of one eye on the phosphate concentration of the intraocular fluids of the fellow eye appeared to be negligible. As indicated by the second group of Table I, the inclusion of vitreous sampling, in many instances, caused a decrease in C_a of phosphate at longer time intervals. In another vitreous-sampled group, not included in the present study, a similar decrease in C_a at 6 hours was found with no change in C_h or C_v of phosphate. The groups are too small for the data to demonstrate conclusively an effect of vitreous sampling on the fellow eye.

Two series of experiments with young animals were used to follow the time course of change of phosphate concentration in posterior and anterior aqueous humors after the administration of the carbonic anhydrase inhibitor, dichlorphenamide. In the first of these series of animals, paracenteses were done 30 minutes to 9 hours after dichlorphenamide administration. Vitreous samples were also obtained. It was possible that the added trauma of vitreous sampling might have altered the dynamics of the contralateral eye. Consequently, a second series of experiments was conducted in which vitreous samples were not obtained and anterior and posterior aqueous humor samples were taken at shorter intervals during the early time period. Paracenteses were done 10 minutes to 9 hours after dichlorphenamide administration. In a third series of experiments, the change in the phosphate concentration in the posterior and anterior aqueous humors of older rabbits was determined 2 to 12 hours after administration of dichlorphenamide.

An average of 7 animals was used for each time period of each series.

The average phosphate concentrations found at different time intervals after dichlorphenamide administration are summarized in Fig. 3, *A* and *B*. The concentration of phosphate in the posterior aqueous humor of animals without vitreous sampling increased more rapidly than that in the anterior aqueous humor. It increased from an initial value of 0.58 to 1.05 mM. per kilogram water and remained elevated some 80 per cent (in many instances 100 per cent) at 6 to 9 hours. The concentration of phosphate in the anterior aqueous humor increased from an initial value of 0.86 to 1.30 mM. per kilogram water and remained elevated some 50 per cent at 6 to 9 hours. The results obtained from animals with vitreous sampling were qualitatively similar. The concentration of phosphate in the vitreous showed a small (10 per cent), but not statistically significant, increase by 9 hours. In older animals (Fig. 3, *B*) it was possible to obtain posterior aqueous samples approximately 2.5 times the volume of those obtainable from young animals. These larger volumes of intraocular fluids of older rabbits probably accounted for the more gradual rise in phosphate concentrations. The concentration in the anterior aqueous humor increased from an initial value of 0.73 to 1.40 mM. per kilogram water at 10 to 12 hours. The posterior aqueous humor concentration increased from an initial value of 0.56 mM. per kilogram water to approximately anterior aqueous humor levels at 8 hours, and averaged a slight excess at 10 to 12 hours. Longer experi-

Table I. Comparison of phosphate concentration (mM./Kg. water) of intraocular fluids of right and left eyes—average ± S.D.

No.	*Hour**	*Concentration in plasma*	*Concentration in posterior aqueous humor*	*Concentration in anterior aqueous humor*	*Concentration in vitreous humor*
6‡	0	1.73	0.57 ± 0.13	0.76 ± 0.18	0.20 ± 0.09
	2	1.85	0.53 ± 0.22	0.76 ± 0.19	0.20 ± 0.09
6†‡	0	1.60	0.58 ± 0.14	0.85 ± 0.13	0.38 ± 0.18
	6	1.68	0.61 ± 0.10	0.74 ± 0.13	0.23 ± 0.05
6‡	0	1.91	0.70 ± 0.09	1.01 ± 0.10	
	4	1.93	0.62 ± 0.07	1.03 ± 0.13	
7‡	0	1.84	0.57 ± 0.07	0.86 ± 0.20	
	6	2.09	0.61 ± 0.10	0.98 ± 0.17	
5‡	0	1.90	0.64 ± 0.08	0.95 ± 0.10	
	0		0.62 ± 0.05	0.95 ± 0.08	
6§	0	1.13	0.56 ± 0.09	0.67 ± 0.07	
	0		0.56 ± 0.13	0.65 ± 0.08	

*Time after paracentesis of the first eye that the contralateral eye was sampled.

†Animals were given inert analogs of carbonic anhydrase inhibitors. One animal had an unusually high C_v of phosphate of the control eye only. Omitting this sample the average C_v was 0.30 ± 0.06.

‡Young immature rabbits.

§Old rabbits.

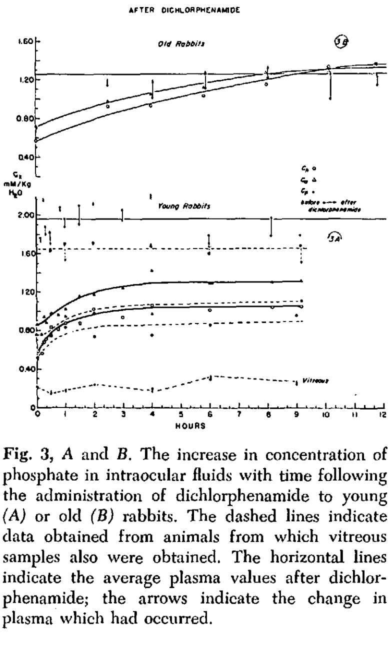

Fig. 3, *A* and *B*. The increase in concentration of phosphate in intraocular fluids with time following the administration of dichlorphenamide to young *(A)* or old *(B)* rabbits. The dashed lines indicate data obtained from animals from which vitreous samples also were obtained. The horizontal lines indicate the average plasma values after dichlorphenamide; the arrows indicate the change in plasma which had occurred.

ments would be necessary to determine the plateau levels in old animals.

Scattergrams of data obtained after the administration of dichlorphenamide to young and older animals (Figs. 4 and 5) showed that approximately two thirds of older animals at 10 to 12 hours had phosphate concentrations in intraocular fluids from 10 to 50 per cent in excess of plasma concentrations. Of the 5 animals which remained in deficit, the plasma concentration of 4 rabbits increased considerably. These scattergrams indicated that in young rabbits approximately 95 per cent of the data obtained from posterior and anterior aqueous humor samples after 4 hours of inhibition of secretion by dichlorphenamide were above the normal average ± 2 S.D.

Several investigators have shown that the rate of change of concentration of a substance in the anterior aqueous humor (C_a)* is proportional to the contribution by flow (secretion) from the posterior chamber ($k_{fa}C_h$) less the loss by flow out of the anterior chamber ($k_{fa}C_a$) and the net gain or loss by diffusional exchange between plasma and anterior aqueous humor ($k_{dpa}[C_p - C_a]$). For determining alterations in the secretion of aqueous, Becker[1] has shown that the change in concentrations at a new steady state may be usefully employed.* Values obtained for k_{fa}/k_{dpa} before and at various time intervals after the administration of dichlorphenamide to young and older rabbits are summarized in Table II. These data indicated that a new steady state was approached in young animals at 6 hours. The decrease in the ratio k_{fa}/k_{dpa} by 52 to 56 per cent from 6 to 9 hours was in excellent agreement with results obtained by Becker[1] from bicarbonate and ascorbate data (51 to 60 per cent). In older animals a decrease in this ratio by 76 to 89 per cent was found at 6 and 8 hours, respectively. At longer intervals, 10 to 12 hours, the data are difficult to interpret because of the maintenance of an excess of phosphate in intraocular fluids of the majority of the rabbits.

Occasionally the concentrations of phosphate in posterior and anterior aqueous humors of old rabbits were very similar. Because of the possibility that the lens may not have provided a water-tight seal between the posterior and anterior chambers during proptosis in these animals, analysis of bicarbonate and ascorbate concentrations were determined simultaneously in a series of 5 animals before and 4 to 6 hours after the administration of dichlorphenamide.

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*$\frac{dC_a}{dt} = k_{fa}\ (C_h - C_a) - k_{dpa}\ (C_p - C_a)$

at steady state, $\frac{dC_a}{dt} = 0$; $\frac{k_{fa}}{k_{dpa}} = \frac{C_a - C_p}{C_h - C_a} = \frac{\text{gradient, anterior aqueous humor to plasma}}{\text{gradient, posterior aqueous humor to anterior aqueous humor}}$

k_{fa} = flow coefficient

k_{dpa} = diffusion coefficient

The use of the formulation as a measure of the flow coefficient at steady state assumes that diffusion has not been altered.

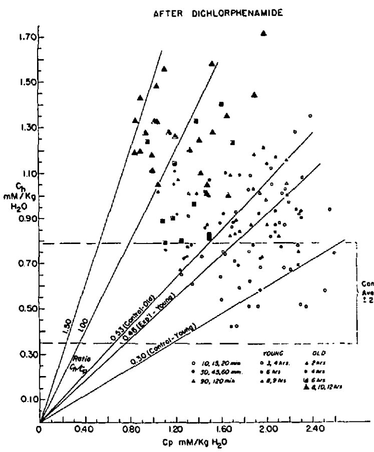

Fig. 4. Scattergram of the concentration of phosphate in the posterior aqueous humor of young and old rabbits at various times after the administration of dichlorphenamide.

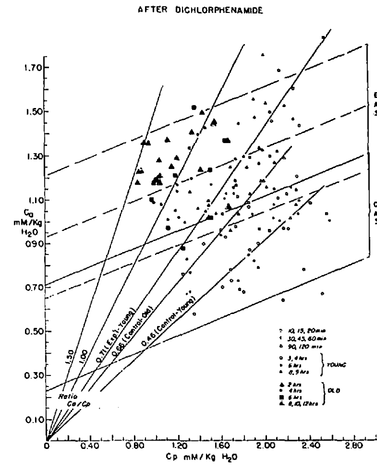

Fig. 5. Scattergram of the concentration of phosphate in the anterior aqueous humor of young and old rabbits at various times after the administration of dichlorphenamide.

These data are given in Table III. It was apparent from the data that suspicions of incomplete separation of chambers aroused because of the ease of obtaining large posterior aqueous humor samples (No. 9 = 90 μl) and the observed relative stiffness of the iris in these older animals were unwarranted. In animal No. 9 the value of the difference, $C_a - C_p$, was 0.01 mM. PO_4 per kilogram water before and 0.04 mM. per kilogram water after dichlorphenamide, whereas the value for bicarbonate concentration was 10.8 mM. and 10.6 mM. per kilogram water, respectively. The agreement in the change in $k_{fa}/_{dpa}$ as calculated from phosphate, ascorbate, and bicarbonate data was very good. The data from the three analyses indicated that animal No. 3 was not approaching a new steady state.

The effect of probenecid on the change in phosphate concentration of intraocular fluids at 4, 6, and 8 hours after the administration of a single dose of dichlorphenamide (30 mg. per kilogram intravenously) was studied. These data are summarized in Table IV. The average concentrations of phosphate in the anterior aqueous humor found in all groups receiving dichlorphenamide were similar and ranged from 1.29 to 1.44 mM. per kilogram water. The increase in concentration of phosphate in the posterior aqueous humor of animals receiving dichlorphenamide alone was 28 to 36 per cent compared to 57 to 100 per cent at comparable time periods in animals which received both dichlorphenamide and probenecid. The increases in phosphate concentration in intraocular fluids occurred in the probenecid-dichlorphenamide groups in spite of a decrease in phosphate concentration in the plasma. The plasma concentration showed an average decrease of 0.46 mM. per kilogram water in 57 per cent of the animals. This decrease in plasma concentration was not seen after probenecid alone or dichlorphenamide alone. Probenecid alone did not significantly alter the concentration of phosphate in the posterior or anterior aqueous humors.

Although the decrease in the ratio $k_{fa}/$

Table II. The inhibition of aqueous secretion following dichlorphenamide administration as indicated by the phosphate concentration of intraocular fluids (mM./Kg. H_2O)

	Young rabbits					Old rabbits				
Time (min.)	*No.*	$C_p - C_a$ *mM.*	$C_a - C_h$ *mM.*	$\frac{k_{fa}}{k_{dpa}}$	$\Delta \frac{k_{fa}}{k_{dpa}}$ *(%)*	*No.*	$C_p - C_a$ *mM.*	$C_a - C_h$ *mM.*	$\frac{k_{fa}}{k_{dpa}}$	$\Delta \frac{k_{fa}}{k_{dpa}}$ *(%)*
0	7	0.94	0.26	3.6						
10		0.98	0.20	4.9	+					
0	8	1.27	0.33	3.9						
15		1.22	0.26	4.7	+					
0	8	0.97	0.27	3.5						
20		0.95	0.17	5.6	+					
0	6	0.88	0.25	3.5						
30		0.78	0.15	5.2	+					
0	7	1.27	0.26	4.9						
45		1.10	0.15	7.3	+					
0	5	1.08	0.24	4.5						
60		0.90	0.15	6.0	+					
0	8	1.08	0.34	3.2						
90		0.84	0.29	2.9	- 9					
0	6	1.31	0.30	4.4		3	0.42	0.12	3.5	
120		1.05	0.15	7.0	+		0.23	0.05	4.6	+
0	8	1.16	0.35	3.3						
180		0.76	0.31	2.5	-24					
0	7	1.34	0.23	5.8		7	0.29	0.21	1.4	
240		0.90	0.28	3.2	-45		0.15	0.12	1.2	-14
0	7	0.91	0.28	3.3		10	0.42	0.17	2.5	
360		0.45	0.28	1.6	-52		0.11	0.18	0.6	-76
0	5	1.06	0.21	5.0		8	0.53	0.10	5.3	
480		0.58	0.26	2.2	-56		0.07	0.11	0.6	-89
0	6	0.96	0.25	3.9						
540		0.48	0.27	1.8	-54					
0						8	0.25	0.12	2.1	
600							-0.12	0.08	-1.5	?
0						7	0.39	0.10	3.9	
720							-0.10	-0.01		?

k_{dpa} (Table IV) was similar in all groups, as was indicated in the foregoing paragraph, this result was not achieved by similar changes in phosphate concentrations. In the dichlorphenamide-probenecid treated animals the difference in concentration of phosphate between anterior and posterior aqueous humors was the same at the new steady state as that in the untreated animal. An analogous situation was seen at 4 hours in animals which received only dichlorphenamide. However, at 6 and 8 hours the latter group showed a marked increase (50 to 78 per cent) in the difference in phosphate concentration between posterior and anterior aqueous humors. Since the concentration of phosphate in the anterior aqueous humor of all groups were similar, the increase in the difference, $C_a - C_h$, seen in the group given only dichlorphenamide, was due to the failure of the phosphate concentration to increase in posterior aqueous humor to the same degree as seen in the dichlorphenamide-probenecid groups. At 8 hours the C_h of phosphate in animals given a single dose of dichlorphenamide was 0.75 mM. per kilogram water compared to 1.00 mM. per kilogram water in the dichlorphenamide-probenecid group. These data do not permit estimates to be made of the flow or diffusion coefficients or estimates of the changes in these coefficients which may have occurred.

The prolonged action of dichlorphenamide in this population of rabbits was confirmed in other animals by tonography. These data are summarized in Table V. The inhibition of secretion by a single dose of dichlorphenamide was found to persist in many animals for 4 hours and in some animals for 6. The administration of probenecid with the dichlorphenamide prolonged the inhibitory action of dichlorphenamide on aqueous secretion as measured by tonography.

The protein concentrations found in the posterior and anterior aqueous humors of many of the groups of animals used in this study are summarized in Table VI. The determination of protein was of considerable value in noting the infrequent animal in which the lens was nicked during the advancement of the needle to obtain posterior aqueous humor samples or in eliminating the occasional animals with abnormal blood-aqueous barriers undetected by the appearance of the conjunctiva or iris. In the former case, the protein concentration increased to approximately 600 mg. per 100 ml. with a much smaller change in protein concentration of the anterior aqueous humor. In three instances in which this occurred in control eyes, the phosphate concentration of the posterior aqueous humor was indistinguishable from the values obtained from other animals with

Table III. Effect of dichlorphenamide on the phosphate, ascorbate, and bicarbonate concentrations (mM./Kg. H_2O) in intraocular fluids

Animal No.	*Hour*	C_p	C_h	C_a	$C_a - C_h$	$\frac{k_{fa}}{k_{dpa}}$	$\Delta \frac{k_{fa}}{k_{dpa}}$ (%)
Phosphate							
9	0	1.03	0.53	0.54	0.01	49.0	
	4	1.32	0.73	0.77	0.04	13.7	-72
3	0	0.98	0.48	0.64	0.16	2.1	
	4	1.11	0.76	1.01	0.25	0.4	-81
4	0	1.03	0.43	0.87	0.44	0.4	
	4	1.33	0.78	1.20	0.42	0.3	-25
1	0	1.05	0.38	0.57	0.19	2.5	
	6	1.50	0.81	1.02	0.21	2.3	-10
2	0	1.38	0.35	0.72	0.37	1.8	
	6	1.50	0.83	1.18	0.35	0.9	-50
Ascorbate							
9	0		1.13	0.96	0.27	5.6	
	4		1.36	0.98	0.38	2.6	-54
3	0		1.34	1.16	0.18	6.5	
	4		1.89	1.35	0.54	2.5	-62
4	0		1.53	1.08	0.45	2.4	
	4		1.45	0.85	0.60	1.4	-42
1	0		1.33*	1.06	0.27	3.9	
	6		1.21	0.85	0.36	2.4	-38
2	0		1.32	1.12	0.20	5.6	
	6		1.66	1.01	0.55	1.6	-71
Bicarbonate							
9	0	25.4	41.1	30.3	10.8	0.5	
	4	22.4	33.6	23.0	10.6	0.1	-80
3	0	27.0	38.0	32.0	6.0	0.8	
	4	16.8	22.8	21.1	1.7	2.6	+
4	0	24.0	38.0	30.5	7.5	0.9	
	4	17.4	24.4	20.2	4.2	0.7	-22
1	0	30.0	40.2	34.4	5.8	0.8	
	6	18.6	26.9	22.4	4.5	0.8	- 0
2	0	26.1	39.9	32.5	6.6	0.9	
	6	18.6	25.3	20.0	5.3	0.3	-66

normal protein concentrations. In animals with disturbed blood-aqueous barriers, the protein concentration was increased in both posterior and anterior aqueous humors to approximately 200 mg./100 ml. or more compared to average values of posterior aqueous humor of normal eyes of some 40 mg./100 ml. The protein concentration of the posterior aqueous humor was approximately 30 per cent less than that of the anterior aqueous humor. The administration of dichlorphenamide frequently caused a small increase and that of probenecid a small decrease in protein concentration of intraocular fluids, but the changes were not statistically significant. The protein concentration in intraocular fluids of older rabbits was similar to that of young animals.

Discussion

The concentrations of phosphate found in the intraocular fluids in the present study are in excellent agreement with values reported by Reddy and Kinsey,[5] with the exception of the lower concentration in the vitreous humor found in our experiments (Table VII). The somewhat higher concentration found in the vitreous humor by Reddy and Kinsey might result from the larger vitreous samples they removed (1 ml. compared to ¼ ml. in the present study). The larger sample probably would include more of the anterior vitreous with its higher phosphate concentration.[6]

The concentration of phosphate in posterior aqueous humor shows little relationship to that of plasma. This observation indicates that phosphate appears to be unique

Table IV. Effect of probenecid on the phosphate concentration (mM./Kg. H_2O) of intraocular fluids following the administration of a single dose of dichlorphenamide

No.	*Hr.*	C_p	C_h	C_a	$C_a - C_h$	$\frac{k_{fa}}{k_{dpa}}$	$\Delta \frac{k_{fa}}{k_{dpa}}$ (%)
Dichlorphenamide only°							
6	0	1.86	0.69	0.99	0.30	2.9	
	4	1.75	0.94	1.29	0.35	1.3	-55
6	0	2.17	0.64	1.03	0.39	2.9	
	6	2.41	0.86	1.44	0.58	1.7	-41
6	0	2.09	0.58	0.90	0.32	3.7	
	8	2.14	0.75	1.32	0.57	1.4	-62
Dichlorphenamide and probenecid (200 mg./Kg. initially)†							
6	0	2.09	0.63	1.07	0.44	2.3	
	4	1.72	1.05	1.42	0.37	0.8	-65
6	0	2.01	0.69	0.92	0.23	4.7	
	6	1.89	1.08	1.35	0.27	2.0	-57
6	0	2.00	0.50	0.85	0.35	3.3	
	8	1.82	1.00	1.38	0.38	1.2	-64
Dichlorphenamide and probenecid (100 mg./Kg. initially)†							
6	0	2.18	0.55	0.80	0.25	5.5	
	6	1.93	1.01	1.29	0.28	2.3	-55
7	0	2.12	0.50	0.87	0.37	3.4	
	8	1.88	1.07	1.36	0.29	1.8	-47
Probenecid only (200 mg./Kg. initially)†							
7	0	2.14	0.70	1.08	0.38	2.8	
	4	2.23	0.67	1.04	0.37	3.2	+14
10	0	2.15	0.55	0.89	0.34	3.7	
	8	2.42	0.61	0.97	0.36	4.0	+ 8

°Dichlorphenamide was given as a single dose of 30 mg./Kg. intravenously.
†Probenecid was given intraperitoneally at an initial dose as stated for the first 6 hours. In 8 hour experiments a second intraperitoneal dose of 100 mg./Kg. was given at 4 hours.

Table V. The effect of a single dose of dichlorphenamide, without and with simultaneous probenecid, on aqueous humor dynamics as measured by tonography

No. eyes	Before drug P_o*	Before drug C†	After drug P_o	After drug C	ΔF‡
Dichlorphenamide§—4 hours					
18	20 ± 2	0.30 ± 0.08	14 ± 3	0.29 ± 0.10	-55‖
Dichlorphenamide—6 hours					
16	19 ± 3	0.27 ± 0.06	16 ± 3	0.27 ± 0.08	-30¶
Dichlorphenamide + Probenecid#—6 hours					
23	20 ± 3	0.28 ± 0.07	14 ± 3	0.26 ± 0.05	-58**

*P_o, intraocular pressure, mm. Hg, average ± S.D.
†C, outflow facility, μl/min./mm. Hg, average ± S.D.
‡ΔF, change in flow of aqueous humor, %, average.
§Dichlorphenamide dose was 30 mg./Kg. I.V.
‖Five eyes showed less than a 30% decrease.
¶Three eyes showed more than a 55% decrease.
#Probenecid dose was 200 mg./Kg. initially plus 100 mg./Kg. at 4 hrs. I.P.
**Three eyes showed less than a 30% decrease.

among the various anions studied in intraocular fluids. No relationship of C_v to C_p is found. A significant correlation of C_a and C_p of phosphate is found which can be expressed by the equation $C_a = 0.21\ C_p + 0.47$. A relationship of C_v to C_p is found which is expressed by the equation $C_v = 0.23\ C_p + 0.11$ and is significant at the 5 per cent level. Scrutiny of data from individual animals indicates that deviation from the predicted values for phosphate concentration in intraocular fluids might be explained in part by differences in the phosphate concentration of the vitreous (Table VII). This possibility is being investigated further in older animals and in young animals in which it has been found possible to alter the concentration of phosphate in vitreous humor by pretreatment with steroids.

Since phosphate is so universally involved in metabolism, it is apparent that studies of the effect of any drug on the secretion of aqueous as measured by phosphate concentration must take this possibility under consideration. The present studies in which a carbonic anhydrase inhibitor was used indicate that for this class of inhibitors of aqueous secretion, changes in concentration of phosphate in intraocular

Table VI. Protein concentrations (mg./100 ml.) in intraocular fluids

No.	Hours	Posterior aqueous humor Before	Posterior aqueous humor After	Anterior aqueous humor Before	Anterior aqueous humor After
Paracentesis only—young rabbits					
6	0 + 4	42 ± 12	40 ± 7	60 ± 12	51 ± 13
5	0 + 6	22 ± 5	23 ± 3	33 ± 6	35 ± 5
5	0 + 0	47 ± 11	43 ± 6	78 ± 19	74 ± 16
Paracentesis only—old rabbits					
3	0 + 0	29 ± 3	30 ± 5	38 ± 8	35 ± 3
Dichlorphenamide—old rabbits					
8	0 to 12	32 ± 8	38 ± 7	44 ± 12	47 ± 12
11	0 to 12	42 ± 6	56 ± 9	58 ± 13	70 ± 17
Dichlorphenamide—young rabbits					
6	0 + 4	46 ± 5	52 ± 12	75 ± 9	76 ± 16
7	0 + 6	33 ± 4	36 ± 7	46 ± 11	59 ± 18
5	0 + 8	39 ± 4	39 ± 6	61 ± 11	81 ± 20
Probenecid—young rabbits					
6	0 + 4	49 ± 5	44 ± 5	71 ± 13	58 ± 23
6	0 + 8	52 ± 14	36 ± 13	78 ± 22	49 ± 20
Dichlorphenamide plus probenecid—young rabbits					
5	0 + 4	50 ± 7	49 ± 4	74 ± 11	76 ± 10
6	0 + 6	40 ± 18	36 ± 10	45 ± 16	52 ± 23
9	0 + 8	47 ± 9	61 ± 18	81 ± 25	82 ± 17

Table VII. Comparison of phosphate concentrations (mM./Kg. water) of intraocular fluids

	C_p	C_h	C_a	C_v
Data of Reddy and Kinsey				
	2.04	0.58	0.89	0.40
Present studies				
	1.86	0.56	0.88	0.23
Animal A				
OD	1.25	0.34	0.41	0.03
OS	1.24	0.25	0.45	0.05
Animal B				
OD	2.05	0.72	0.99	0.33
OS	2.25	0.97	1.10	0.35

fluids give results for calculated inhibition in excellent agreement with results obtained from ascorbate and bicarbonate data. It would appear that under these conditions the contributions by metabolism to alteration in phosphate concentration are small compared to that caused by the inhibition of secretion by dichlorphenamide. The effect of other inhibitors (as determined by tonography) is under investigation.

After dichlorphenamide administration to young animals, the phosphate concentration in anterior aqueous humor increased to approximately 70 per cent of the plasma level. In older animals, the number of animals in which the C_a was more than 10 per cent in excess of plasma concentration after treatment was striking. This observation indicates that phosphate may differ from other anions, although studies with older animals have received little attention. If one lowers the plasma phosphate of young animals by pretreatment with cortisone to the level found in old animals, then a number of young animals show phosphate concentrations of anterior aqueous humor in excess of plasma after dichlorphenamide administration. It has not been feasible yet to achieve the same excess as found in older animals. A number of factors might be influencing these results: the larger volumes of aqueous chambers in older animals would require longer periods for concentrations to reach a new steady state; the metabolic activity of the retina might be altered so that the usual metabolic drain from the posterior portion of the eye is not functioning properly; or the tissues of the eye under different metabolic conditions may be contributing more significantly to the concentrations in intraocular fluids than they normally do. Further study is necessary to provide an explanation for the excess of phosphate found in intraocular fluids of older animals which have been given dichlorphenamide.

In an earlier report, Kinsey[7] found the diffusion coefficient for phosphate to be 0.011. Based on his more recent data and the present studies which show an average value of k_{fa}/k_{dpa} of 3.5, assuming an average k_{fa} of 0.016[1, 7] k_{dpa} averages 0.0046. Ascorbic acid is the only other electrolyte studied to date which shows a similarly low k_{dpa} (0.006).[1, 7]

Probenecid combined with a single dose of dichlorphenamide causes a striking decrease in plasma phosphate concentration in many animals. This effect is not seen after probenecid alone and has not been observed when probenecid was given along with 5 ml. of 3 per cent ammonium chloride per kilogram of body weight intraperitoneally. It may be that experiments to date have not resulted in the same degree of acidosis as found after dichlorphenamide.

In patients with normal parathyroids, probenecid has no significant effect on urinary excretion of phosphate.[8] Although a lowering of plasma phosphate concentration and an increase in renal excretion is found in patients with pseudohypoparathyroidism, the effect is quite variable. A similar, but variable effect, is found with acetazolamide.[9] The two drugs were not used in combination as in the present studies with rabbits.

One factor which is not corrected for in these experiments is the "bound" plasma phosphate. If rabbit plasma is similar to that of humans, plasma binding would result in a correction factor, a decrease of

17 per cent.[10] If one then corrected for the theoretical Donnan ratio, an increase of 9 per cent,[10] there would remain a net decrease of plasma concentration from presently reported values of 8 per cent. With this correction, almost all old animals would show an excess of phosphate in posterior and anterior aqueous humors after 8 hours of dichlorphenamide administration.

Although the protein concentration of intraocular fluids is more variable after dichlorphenamide administration and frequently increased in individual animals, group averages of experimental eyes are not statistically different from those of control eyes. Probenecid, more often than not, caused a decrease in protein concentration, but this cannot be considered significant.

Addendum

As a result of discussion of this paper, plasma binding in rabbits was determined at 25° C. by use of 10 mμ Millipore filters. An average of 12 per cent bound phosphate (7 to 16 per cent) was found in plasma of 8 young rabbits with an average total phosphate concentration of 1.99 mM. per liter. An average of 3 per cent bound phosphate (0 to 8 per cent) was found in 5 older rabbits with an average total phosphate concentration of 0.96 mM. per liter. Plasma values in graphs and tables have not been corrected with this average binding value since physiologic changes and drugs alter these results and they were not done on the same populations. For example, insulin, strophanthidin, and probenecid altered plasma phosphate concentrations to a degree which appears to be dependent in part on the initial concentration. In addition, in 4 rabbits with an average binding of 12 per cent (9 to 14 per cent), ammonium chloride (150 mg. per kilogram) has been found not to alter significantly the plasma concentration of phosphate, but did increase the amount bound to an average of 22 per cent (19 to 24 per cent).

As correctly pointed out by Dr. Winston Barber, a regression line to the ordinate implied a mechanism of events. Consequently, the regression line has been partially blotted out. However, the use of the equation for such a line, necessarily including an intercept, provided evidence that groups having intraocular concentrations different from predicted values may show abnormal concentrations in the vitreous humor. This clue has led to the possibility of altering concentrations in vitreous humor, although the mechanisms for these changes remain obscure.

Dr. Barber also noted the paradox that the protein concentrations in intraocular fluids did not increase as did phosphate concentrations after the use of carbonic anhydrase inhibitor. An equally puzzling observation was the probable decrease in protein concentration after probenecid. Consequently, it is important to emphasize that the methodology used for protein determination was not specific and was used as an indication of the presence of gross changes due to technique or physiopathologic changes in the eye. Thus, the protein precipitates were not washed to free them of interfering substances such as free amino acids or of reducing substances, although most of these substances were removed with the supernatant. The probable failure of protein concentration to change in the direction anticipated remains to be explained.

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