LXVII. A STUDY OF THE ACID-BASE EQUILIBRIUM OF THE OCULAR HUMOURS.

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So long as the fluids in the eye were regarded as the secretory product of the ciliary body no direct relationship between them and the blood stream was postulated. A large amount of experimental work, however, has recently been brought forward which tends to show that this fluid is a dialysate of the capillary plasma [see Duke-Elder, 1927, 1], and if this theory is correct the relationship between the intra-ocular fluid and the blood-plasma should be very intimate. Most of the evidence which has been brought forward in support of the newer theory is either chemical in nature, dealing with the constitution of normal and abnormal aqueous humour, or physical, dealing with the hydrostatic, osmotic and electrostatic forces involved. It may be of value therefore to consider how far the relationship of the acid-base equilibrium of the fluids in the eye with that of the plasma brings support to the more recent conceptions.

It was considered desirable to estimate the hydrogen ion concentration of the vitreous and aqueous humours, the carbon dioxide content of the aqueous humour, and the carbon dioxide content and capacity as well as the dissolved oxygen in the vitreous humour. The eyes were obtained immediately after death and the aqueous humour was abstracted by means of a syringe and kept under paraffin oil. The vitreous humour was then shelled out through a posterior incision and was kept in a paraffin-covered tube.

Since the vitreous humour is difficult to measure accurately on account of its jelly-like consistency, it was used after liquefaction by forcing it through the perforated plunger of a syringe the opening of which had been plugged.

p_H DETERMINATION.

Cullen's [1922] colorimetric method as applied in Myers's [1924] technique was employed. Since both humours are only slightly buffered in comparison with the blood-plasma, the p_H determinations were carried out without any dilution. The values are only comparative in respect to the two humours, since no corrections were made for temperature (Tables I and II).

Table I. *Aqueous humour.*

p_H values of the aqueous humours of cattle varying in age from 5 weeks to 2 years.

No. of animal	Age of animal	Room temp.	p_H		
			Eye 1	Eye 2	Average
1	5 wks.	26°	7.20	7.25	7.23
2	2 mths.	27	Mixture		7.22
3	4 "	30	7.43	7.33	7.38
4	4 "	27.5	7.40	—	7.40
5	4 "	27	7.27	7.32	7.30
6	4 "	27	Mixture		7.41
7	8 "	27	7.33	7.44	7.39
8	1½ yrs.	24	7.42	7.44	7.43
9	1½ "	24	7.40	7.41	7.41
10	1½ "	24	7.38	—	7.38
11	1½ "	25.5	*7.35	7.35	7.35
12	1½ "	25.5	*7.10	7.20	7.15
13	1½ "	25.5	*7.35	7.25	7.30
14	1½ "	25.5	*7.42	7.36	7.39
15	1½ "	26	*7.25	7.20	7.23
16	1½ "	26	*7.36	7.20	7.28
17	1½ "	26	*7.35	7.33	7.34
18	2 "	27.5	7.28	7.26	7.27
19	2 "	27.5	7.35	7.34	7.35
20	2 "	26	7.31	7.40	7.36
21	2 "	26	7.20	—	7.20
22	2 "	26	Mixture		7.40
23	2 "	26	7.58	7.48	7.53
24	2 "	26	7.48	7.58	7.53
25	2 "	25.5	7.39	7.44	7.32
26	2 "	25.5	7.51	7.42	7.47
27	2 "	25.5	7.45	7.45	7.45
28	2 "	25	*7.28	7.33	7.31
29	2 "	25	*7.20	7.25	7.23
30	2 "	25	*7.27	7.25	7.26
31	2 "	25	*7.18	—	7.18
32 (Sheep)	Age unknown	27	7.23	—	7.23

* The humours were allowed to remain in the extracted eye in the refrigerator half a day. All other results were obtained from strictly fresh humours.

When the ocular humours are allowed to remain in the excised eyes for some hours, they become more acid (see Tables III and IV), despite a possible loss of carbon dioxide, which may be due to autolytic decomposition of protein. Unpublished earlier analyses by the author (1927), in confirmation of the findings of other workers, revealed that the vitreous humour under these conditions suffers a great increase in protein content. This increase in protein content is thought to be due to transudation from the surrounding capillaries [Duke-Elder, 1927, 2].

A comparison of the average p_H values of aqueous humours of cattle for different age groups with those of vitreous humours for similar age groups shows that the aqueous humour is slightly less alkaline than the vitreous humour. This is illustrated in Fig. 1.

Table II. *Vitreous humour.* p_H values of the vitreous humours of cattle varying in age from 3 months to 2 years.

No. of animal	Age of animal	Age of specimen under oil (days)	Room temp.	p_H			Average
				Determinations			
				1	2	3	
1	3 mths.	Fresh	27°	7.27	7.20	7.31	7.26
2	4 "	"	27.5	7.40	7.41	—	7.40
3	8 "	"	27	7.36	7.43	—	7.40
4 Eye 1	1½ yrs.	15	24	*7.36	7.33	—	7.35
Eye 2	1½ "	15	24	*7.33	7.31	—	7.32
5	1½ "	15	24	*7.30	7.30	7.15	7.30
6	1½ "	16	22	*7.14	7.20	7.15	7.16
7	1½ "	4	22.5	*7.24	7.24	—	7.24
8 Eye 1	1½ "	4	22.5	*7.24	7.24	—	7.24
Eye 2	1½ "	4	22.5	*7.25	7.30	—	7.27
9	1½ "	3	25	7.45	—	—	7.45
10	1½ "	3	25	7.54	—	—	7.54
11	1½ "	3	25	7.49	—	—	7.49
12	1½ "	3	25	7.44	—	—	7.44
13	1½ "	3	25	7.40	7.40	—	7.40
14	1½ "	3	25	7.40	—	—	7.40
15	1½ "	3	25	7.35	7.36	—	7.36
16 Eye 1	2 "	Fresh	25	7.44	7.38	—	7.41
Eye 2	2 "	"	25	7.38	7.42	—	7.40
17 Eye 1	2 "	1	24	7.44	7.40	7.45	7.43
Eye 2	2 "	1	24	7.40	7.40	7.38	7.39
18 Eye 1	2 "	2	26	7.49	7.54	7.54	7.52
Eye 2	2 "	2	26	7.49	7.56	7.54	7.53
19	2 "	2	26	7.66	7.65	7.65	7.65
20 Eye 1	2 "	2	26	7.64	7.58	7.64	7.61
Eye 2	2 "	2	26	7.63	7.61	—	7.62
21 Eye 1	2 "	6	25.5	*7.49	7.54	—	7.51
Eye 2	2 "	6	25.5	*7.24	7.33	7.38	7.32
22 Eye 1	2 "	6	26	*7.40	7.43	—	7.41
Eye 2	2 "	6	26	*7.32	7.33	—	7.33
23	2 "	6	26	*7.46	7.36	—	7.41
		6	26	*7.35	7.40	7.35	7.37

* The humours were allowed to remain in the excised eye for half a day in the refrigerator.

Table III. p_H values for fresh aqueous humours and for those kept in the excised eye, in the refrigerator, for half a day. (The specimens were handled under oil.)

Fresh aqueous		Aqueous kept in the excised eye for half a day	
No. of animal	p_H averages	No. of animal	p_H averages
Animals 6 months old.			
8	7.43	11	7.35
9	7.41	12	7.15
10	7.38	13	7.30
—	—	14	7.39
—	—	15	7.23
—	—	16	7.28
—	—	17	7.34
Average	7.41		7.29
Animals 2 years old.			
18	7.27	29	7.28
19	7.35	30	7.20
20	7.36	31	7.27
21	7.20	32	7.18
22	7.40	—	—
23	7.53	—	—
24	7.53	—	—
25	7.42	—	—
26	7.47	—	—
27	7.45	—	—
Average	7.40		7.23

Table IV. p_H values for fresh vitreous humours and those kept in the excised eyes, in the refrigerator, for half a day. (All were handled under oil.)

Vitreous removed immediately		Vitreous kept in the extracted eye for half a day	
No. of animal	p_H averages	No. of animal	p_H averages
Animals 1½ years old.			
9	7.45	4	7.35
10	7.54	4	7.32
11	7.49	5	7.30
12	7.44	6	7.16
13	7.40	7	7.24
14	7.40	8	7.24
15	7.36	8	7.24
Average	7.44		7.27
Animals 2 years old.			
16	7.41	21	7.51
16	7.40	21	7.32
17	7.43	22	7.41
17	7.39	22	7.33
18	7.52	23	7.41
18	7.53	23	7.37
19	7.65		
20	7.61		
20	7.62		
Average	7.51		7.39

Fig. 1. Rate of increase in alkalinity of the ocular humours of cattle with advancing age. (Figures in parentheses denote the number of eyes on which the averages were based.)

CARBON DIOXIDE DETERMINATIONS.

Analyses for carbon dioxide were made by the method of Van Slyke and Neill [1924], in which 2 cc. of the fluid were used.

The vitreous humour only was studied in respect to the relationship of the carbon dioxide content to the age of the animal. The carbon dioxide content of the vitreous humour decreases with increasing age. This is shown by the arrangement of the data in Table V and particularly by the average values for various ages presented in Table VI.

The aqueous humour was analysed for its carbon dioxide content only (see Table VIII). In the vitreous humour both carbon dioxide content and capacity were studied. The carbon dioxide capacity determination differed from the

Table V. *Carbon dioxide content of the vitreous humours from cattle varying in age from 6 weeks to 2 years.*

No. of animal	Age of animal	No. of eye	Age of specimen under oil (days)	Vol. % CO ₂ content			Average
				Determinations			
				1	2	3	
1	6 wks.	1	Fresh	58.7	58.5	59.5	58.9
		2	"	58.1	—	—	58.1
2	2 mths.	Mixture	2	74.1	74.3	—	74.2
3	3 "	"	Fresh	60.8	60.1	—	60.5
4	4 "	"	"	52.8	52.1	52.0	52.3
5	8 "	"	"	59.5	59.9	—	59.7
6	1 yr.	"	"	54.9	—	—	54.9
7	1 "	"	"	54.9	—	—	54.9
8	1 "	"	"	62.3	—	—	62.3
		"	3	60.9	59.9	—	60.4
		"	7	60.8	—	—	60.8
9	1½ yrs.	"	4	46.2	46.5	—	46.4
10	1½ "	"	"	44.1	46.2	44.7	45.0
11	1½ "	"	"	41.2	42.2	—	41.7
12	2 yrs.	1	Fresh	51.3	51.5	50.7	51.1
		2	3	52.4	—	—	52.4
13	2 "	1	1	45.4	44.3	44.5	44.7
		2	"	45.1	45.1	—	45.1
14	2 "	1	Fresh	46.3	46.7	—	46.5
15	2 "	1	"	46.3	46.0	—	46.2
		2	"	44.7	—	—	44.7
16	2 "	1	"	45.6	46.4	—	46.0
17	2 "	1	"	46.3	—	—	46.3
		2	"	47.1	46.3	—	46.7
18	2 "	1	15	38.0	37.3	—	37.7
		2	15	37.3	37.3	—	37.3
19	2 "	1	15	38.3	—	—	38.3
20	2 "	1	16	40.8	40.6	—	40.7
21	2 "	1	2	48.5	—	—	48.5
		2	2	54.5	—	—	54.5
22	2 "	1	2	55.4	—	—	55.4
23	2 "	1	2	43.5	—	—	43.5
24	2 "	1	6	42.7	40.8	—	41.8
25	2 "	1	6	44.8	—	—	44.8
26	2 "	1	6	43.7	—	—	43.7
27	2 "	1	6	44.7	—	—	44.7
28	2 "	1	6	47.1	—	—	47.1
29	2 "	1	Fresh	47.5	47.4	—	47.5
		2	"	45.7	47.1	—	47.4
30	2 "	1	1	43.8	42.3	—	43.1
		2	1	45.2	45.6	—	45.4

Table VI. *Average values of the carbon dioxide content of vitreous humours of different ages.*

Age	6 wks.	2 mths.	3 mths.	4 mths.	8 mths.	1 yr.	1½ yrs.	2 yrs.
Vol. % of CO ₂	58.7	74.2	60.5	52.3	59.7	59.0	44.4	42.9
No. of animals used	1	1	1	1	1	3	3	19

carbon dioxide content determination in that in the former analysis the vitreous humour was equilibrated with alveolar air before the carbon dioxide was withdrawn. Under these conditions the vitreous humour took up no additional carbon dioxide. Therefore the carbon dioxide content and capacity of the vitreous humour are alike. The results are given in Table VII.

Table VII. *The carbon dioxide content compared with the carbon dioxide capacity of the vitreous humour.*

No. of animal	Age of animal	Age of specimen	Vol. % CO ₂ content				Vol. % CO ₂ capacity		
			Determinations			Average	Determinations		Average
			1	2	3		1	2	
4	4 mths.	Fresh	52.8	52.1	52.3	52.3	51.7	52.3	52.0
12	2 yrs.	3 days	52.4	—	—	52.4	51.4	51.9	51.7

Table VIII. *Aqueous humour.*

The carbon dioxide content of fresh aqueous humour compared with that previously used in a colorimetric p_H determination. (The humours in both cases were obtained from cattle 1½–2 years old, and handled under oil.)

Vol. % CO ₂ content						
After p_H determination						
No. of animal	Original sample	Determinations			Average	
		1	2	3		
1	49.2	—	—	—	—	
2	49.4	47.8	—	—	47.8	
3	47.6	47.5	—	—	47.5	
4	48.9	—	—	—	—	
5-8	40.3	36.8	—	—	36.8	
9-11	—	37.9	38.7	38.0	38.2	
12-13	45.1	39.5	39.7	—	39.6	
14-16	47.9	—	—	—	—	
14	—	38.9	—	—	38.9	
15	—	50.4	—	—	50.4	
16	—	40.2	—	—	40.2	
17	—	42.4	—	—	42.4	
17-18	—	40.8	—	—	40.8	
19	53.5	—	—	—	—	
20	45.7	—	—	—	—	
Average	47.5				42.3	

Table IX. *Vitreous humour.*

The carbon dioxide content of fresh vitreous humour compared with that of vitreous humour previously used in a colorimetric p_H determination. (The humours in both cases were obtained from cattle 1½–2 years old, and handled under oil.)

No. of animal	Age of specimen (days)	Vol. % CO ₂ content; averages	
		Original	After p_H det.
21	2	48.5	46.3
21	2	54.5	56.0
22	2	55.4	53.4
23	2	43.5	42.4
24	6	41.8	38.2
25	6	44.8	44.5
26	6	43.7	41.5
27	6	44.6	43.5
27	6	47.1	42.3
Average		47.1	45.3

Carbon dioxide content was determined on several samples of both humours after p_H determinations had been carried out on them, and the results were compared with those obtained on the same humours in their original fresh state. The results given in Tables VIII and IX show that both aqueous and vitreous humours tend to lose their carbon dioxide, if disturbed, even under oil, the aqueous humour more so than the vitreous humour.

Several determinations for carbon dioxide content of the plasma and the vitreous humour in the same animal show that the carbon dioxide content of the vitreous humour is lower than that of the plasma.

Table X. *The carbon dioxide content of ox-plasma as compared with the carbon dioxide content (or capacity, see Table VII) of the vitreous humour of the same animals. The figures express average values.*

No. of animal	Age of animal (yrs.)	Vol. % CO ₂ plasma	Vol. % CO ₂ vitreous
12 and 13	2	59.1 (2)*	47.8 (9)*

* This figure indicates the number of determinations on which the average is based.

Comparing the figure 47.5 vol. % obtained on fresh aqueous humour of cattle 1½–2 years old (Table VIII) with 43.7 vol. %, the average figure for carbon dioxide content of fresh vitreous humour of cattle of the same age (Table V), it is seen that the carbon dioxide content of the aqueous exceeds that of the vitreous humour by 3.8 vol. %. Hence the order of increasing values of carbon dioxide content is represented in the ocular humours and the blood-plasma of cattle of the same age, as follows: vitreous, 43.7 vol. %; aqueous 47.5 vol. %; plasma 59.1 vol. %.

Table XI. *The carbon dioxide content of fresh vitreous humour as compared with vitreous preserved in the refrigerator under and without oil.*

No. of animal	Age of animal (yrs.)	Vol. % CO ₂ content; averages		Days in refrigerator
		Fresh	Preserved under oil	
8	1	62.3	60.5	3–7
12	2	51.1	52.4	3
			Preserved without oil	
14	2	46.5	46.7	3–7

The carbon dioxide content of the vitreous humour was studied also after the humour had been kept in the refrigerator for several days, both without oil and under oil. The figures presented in Table V, and the results shown in Table XI, indicate that the vitreous humour, whether under oil or exposed, can be kept in the refrigerator for about a week without loss of carbon dioxide. The average of the nine determinations made on the fresh vitreous humours from 2-year old animals in Table V is 46.9 vol. %. The average of the fourteen determinations on similar vitreous humours that had been kept in the refrigerator from one to six days is 46.1 vol. % (Table V). A detectable loss of

carbon dioxide occurs, however, when the humour, even under oil, is allowed to stand for longer than a week, as shown by the results on animals 18, 19 and 20 in Table V.

Oxygen determinations on the vitreous humour.

The analyses were made by the Van Slyke and Neill [1924] method, in which 2 cc. of the fluid were employed.

It was thought probable that the vitreous humour would contain little, if any, oxygen, except the traces in physical solution. Table XII proves this conjecture to be correct.

Table XII. *Analysis of the vitreous humour for dissolved oxygen.*

No. of animal	No. of determinations	Mm. Hg before absorption of O ₂	Mm. Hg after addition of Na ₂ S ₂ O ₄	Difference in mm. Hg
1	1	116.5	116.2	+0.3
1	2	114.0	116.5	-2.5
1	3	109.0	110.8	-1.8
1	4	108.5	111.0	-2.5
2	5	115.0	118.0	-3.0

A blank determination gave a figure of 0.5 mm. Hg. When this figure is subtracted from those of the last column, a negative value is obtained for oxygen in each case. Any amount of oxygen larger than the equivalent of experimental error would be represented under the assumed conditions by a positive value. The fact that all the five values happen to be negative suggests that some other gas was evolved on addition of Na₂S₂O₄ and proves (assuming the amount of this gas to be negligible) that the vitreous humour contains no dissolved oxygen. The gas evolved was identified, by its odour, as sulphur dioxide.

DISCUSSION.

The carbon dioxide content in order of decreasing values is represented by the fluids of cattle 1½–2 years old as follows: plasma, 59.1 vol. %; aqueous humour, 47.5 vol. %; vitreous humour, 43.7 vol. %.

No direct experimental data are available which would show whether changes in the hydrogen ion concentration of the blood are reflected in the ocular humours as shown in respect to sugar and carbon dioxide. The present work brings out the fact that the vitreous humour is slightly more alkaline than the aqueous humour. This is to be explained from the fact that the vitreous humour contains at least twice as much protein as the aqueous humour. Duke-Elder [1927, 3] has shown that the aqueous humour is particularly rich in the acid radicals (Cl⁻, PO₄⁼, SO₄⁼) of the inorganic salts present in the body fluids, whereas the blood-plasma exceeds the aqueous humour in respect to the cations (Na⁺, K⁺, Ca⁺⁺, Mg⁺⁺) of the same salts. It is an accepted fact that the cations are held more or less in combination by the blood-proteins, and thus partially withdrawn from body fluids that contain less protein toward

those that contain more. It is to be expected therefore that the plasma, since it contains more protein than the vitreous humour, must be more alkaline than the latter. According to data obtained by Baurmann [1927], the p_H values of human aqueous humours vary between 7.20 and 7.35, with an average of 7.27. These figures are definitely lower than those for blood, which are 7.35 to 7.50. The data of the present work show, on the whole, smaller differences between the aqueous and vitreous humours of cattle than Baurmann observed between plasma and aqueous humour in man. It is clear that the order of increasing values of the hydrogen ion concentration (or decreasing alkalinity) on the p_H scale is represented by the ocular humours and the plasma as follows: p_H of plasma $>$ p_H of vitreous humour $>$ p_H of aqueous humour.

In view of the foregoing, there is sufficient reason to postulate that changes in the hydrogen ion concentration of the blood would be readily reflected in the ocular humours, particularly in the aqueous humour.

SUMMARY.

(1) Carbon dioxide content and capacity are identical for the vitreous humour. The amount decreases, with a few fluctuations, on the average from 58.7 vol. % in a calf 6 weeks old to 42.9 vol. % in cattle 2 years old. Plasma exceeds the vitreous humour in carbon dioxide capacity by an average of 11 vol. % in 2-year old cattle. The vitreous humour, in its natural state, can be kept under oil in the refrigerator for about 1 week without loss of carbon dioxide. If, however, its structure is destroyed by mechanical liquefaction, it tends to lose its carbon dioxide much more readily.

(2) No dissolved oxygen could be demonstrated in the vitreous humour.

(3) The aqueous humour of cattle 1½–2 years old contained an average of 47.5 vol. % carbon dioxide. On the other hand, an average figure of 42.3 vol. % was obtained on the same or similar humours after they had been used in a colorimetric p_H determination. Hence, the aqueous humour loses its carbon dioxide very readily if disturbed, even under oil.

(4) The carbon dioxide content of the aqueous humour exceeds that of the vitreous by 3.8 vol. % for cattle 1½–2 years old, the average figures being 47.5 vol. % and 43.7 vol. % respectively.

(5) There is a distinct decrease in the hydrogen ion concentration of the aqueous humour of cattle with advancing age. The relative p_H values increase from 7.23 in a 5-week old calf to 7.40 in 2-year old cattle. (No correction was made for temperature.)

(6) The relative p_H values of the vitreous humour of cattle increase, on the average, from 7.29 in 3-month old calves to 7.50 in 2-year old cattle.

(7) The aqueous humour is slightly, but consistently, more acid than the vitreous humour.

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