timing of the BHDB treatment and first repeated their experiment in duplicate. One group showed no decrease of urinary iodide output, whilst the other showed appreciable diminution. The effect of conditions intermediate between those used in our experiments and those of the French workers was investigated in another experiment in which the BHDB treatment was stopped 24 hr. after the thyroxine injection. During the first 2 days the inhibitory effect of BHDB was evident (Fig. 2), but later the iodide output increased in a manner similar to that reported by Roche *et al.*

The change in the rate of iodide excretion in this experiment coincided with the onset of severe constipation in both groups of BHDB-treated rats. Instead of the normal 10–20 g./day of faeces these rats excreted less than 0·5 g./day. The constipation appeared to be due to loss of appetite rather than to a direct effect of the withdrawal of BHDB. A study of the faecal radioactivities (Table 1) suggests that small doses of thyroxine (2·5 $\mu$g./rat) were insufficient to overcome the systemic effects of 3 days' treatment with BHDB, whilst larger doses of thyroxine were capable of preventing loss of appetite and the resulting constipation.

In a relative degree of intestinal stasis, thyroxine and its metabolic products excreted in the bile would undergo a much greater degree of re-absorption than in a normal gut. These re-absorbed substances would then be subjected to further de-iodination and more iodide would be available for excretion by the kidney. Thus the net result of severe constipation would be the diversion of radio-activity to the urine. Such an effect might account for the results of Roche *et al.* (1953) for in a footnote they report that in their experiments the faecal radioactivity was minimal.

We therefore suggest that BHDB reduces the rate of de-iodination of thyroxine in thyroidectomized as well as in intact rats, but the use of urinary iodide output as a measure of this process is only possible when the administration of the BHDB is correctly timed and when complications due to constipation do not occur.

## SUMMARY

1. The output of urinary iodide by thyroidectomized rats after the injection of [3′:5′-$^{131}$I]thyroxine was substantially reduced by the simultaneous administration of butyl 4-hydroxy-3:5-diiodobenzoate.

2. A probable explanation of some conflicting results reported in the literature is given.

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# The Hydration of the Cornea

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In the dead eye the cornea swells, at the same time losing its transparency; similar changes are observed when the isolated cornea is kept in physiological saline. These, and other, observations suggest that the cornea, *in vivo*, has a constant tendency to absorb water from its surrounding fluids, a tendency that is opposed by dehydrating factors which cease to operate on the death of the animal. Essentially, the cornea consists of a number of collagenous laminae—the stroma—separated from the tears by the epithelium, and from the aqueous humour by the endothelium (Fig. 1); it is only at the limbus, through the conjunctival capillaries, that the cornea comes into direct relationship with the blood. Under the electron microscope the structural units of the laminae are

visible as banded collagen fibrils; these are finer than those in tendon and sclera, and are heavily embedded in a ground substance that is probably mucoid (Schwarz, 1953). It is probably the high concentration of mucoid in the cornea that determines its transparency, and also its remarkable tendency to post-mortem swelling, both these characteristics being absent from the sclera. In the living eye, fluid and salt exchanges are theoretically possible across the epithelium and endothelium, and also, in the limbal region, across the limbal capillaries, so that turgescence of the cornea may conceivably result from the absorption of tears, aqueous humour, and limbal interstitial fluid. The cause of the turgescence would be the colloid-osmotic pressure exerted by the colloidal anions of the stroma (Davson, 1949*b*). It has been claimed by Cogan & Kinsey (1942) that, *in vivo*, the tendency to hydrate is opposed by the osmotic withdrawal of water into the supposedly hypertonic aqueous humour and tears. The theoretical objections to this mechanism have already been put forward (Davson, 1949*a*, *b*; Maurice, 1951), but it was considered desirable to provide experimental evidence that would permit of an unequivocal decision between this mechanism and an obvious alternative, namely that the normal state of hydration of the cornea represents a balance between the colloid-osmotic forces favouring the uptake of fluid and salt, and an active-transport mechanism that drives water and salts against the gradient of electrochemical potential. The cells responsible for this active transport could be either those of the epithelium, of the endothelium, or of both.

To appreciate the significance of the experiments to be described, the following introductory considerations will be of value. (*a*) In the excised eye, any increase in hydration consists essentially in the absorption of aqueous humour (Davson, 1949*b*), and its rate will be determined by the permeability of the endothelium to salts and water, on the one hand, and on the efficacy of the hypothetical active-transport mechanism that opposes this uptake. Cooling the eye will have two opposing effects on such a system; by lowering the permeability of the endothelium it will slow the rate of hydration; on the other hand, by decreasing the rate of metabolism it will impair the efficiency of the active-transport mechanism, thereby favouring hydration. By analogy with other active-transport systems, e.g. the erythrocyte (Harris, 1940), we may expect the effect on metabolism to be the more important. Consequently, on comparing two excised eyes, the one maintained at the normal corneal temperature of about 31°, and the other at 7°, we may expect corneal hydration to be greater at the lower temperature. (*b*) As a corollary to the above, we may expect that when a cornea has become heavily hydrated as a result of maintaining it at 7°, warming it to 31° should inhibit further hydration and actually reverse the process, so that fluid is driven out of the cornea. (*c*) If the epithelium contributes to the active-transport process, removal of this layer may be expected to favour corneal hydration in the excised eye maintained at 31°, but to have a negligible effect at 7°, when metabolism is very small. (*d*) If the endothelium also contributes to the active process, the hydration of a cornea maintained, without epithelium, at 31° may be less than that of a normal cornea maintained at 7°. (*e*) If the physical removal of water (by osmosis into the evaporated tears in the normal intact eye) is an insignificant factor in maintaining normal hydration, then in the excised eye we may expect that variations in the relative humidity of the surroundings will be without significant effects on the corneal hydration. (*f*) Interference with metabolism, for example by removal of oxygen, might be expected to increase hydration.

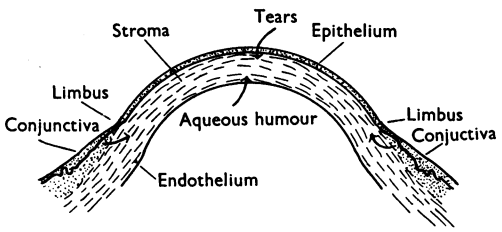


Fig. 1. Illustrating fluid relationships of the cornea; the arrows indicate direction of fluid intake.

It will be seen that all these predictions are verified by experiment; they are all inconsistent with the purely physical mechanism mentioned above.

## METHODS

Eyes, freshly excised from rabbits given a lethal dose of Nembutal (sodium pentobarbitone), were employed throughout this work. They were placed, for ease of handling, on shallow Perspex cups so that the cornea and part of the sclera were exposed to the atmosphere. The cups were placed on a tray in a Perspex box of about 1 l. capacity; the humidity in the box was controlled by passing moist air through it at a rate of about 100 ml./min. The air, before passing through the box, bubbled through two Winchester quart bottles in series, containing 0·9% (w/v) NaCl. The whole system was immersed in a water bath at 31°, the temperature being maintained constant to within about 0·02°. After some 16 hr. the eyes were successively removed and from each the cornea was excised rapidly and blotted, and its water content was determined by weighing before and after drying at 110°. When it was necessary to study pairs of eyes at the same temperature, but under different atmospheric conditions, a Perspex box divided into two compartments was used; each compartment had its own inlet and outlet tube. For maintenance of eyes at 7° it was

not, in general, considered necessary for the purposes of these experiments to control the humidity, so for most of these experiments the eyes were kept in a closed box containing cotton wool moistened with 0·9% NaCl, the box being placed in a refrigerator. Usually, to check on the degree of evaporation that took place during the experimental period, the eyes were weighed before and after their sojourn in the box. The temperature of 7° mentioned throughout this work must be interpreted as the temperature aimed at, rather than the true temperature in any given experiment, since an ordinary commercial refrigerator was employed, the internal temperature of which varied between about 5° and 10°.

## RESULTS

*The normal hydration.* The corneae from ten separate animals were excised immediately after death and their water contents determined. The range of values so obtained extended between 73·7 and 77·5 %, with a mean of 75·9. We may note that the percentage of water in a tissue is usually an insensitive index to any changes in its hydration, and it is preferable to indicate the weight of water per unit weight of solid matter; thus the range of hydration indicated above, expressed in this way, is 2·8–3·45 g./g. and the mean is 3·15 g./g. In the following description the hydration will be expressed in both ways whenever convenient.

*Eyes maintained at* 31 *and* 7°. The results on four pairs of eyes, each pair from the same animal, are shown in Table 1; in every case the hydration was considerably less in the eye maintained at normal temperature. During the course of this work, eyes from twenty-four separate animals were maintained at 31° for periods ranging from 17 to 23 hr., whilst twelve eyes were maintained at 7°; the average water content of the eyes maintained at 31° was 77·5 % ± 0·14 (S.E.) or 3·45 ± 0·04 g./g., whilst that of the eyes maintained at 7° was 82·2 % ± 0·3 or 4·6 ± 0·1 g./g. These results show that the cornea of an excised eye, stored at 31°, is less heavily hydrated than one stored at a low temperature. It is interesting, moreover, that of the twenty-four eyes kept at 31° under these conditions, the corneae of twelve had a hydration within the normal range of 73·7–77·5 %.

*Reversal of hydration.* Table 2 shows the effect of warming eyes that had previously been maintained at 7°. Pairs of eyes from the same animals were kept at 7° for 15–18 hr.; the cornea from one eye was excised and its water content determined, whilst the other eye was transferred to the box at 31° for a period of 6–8 hr., when its corneal water content was likewise determined. In every experiment the period at 31° resulted in a decrease in the hydration.

*Removal of the epithelium.* In these experiments the epithelium was scraped off the cornea of one eye of each pair; both eyes were maintained at the stated temperature for periods ranging from 6 to 19 hr. The results are summarized in Table 3. In all cases the eye without its epithelium showed the greater degree of hydration, but the effect was much more marked at the normal temperature. The average water content of these stripped corneae, maintained at 31°, was 79·4 % ± 0·4 (S.E.) or 3·85 ± 0·1 g./g. If we compare this hydration with the average for normal eyes maintained at 7°, namely 82·2 % ± 0·3 or 4·6 ± 0·1 g./g., we see that it is quite significantly less. If the endothelium were acting merely as a passive membrane, we should expect the hydration at 31° to be considerably higher than that of a normal eye at 7°; this finding

Table 1. *Comparison of eyes maintained at normal and low corneal temperatures*

| Expt. no. | Temp. (°) | Time (hr.) | Water content (g./100 g. tissue) | Water content (g./g. solid) |
|---|---|---|---|---|
| 1 | 7 | 15 | 82·8 | 4·8 |
| | 31 | — | 77·2 | 3·4 |
| 2 | 7 | 15 | 82·8 | 4·8 |
| | 31 | — | 77·0 | 3·35 |
| 3 | 7 | 17 | 82·1 | 4·65 |
| | 31 | — | 78·2 | 3·6 |
| 4 | 7 | 7 | 78·5 | 3·65 |
| | 31 | — | 77·8 | 3·5 |

Table 2. *The effect of subsequent warming on eyes maintained for* 15–18 *hours at* 7°

Column *A* gives the water content after the period at 7°; column *B* the water content after a further period of 6–8 hr. at 31°.

| Expt. no. | Water content (g./g. solid) (*A*) | (*B*) | Change (%) |
|---|---|---|---|
| 1 | 4·35 | 3·3 | 24 |
| 2 | 5·1 | 3·0 | 41 |
| 3 | 4·45 | 3·7 | 17 |
| 4 | 4·65 | 3·9 | 16 |

Table 3. *The effect of removal of the epithelium on the maintainance of corneal hydration*

| Temp. (°) | No. of expts. | Average time (hr.) | Water content (g./g. solid) Control | Epithelium removed | Mean difference (Test – Control) |
|---|---|---|---|---|---|
| 31 | 7 | 12·5 | 3·4 | 3·85 | 0·45 ± 0·09 (S.E.) |
| 7 | 3 | 14 | 4·15 | 4·3 | 0·15 ± 0·05 (S.E.) |

suggests, therefore, that the endothelium is capable of resisting to some extent the tendency of the cornea to absorb aqueous humour.

*The effect of evaporative loss.* In spite of the precautions taken to minimize losses due to evaporation at 31°, it was only rarely that the eyes, maintained at this temperature for 16 hr. or so, preserved a constant weight; the average loss was some 0·5 % of the total weight of the eye. It might be argued that this was a significant factor in maintaining the approximately normal hydration that was so frequently observed at 31° by comparison with the grossly abnormal hydration at 7°. So far as this comparison is concerned, however, this consideration is groundless, since in all cases where the weights were measured it was found that the loss by evaporation was considerably greater at the lower temperature; moreover, by deliberately increasing the rate of evaporation at 7°, by passing through the box air that had been bubbled through 10 % (w/v) NaCl, the corneal hydration was no different. As a further check on the significance of this factor, pairs of eyes were placed in the two-compartment box at 31°. Through one compartment was passed air that had been bubbled through distilled water (or, in three experiments, through 0·9 % (w/v) NaCl) and through the other air that had been bubbled through 10 % (w/v) NaCl. The results on the corneal hydration are summarized in Table 4, from which it will be seen that, in spite of the quite large difference in evaporative loss, the average hydration was the same under the two conditions, due presumably to the fact that any evaporative loss from the cornea is rapidly compensated by an influx of water from the aqueous humour. It will be noted that the evaporative loss with air bubbled through water or 0·9 % (w/v) NaCl was quite high in these experiments; this was because the compartments in the box employed were much smaller than the box employed for the other experiments. In consequence, the rate of flow of air over the eyes was greater. Although the air was dispersed as fine bubbles by passing through porous stone, it was apparently not completely saturated.

Table 4. *The effect of increasing the rate of evaporative loss from the eye, maintained for* 16 *hours at* 31°, *on the hydration of the cornea*

Mean of seven experiments. Limits are standard errors.

| Water content | | Loss in wt. of whole eye |
|---|---|---|
| (g./100 g. tissue) | (g./g. solid) | (%) |
| Air bubbled through distilled water or 0·9 % (w/v) NaCl | | |
| 77·4±0·16 | 3·4±0·03 | 0·8 |
| Air bubbled through 10 % (w/v) NaCl | | |
| 77·6±0·44 | 3·5±0·09 | 3·1 |

*Effect of anoxia.* Pairs of eyes were placed in the two-compartment box; over one, moist air was passed, over the other, moist $N_2$. The effect of anoxia was so unequivocal that only two experiments were performed. The results were as follows: Hydration of cornea in air (1) 3·4 g./g.; (2) 3·7 g./g. Hydration of cornea in $N_2$ (1) 8·1 g./g.; (2) 7·8 g./g. Thus in each case the cornea in $N_2$ was more than twice as hydrated as that in air.

## DISCUSSION

The results described here leave no doubt as to the importance of a normally functioning metabolism in maintaining the normal corneal hydration, and are consistent with the view that the tendency for the cornea to absorb fluid and crystalloids from its surroundings is opposed by an active-transport mechanism. On this basis, then, we may discuss very briefly the salient features of the water economy of the cornea. As indicated earlier, a slow uptake of fluid and crystalloids may be expected from the tears and aqueous humour, its speed being determined by the low permeability of the epithelium and endothelium to salts. In the long run, this uptake will be independent of the tonicities of the tears and aqueous humour, although fluctuations in these values may have temporary effects (von Bahr, 1948). A further uptake is possible in the limbal region, directly from the blood capillaries; but, although this is unimpeded by any selective membranes, its extent will be limited by the relatively large distances through which diffusion must take place. It is necessary, therefore, to postulate a mechanism that will efficiently oppose the entry of both water and crystalloids. Two main possibilities present themselves (Maurice, 1951): first, there may be an active transport of water into the tears and aqueous humour by the epithelium and endothelium respectively; this would lead to a rise in the concentration of crystalloids in the stroma which would then favour their outward diffusion. Alternatively, we may postulate an active extrusion of a crystalloid, e.g. $Na^+$, which would lead, in a similar way, to a passive removal of water. At present there is no evidence to permit a decision between the alternatives. We may note that the active transport of water, to be efficient, must occur across both the epithelium and endothelium; otherwise the removal of water at one side of the cornea would, to a large extent, be nullified by an osmotic entry at the other, since the diffusion of salt through epithelium or endothelium is slow by comparison with that of water. This is consistent with the findings, described in this paper, indicating that both the epithelium and endothelium oppose the entry of fluid into the cornea of the excised eye maintained at 31°. Variation in the tonicity of the

tears, determined by evaporation, will, if the active transport of water by the epithelium is independent of this factor, be largely self-compensating; an increase in evaporative loss, for example, will be fairly rapidly balanced by osmotic withdrawal from the aqueous humour. Thus the mechanism is consistent with the observation, reported here, that the state of hydration is independent of the atmospheric conditions, at any rate within the limits described. It is also consistent with the common observation that the eye may be maintained closed for days without any obvious increase in thickness, or loss of transparency, of the cornea.

The source of energy for the hypothetical active-transport mechanism would probably be largely the oxidative conversion of glucose and glycogen into carbon dioxide and water (Hermann & Hickman, 1948). We may expect interference with metabolism, by robbing the postulated active-transport mechanism of its energy, to cause an increase in hydration. The present work leaves little doubt that anoxia causes a large increase in the hydration in a period of 16 hr.; similar *in vivo* observations may be found scattered through the literature of this subject (e.g. Langham, 1952), and it would seem that the corneal hazing that results from the wearing of contact lenses is due to an impairment of the oxygen supply to the cornea (Smelser & Ozanics, 1953). It might be argued, however, that the results of anoxia are due to the accumulation of abnormal metabolites which influence the state of the corneal colloids. This raises a point that has so far been ignored, namely whether the post-mortem swelling is really an expression of a normal tendency for the corneal colloids to swell *in vivo*, or whether it is secondary to metabolic interference. In the present paper I have accepted the thesis of Cogan & Kinsey (1942) that the corneal colloids do, indeed, tend to swell *in vivo*, and the purpose of this paper was primarily to test the validity of the purely physical mechanism, advanced by these authors as the mode in which this swelling is prevented. The results quite unequivocally dispose of this hypothesis, but they do not necessarily prove that the cornea *in vivo* has any tendency to swell, or, to put the matter more correctly, that the colloid-osmotic pressure exerted by the stroma is not adequately resisted by the mechanical rigidity of the system. A definite answer to this question cannot yet be given; nevertheless, the results of the several different types of experiment described here, whilst each in itself does not give a conclusive answer, when taken together, do provide strong presumptive evidence for the existence of an active-transport mechanism opposing the entry of fluid into the stroma.

## SUMMARY

1. The swelling of the cornea in the excised rabbit eye has been studied with a view to establishing whether, in the living eye, there is an active-transport mechanism that normally prevents the uptake of aqueous humour, tears, and limbal capillary filtrate.

2. The following results are described: (*a*) The corneae of eyes maintained for 16 hr. at 31° are far less heavily hydrated than those maintained at 7°, in fact half of the eyes kept at 31° had a hydration within the normal range. (*b*) Subsequent keeping of eyes at 31°, after maintaining them at 7° for 16 hr., causes a reversal of the hydration that takes place at the lower temperature. (*c*) Removal of the epithelium causes the hydration to increase at 31° but has a very small effect at 7°. The hydration of corneae maintained without epithelium at 31° is less than that of intact corneae at 7°, indicating that the endothelium resists the uptake of fluid. (*d*) The state of hydration is apparently independent of the rate of evaporation from the eye. (*e*) Anoxia causes a very marked increase in hydration.

3. All the results are consistent with the existence of an active-transport mechanism but quite inconsistent with the view that the hydration *in vivo* is prevented by an osmotic withdrawal of fluid into the supposedly hypertonic tears and aqueous humour.

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