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Formation of Intra-Ocular Fluid and Permeability of the Ciliary Body.*

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Previous investigations have shown that there is a continuous circulation of the intra-ocular fluid, the chief source being the ciliary body, the chief exit being the canal of Schlemm.[1] The ciliary body exhibits an irreciprocal permeability to water and to methylene blue, both of which pass readily from stroma to epithelium but not in the reverse direction.[2] The present study was directed toward the discovery of the physico-chemical factors involved in this irreciprocal permeability. We have found that the tissue is permeable in both directions to acid and neutral crystalloid dyes, but impermeable in both directions to non-reducible basic crystalloid dyes. The stroma is capable of reducing methylene blue while the epithelium is capable of oxidizing methylene white. The passage of methylene blue from stroma to epithelium occurs therefore in its reduced, neutral state. By using a series of oxidation-reduction indicators, we found that the difference in oxidation-reduction potential between the epithelium and the stroma amounts to 100 millivolts.

At a pH of 5.3-5.5 the charge on the membrane barrier between epithelium and stroma is reversed, at lower pH the membrane barrier is permeable to basic dyes, impermeable to acid dyes, at higher pH the reverse is true. The membrane, therefore, is negatively charged at normal pH. This conclusion was confirmed by the production of an artificial electro-endosmotic flow in the proper direction through the tissue by means of an externally applied E.M.F.

In order that there should be a unidirectional flow of water by electro-endosmosis through a charged membrane there must be an electron conductor in the system. It is possible that a reversible oxidation-reduction system within the membrane could act as such an electron conductor. If the charge on the membrane is reversibly

* The details of this study will be published in the *Archives of Ophthalmology.*

1 *Arch. of Ophth.*, 1932, **7**, 538; 1932, **8**, 9.

2 *Trans. Amer. Ophthal. Soc.*, 1933, **31**, 143.

altered by the addition of oxidants or reductants at a fixed pH, the membrane must contain a reversible oxidation-reduction system. By operating at a pH of 5.7 we were able to reverse the charge on the membrane by the addition of oxidants, and to regain the normal charge by subsequent reduction. *In summary,* the physiological flow of water from stroma to epithelium, *i. e.,* from blood capillaries to intra-ocular cavity, is the result of electro-endosmosis. The mechanism for this electro-endosmosis is a difference in oxidation-reduction potential across a charged membrane. It is postulated that the reversible oxidation-reduction system within the membrane acts as the electron conductor necessary to complete the electrical circuit.

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Differentiation of Pigment Cells in Tissue Cultures of Chick Neural Crest.

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The experiments of DuShane[1, 2] have proved conclusively that the dermal melanophores of the amphibia originate in the neural crest, as suggested by Harrison,[3] and later by Weidenreich[4] and Holtfreter.[5] The differentiation of large numbers of typical pigment cells in tissue cultures of neural crest taken from embryos of pigmented fowls indicates the probability of a similar source of origin in the chick.

Embryos of various breeds have been used, ranging in stage between 6 and 10 somites. Thin strips of the rising folds anterior to the first somites were removed with fine scissors and explanted in a medium consisting of equal parts of plasma and embryonic extract. For controls, whole embryos minus the neural crest were cut into small pieces and explanted on a single slide; or specific areas, such as the eye, the brain floor with underlying endoderm,

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1 DuShane, G. P., *Anat. Rec.*, 1934, **60**, 62.

2 DuShane, G. P., *J. Exp. Zool.*, 1935, **72**, 1.

3 Harrison, R. G., *J. Exp. Zool.*, 1910, **9**, 787.

4 Weidenreich, F., *Z. f. Morphol. u. Anthropol.*, **2**, 59.

5 Holtfreter, J., *Roux' Arch.*, 1933, **127**, 619.