We conclude that the nuclear membrane is freely permeable to a great variety of substances both crystalloidal and, at least, fine colloidal. How far this may be generalized further researches will show.

8028 P

Disposal of Dyes by Proximal Tubule Cells of Chick Mesonephros in Tissue Culture.

ROBERT CHAMBERS.

From Washington Square College, New York University.

Dyestuffs have been used extensively for studying renal activity. However, from evidence in the literature there seems to be no rule governing intake of the various kinds of dyes by the tubule cells. For example, some lipoid-soluble dyes, to which cells are known to be permeable, are not passed into the tubular urine while, of the lipoid-insoluble dyes, some are known to be passed into the urine and others are not, with no apparent reference to their chemical constitution.

By means of the tissue culture method we have been engaged in testing the behavior of the proximal tubules to a series of dyes. Aqueous solutions of the dyes in various concentrations are mixed with the usual tissue culture medium in which are planted fragments of the functioning mesonephros of a 9-11-day chick. This method affords a means of studying the problem in a more direct manner than hitherto possible. Moreover, it enables one to restrict the problem to the proximal tubules which remain alive and functional for days in the explant. Isolated segments of the tubules regenerate their cut ends and become converted into closed sacs into which the progressive accumulation of color can be observed microscopically.

In this report the results of experiments are given on the use of the following dyes. They are the lipoid-soluble basic dyes, Neutral red, No. 825; and Nile blue sulfate, No. 913; which are in general use as vital stains, and the lipoid-insoluble acid dyes, Xylene cyanol FF, No. 715*; Amaranth, No. 184; Acid fuchsin, No. 692; and Orange G No. 27. These acid dyes resemble the sulphonephthaleins in forming highly dissociated, sulfonated compounds in

* The numbers appended to the names of the dyes are those given in Rowe's Colour Index, 1st edition (Society of Dyes and Colourists, Bradford, Yorkshire).

aqueous solutions but differ from them in not being passed by the tubular epithelium into the tubular urine.

It was found that the acid dyes, although they do not vitally stain the cells in general, nevertheless will color the proximal tubule epithelium in such a way that, in the early stages, the color closely resembles the usual vital stains. However, in their staining ability these dyes differ radically from Neutral Red and Nile Blue Sulfate in at least 2 ways.

First, the fine granule-like, colored bodies in the cells coalesce in time to form deeply stained vacuoles which vary in size and may become so large as to occupy the greater part of the interior of the cell. The size of these vacuoles varies with the different dyes used, for example, with cyanol, the vacuoles tend to remain small while those produced with orange G and with Acid fuchsin become so large that the protoplasm of the cell with its nucleus may become converted into a thin peripheral layer similar to the protoplast which encloses the large sap vacuoles in typical plant cells.

Secondly, cold (3-6°C.), which lowers the metabolic activity of the epithelium and prevents the passage of the sulfonephthaleins into the tubules,[1] suppresses the coloration of the cells by the acid dyes. On the other hand this low temperature has no effect on vital staining with the basic dyes.

The results of these experiments are of interest because they show that the cells of the proximal tubules may take up dyes in one of 2 ways. One way appears to be a passive infiltration of a lipoid soluble dye which takes place regardless of temperature changes within viable limits and the other, which depends upon the metabolic activity of the cells. The experiments also show a definite relation between the segregation of fluids into intracellular vacuoles and that type of renal secretion which consists in the transference of materials through the cells into the lumina of the tubules.

It has been shown that a group of sulfonated dyes which are actively taken up by renal cells can be arranged in a series according to the manner in which the cells finally dispose of the dye. At one end of the series are those which pass right through the cell into the lumina of the tubules without any sign of being segregated, even temporarily, within the cell. At the other end of the series are those which are collected in segregation vacuoles in the cells and very little, if any, is passed on into the lumina of the tubule.

[1] Chambers, R., and Kempton, R. T., *J. Cell. and Comp. Physiol.*, 1933, **3**, 131.