4489

Function of the Columnar Epithelium of the Ciliary Body of Albino Rabbits.

A. PILLAT AND W. C. MA.

From the Departments of Ophthalmology and Anatomy, Peking Union Medical College, Peping.

The assumption of secretory function of the non-pigmented epithelium of the ciliary body (Leber,[1] Seidel[2]) has not been satisfactorily confirmed. Some clinical experience of quick absorption of vitreous hemorrhages, however, seems to point to a possible absorbing function; and Meller[3] was able to show blood pigments in the inner layer of ciliary epithelium in such a case. Experiments were therefore attempted on albino rabbits to investigate this question.

The ciliary body of the rabbit is covered by an inner and an outer layer of epithelium. The inner epithelial cells are not uniform, some being columnar, others cubic or flat, while those of the outer layer are uniformly cubic. Each of the columnar cells, which are concerned in this study, is limited sidewise by a very thin membrane and outwardly by a definite thick membrane closely similar to the cuticular membrane of the intestinal columnar cells. Further, the intracellular arrangement of mitochondria and Golgi apparatus is identical to that of the intestinal absorbing cells.

These structural similarities led to the following absorption tests. The foreign material was introduced into the posterior chamber of one eye, the other serving as a control. A fine needle was inserted in the upper half of the eyeball obliquely through conjunctiva and solera to prevent outflow of the injected material after removal of the needle. Two hours after injection, the lower sector of the ciliary process, *i. e.*, the sector opposite the place of injection, as well as the ciliary body of the control eye were fixed and prepared for study.

Indian ink injection: From 0.1 to 0.2 cc. Indian ink was given. Specimens were fixed in 10% formalin and stained with hematoxylin-eosin.

At the end of 2 hours, fine black granules appeared in the intercellular spaces and columnar cells. The distal portion of the latter were somewhat injured judging from the flattening of the

[1] Leber, Th., *Graef. Arch. f. Ophthlmol.*, 1873, xix, 87.

[2] Seidel, E., *Graef. Arch. f. Ophthlmol.*, 1918, xcv, 1.

[3] Meller, J., *Arch. of Ophthl.*, 1928, lvii, 134.

cell and uneven staining. Occasionally ink granules were found in the connective tissue of the ciliary body, probably being carried there by wandering cells which are visible in different sections.

If the ink was kept 1 or 2 days in the posterior chamber, more ink granules were found evenly distributed in the epithelial cells which became eventually flattened as if the inner layer had been "shed". Even the nuclei became loaded with ink granules. It usually took 9 days for the gradual disappearance of the ink granules.

The ink granules might appear in the covering cells of the anterior surface of the iris, but very seldom in that of the posterior surface. The columnar ciliary epithelium near the *ora serrata* did not contain any ink granules, but a few were found in the chamber angle and Schlemm's canal one day after injection. The outer layer of ciliary epithelium and endothelium of the posterior surface of cornea were always free from ink particles.

Prussian blue reaction: Two drops of a fresh solution of equal parts of 1% sodium citrate and 1% ammonium ferricyanide were employed. Specimens were fixed in Carnoy's fluid and stained with haematoxylin-eosin.

The columnar epithelial cells were not injured with this injection as with the ink. The cytoplasm was stained red, containing a great number of deep blue or green granules around each of which there was a clear space. The color of the granules faded easily at the outer part of the cell. They were not found in the choroid, chamber angle or Schlemm's canal.

As the original solution injected was yellow, the presence of blue or green granules in the cell should indicate a previous absorption of the solution. The surrounding clear area may be secondary to precipitation of the granules. If the ferricyanide was kept longer in the eye, the green or blue granules would disappear, leaving behind clear spaces or vacuoles.

Fat absorption: A few drops of cod-liver oil were used. Specimens were fixed in Altman's osmic bichromate mixture or Bensley's acetic osmic bichromate mixture. Paraffin sections were stained with aniline acid Fuchsin and differentiated with alcoholic picric acid. The mitochondria were stained red, fat droplets black, nuclei yellow, and cytoplasm yellowish red.

The fat granules were found throughout the cytoplasm of the columnar cells. They might be seen in the swollen part of the filamental mitochondria and sometimes might be connected with one another in the form of chain embedded in the filaments. They

were also found in large amounts in the connective tissue of the ciliary body and choroid. None has been observed, however, in the cells of the anterior iris, Schlemm's canal and retina.

The fat might disappear altogether from the ciliary epithelium 8 to 12 hours after injection, but became abundant in the choroid probably carried there by lymph streams and wandering cells.

A comparative study of fat absorption of the intestinal cell was made side by side. Mice and rabbits were fed frequently with cod-liver oil and killed after 2 to 4 hours. The mucous membrane of the stomach and intestine was removed and treated according to the same technique as used for the ciliary epithelium. The distribution of the fat granules in the intestinal absorbing cells was similar to that of the ciliary columnar epithelium. More significant is the fact that the fat granules were never found in any type of the secretory cells of the stomach and intestine.

The above experiments tend to show that the ciliary columnar epithelium has an absorbing function.

4490

Preparation of Globin.

HSIEN WU.

From the Department of Biochemistry, Peking Union Medical College, Peping.

Schulz[1] in 1898 prepared globin, the protein moiety of hemoglobin, by extracting an acidified solution of oxyhemoglobin with a mixture of ether and alcohol. Most of the pigment went into the ether-alcohol layer, leaving the globin in the aqueous layer. Upon neutralization with ammonia the globin was precipitated which was redissolved in dilute acetic acid and dialyzed. This method is very convenient, but the globin thus obtained is not natural, but denatured, globin, since the ether and the alcohol rapidly denature the protein in the presence of the acid.

Hill and Holden[2] have recently succeeded in preparing some globin which is soluble in neutral solution and which may be natural globin. They did not start with pure hemoglobin, but with washed cells. They observed that the stroma proteins, when swollen with ether, had a great power of absorbing hematin, and they utilized this

[1] Schulz, F. N., *Z. Physiol. Chem.*, 1898, xxiv, 449.

[2] Hill, R., and Holden, H. F., *Biochem. J.*, 1926, xx, 1326.