A THREE MONTHS OLD STRAIN OF EPITHELIUM.

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PLATES 11 TO 14.

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The purpose of the experiments described in this paper was to obtain a pure strain of epithelium and to keep it permanently *in vitro*, as has been done with connective tissue. Epithelium taken from chickens, dogs, human beings, etc., has already been grown *in vitro* for a short period of time and its characteristic growth described.[1] Ruth,[2] Oppel,[3] Holmes,[4] and Uhlenhuth[5] have cultivated epithelium from cold-blooded animals. Carrel[6] attempted several times to obtain a permanent strain of epithelium from the skin of chick embryos, but after 2 or 3 weeks the cultures were invaded by fibroblasts and the epithelial cells progressively disappeared. The transformation of the cultures of epithelium into cultures of connective tissue was probably not caused by dedifferentiation of epithelial cells into fibroblasts, as has been stated by Champy,[7] but by contamination of the cultures by fibroblasts. The fibroblastic contamination was due to the difficulty of obtaining for culture epithelium completely free from connective tissue cells. Ebeling[8] succeeded in keeping pure cultures of corneal epithelium which spontaneously ceased to grow after a few weeks. Although the cultivation of epithelium appeared to be more difficult than that of connective tissue, it seemed probable

[1] Carrel, A., and Burrows, M. T., *J. Am. Med. Assn.*, 1910, lv, 1379; *J. Exp. Med.*, 1911, xiii, 416.

[2] Ruth, E. S., *J. Exp. Med.*, 1911, xiii, 422.

[3] Oppel, A., *Anat. Anz.*, 1913–14, xlv, 173.

[4] Holmes, S. J., *Univ. California Pub. Zool.*, 1913, xi, 155.

[5] Uhlenhuth, E., *J. Exp. Med.*, 1914, xx, 614.

[6] Carrel, A., unpublished experiments.

[7] Champy, C., *Bibliog. Anat.*, 1913, xxxiii, 184.

[8] Ebeling, A. H., unpublished experiments.

that if epithelial cells could be obtained free from connective tissue cells, they could be kept in pure culture indefinitely. The strain of epithelium described in this article was isolated from chick embryo eyes. Fragments of tissues were taken from different parts of the eye, tapetum layer, cornea, and lens, in the hope of obtaining pure epithelium. Only a few cultures from the lens produced pure epithelium, and by repeating the explantation of different tissues from the eye, it appeared that only a certain kind of supposed lens cultures produced a pure outgrowth of epithelium, namely, the peripheral portion which had a little brim of iris epithelium attached. The lens tissue itself did not grow at all, but the little brim of iris which sticks to the lens when it is enucleated grew out apparently as pure epithelium. After 3 months cultivation *in vitro* it still looks as pure as when it was observed on the 1st day. Now and then a few elongated, spindle-shaped cells resemble fibroblasts, but under high magnification they are easily recognized as epithelial cells on account of their similarity to the other cells in the culture, the pigment which the iris epithelium contains, and their characteristic way of growing in close contact with the neighboring cells.

I.

EXPERIMENTAL.

1. Preparation of the Tissue.—Epithelial tissue for cultures is obtained from the eye by taking out the lens with a cataract knife. In most cases a very thin black brim of the iris adheres spontaneously to the peripheral part of the lens. The lens may be cut in three or four parts and placed in the ordinary plasma-tissue juice culture medium. The lens itself does not grow, but sometimes the outgrowth of epithelium from the iris can be observed after 48 hours, although frequently not until after several passages. If any fibroblasts grow out in the same culture, which will be noticed readily, there is little hope of obtaining pure epithelium from it. If parts of the iris are allowed intentionally to remain on the lens, the growth will be largely composed of fibroblasts. The extirpation is carried out as if only the lens is to be removed, and only that part of the iris which spontaneously remains is suitable for obtaining a pure epithelial culture.

2. Nature of the Medium.—The fact that the epithelial cells liquefied the plasma clot much more extensively than fibroblasts suggested that if the cells were cultivated under conditions in which a kind of liquefaction has already taken place, a more extensive and uniform growth would result. An attempt was then made to place the tissue on the free surface of the already coagulated plasma (one volume of plasma and one volume of embryonic juice), and to cover it with a small drop of embryonic tissue juice. This method of cultivation appeared to be the most satisfactory. As a rule, the growth took place in one delicate, continuous layer on the surface of the clot.

3. Preparation of the Cultures.—Equal volumes of plasma and embryonic tissue juice are mixed on the cover-slip and allowed to coagulate. After coagulation commences, which can easily be ascertained with the point of the knife, the tissue is placed on the free surface of the nascent clot. If this precaution is not taken, the tissue will float in the tissue juice which is afterward added, and no growth will occur. It is important to place the tissue on the clot at the proper moment; if too early, the tissue will be embedded, and if too late, it will not adhere. Fixation is the desired object. After the fragment has been properly placed on the clot, a small drop of embryonic juice is spread evenly, in a thin layer over the tissue and beyond its margin, to afford a moist surface for proliferation. When transferring the culture, the excess fluid is removed by means of a piece of sterile filter paper, and the culture cut in the usual way. Because of the retraction of the tissue, it is most important to extirpate the tissue in such a way that no part of the plasma clot remains in the periphery of the excised culture. Otherwise, the cells will be embedded in old clot and further growth will be prevented.

It is advisable to embed the cells in the medium during the first few passages. If connective tissue cells should be present, they are much more easily distinguished, because of their characteristic appearance when they grow in the middle of the clot. When no fibroblasts can be detected, the cultures are allowed to grow on the free surface of the clot, covered only by a small drop of tissue juice.

II.

RESULTS.

The outgrowth of new cells appears in a fine mosaic structure. The size of the epithelial layer of conglomerated cells depends very much on the consistency of the media. When cultivated on the free surface of the plasma clot, the new growth appears as a continuous sheet of cells in pavement formation (Fig. 1). When cultivated in the middle of the clot, smaller and larger peninsulas of cells grow out, and sometimes a few single cells.

For some while there has been a discussion as to the reason for the disappearance of epithelium when cultivated *in vitro*. This fact has been brought out in connection with an assumption by Champy[7] that a dedifferentiation of the epithelial cells to the type of fibroblasts takes place *in vitro*. This does not seem to be correct, and is more an apparent phenomenon. Several investigators, among them Uhlenhuth,[9] have shown that the shape of the cells cultivated *in vitro* depends upon the structure and consistency of the culture medium; in other words, on purely mechanical facts. By taking the precaution of cultivation under the same mechanical conditions, namely on a moist surface of plasma clot, as herein described, the cultures present as typical epithelial characteristics after 3 months cultivation as they did on the 1st day. Whenever it is desired, the epithelial cultures can be embedded in the solid plasma clot and the supposed dedifferentiated epithelial cells can be reconstructed; and repeated cultivation on the surface brings the fusiform cells back to the polygonal type of epithelium. It is purely a matter of mechanical conditions. In the periphery of ordinary, well grown epithelial cultures, made on the surface of the clot, some more or less elongated, spindle-shaped cells appear. These are found mostly when the outlines of the growth reach the outlines of the moist surface; *i.e.*, the border of the area which is covered by the tissue juice. Then they embed themselves in the solid clot and become elongated because of the dense medium. This same phenomenon has been described by Uhlenhuth[5] in his experiment on epithelial cultures from frog skin. He seems to have found just the reverse

[9] Uhlenhuth, E., *J. Exp. Med.*, 1915, xxii, 76.

of the observation stated above; namely, that the elongated, fusiform, epithelial cells grow out only in the very "soft medium," and in compact sheets when cultivated in a "firm medium." This may be explained in the following way: when the culture medium is made as soft as in Uhlenhuth's[9] experiments, but not quite fluid, the fibrin fibrillæ are so sparsely present that the few cells which grow out stick to them as the only support, and become spindle-shaped for that reason. The same phenomenon occurs when tissue is cultivated in a fluid medium in which cotton threads[10] or a spider net (Harrison[11]) is used for framework. In some cases, when cultures were made in the firm clot, it may be that the tissue cells found their support dense and homogeneous. In others, the transplanted fragment of tissue may have been more or less near the surface of the clot and, for that reason, the cells may have grown out on the surface in continuous layers.

The epithelial strain now under cultivation is very easily multiplied. The strain took its origin from five cultures, and in the elapsed time they have been increased to between 50 and 60, though the speed of the growth seems to be slower than that of fibroblasts. The ability to proliferate seems also to have increased recently; whether this is due to an improved technique in making the cultures or whether the cells have become better adapted to the life *in vitro* is difficult to say at present. It is noteworthy that the cultures made from fresh tissue are often continued for several passages before the epithelium begins to grow. A similar stage of latency has been observed by Uhlenhuth[9] in epithelial cultures.

The epithelial cells in cultures cause a liquefaction of the plasma clot to a higher extent than do those of connective tissue cells, and the epithelial tissue itself when excised from the culture has a peculiar mucous character and retracts easily, as does a drop of mucus. The most characteristic feature about the growth of epithelium is that the cells keep close together and grow in pavement formation. Numerous mitoses are found in the cultures and different stages of amitotic cell division (Figs. 2, 3, and 5). Most of the investigators who have cultivated epithelial cells *in vitro* state that they did not

[10] Fischer, A., unpublished experiments.

[11] Harrison, R. G., *J. Exp. Zool.*, 1914, xvii, 521.

observe any mitosis. After cell division, the new cells do not seem to move very far from the point where the division took place. The photographs show such an ability to form a continuous layer (Figs. 1 to 4). Very often in looking at the cultures, one is reminded of a cross-section of epithelial glandular tissue; that is, not only do the cells grow in pavement formation, but they seem to have a tendency to arrange themselves in a structure resembling the acini of glandular tissue (Figs. 2, 4, and 6). No dedifferentiation such as that suggested by Champy[7] has taken place after 3 months cultivation *in vitro*. The appearance of the cells at the present time is identical with that observed after the first passage. The rate of proliferation is increasing. Every culture doubles in size after 3 or 4 days.

III.

CONCLUSIONS.

1. A strain of pure epithelial cells was obtained from the part of the iris which adheres to the extirpated lens.

2. The optimum condition under which epithelial cells grow *in vitro* is on the free surface of the plasma clot under a film of embryonic tissue juice.

3. The epithelial cells did not dedifferentiate. Although the strain is 3 months old, they grow as a pavement membrane and have kept their epithelial characteristics.

EXPLANATION OF PLATES.

PLATE 11.

FIG. 1. Photograph of a living culture of epithelium 6 weeks old. Shows the characteristic growth for epithelial cells. × about 135.

FIG. 2. Stained culture of epithelial cells, 6 weeks old. Shows the epithelial pavement formation; mitosis may be seen. A few proliferation centers may be noticed. × about 660.

PLATE 12.

FIG. 3. Stained culture of epithelial cells, 6 weeks old. To the left may be seen the beginning of an amitotic cell division. × 1,100.

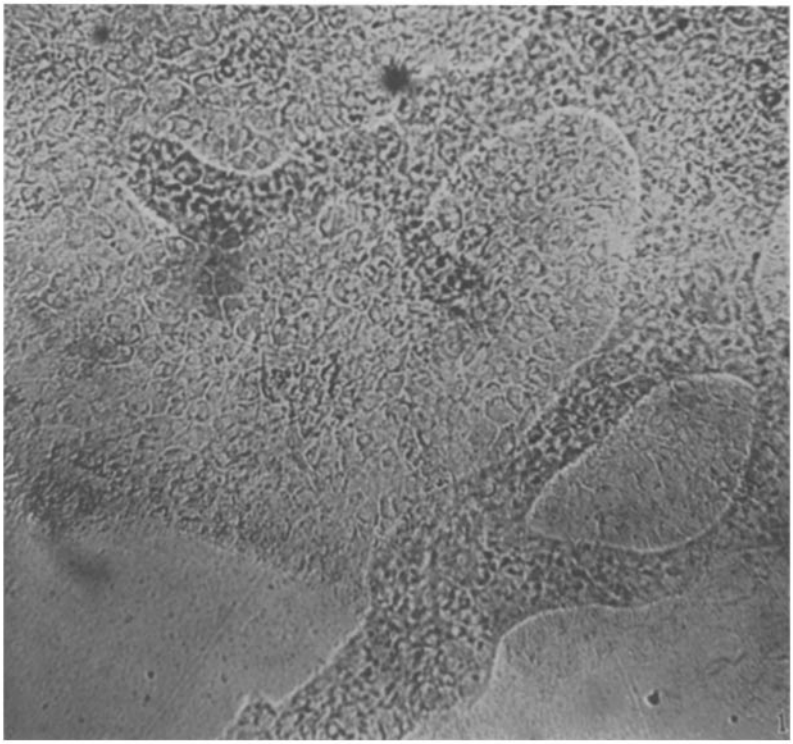
FIG. 4. Stained culture of epithelial cells 2 months old. × 1,425.

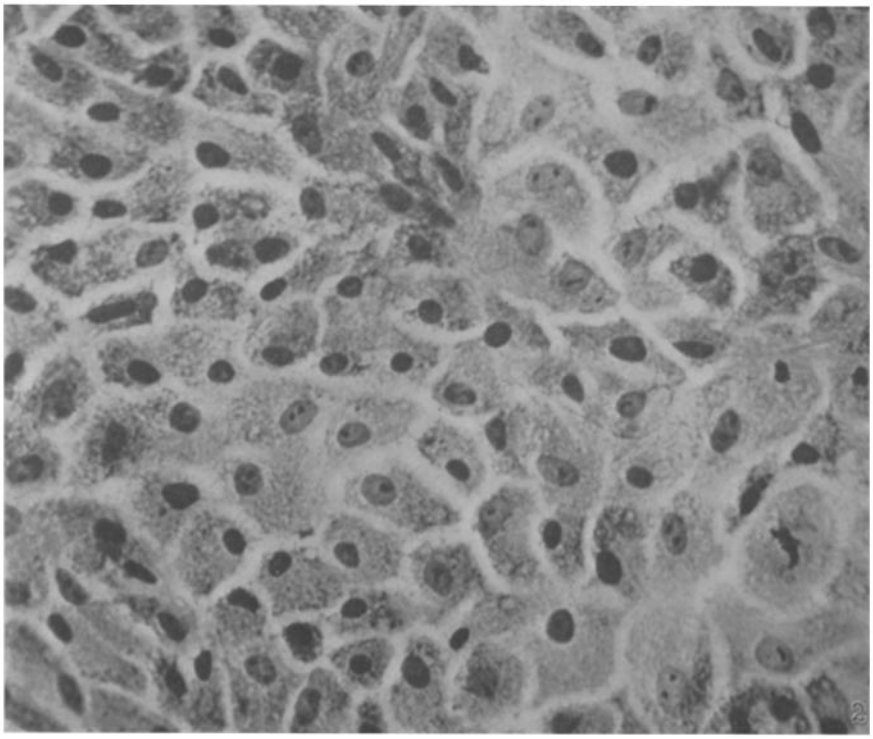
PLATE 13.

FIG. 5. Different stages of mitosis of epithelial cells in cultures.

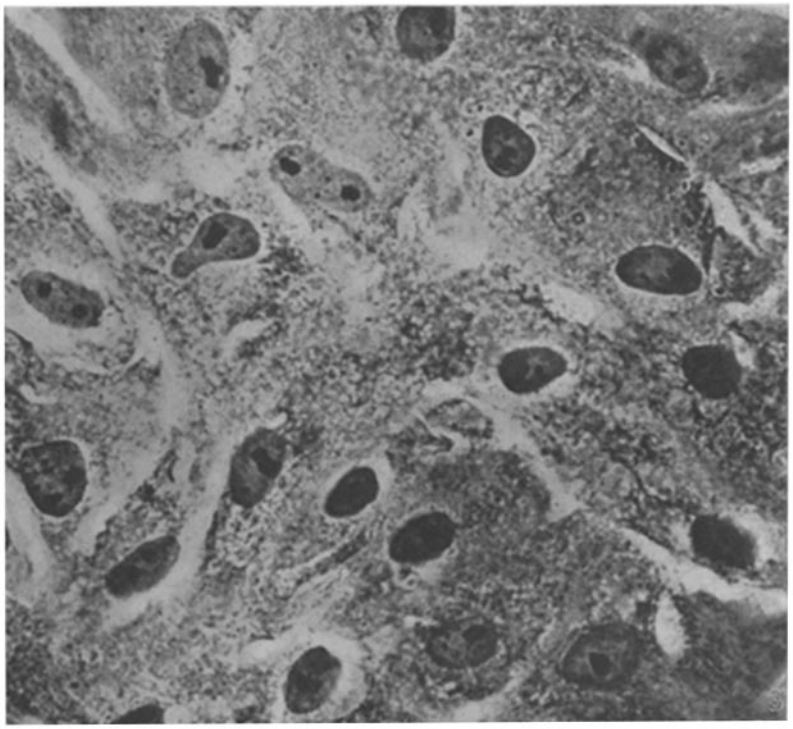
PLATE 14.

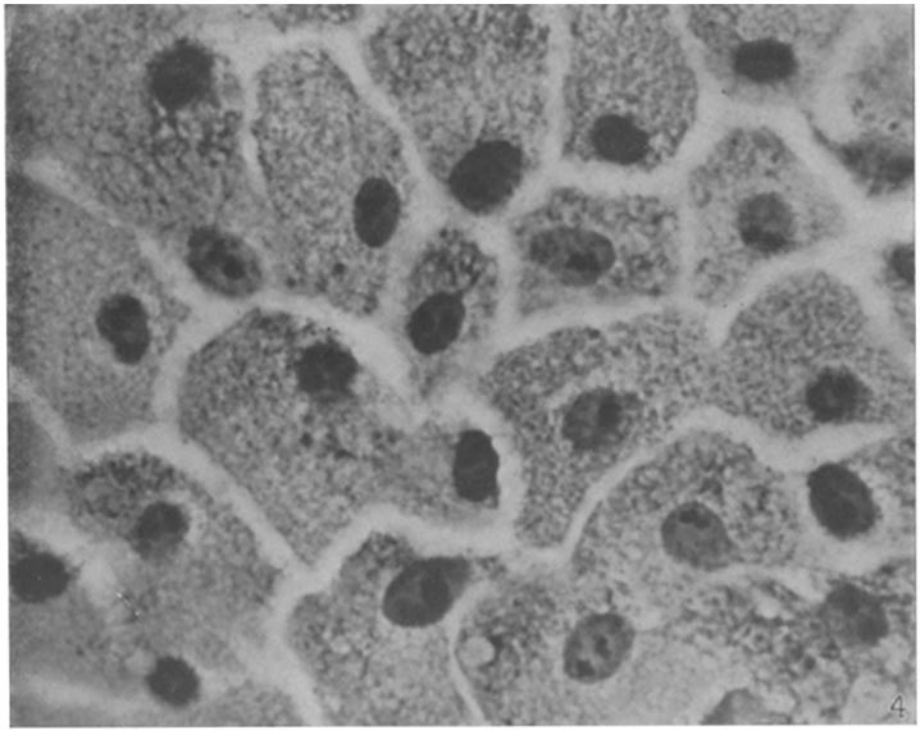
FIG. 6. Typical cell arrangement in epithelial cultures. × 700.





(Fischer: A strain of epithelium.)





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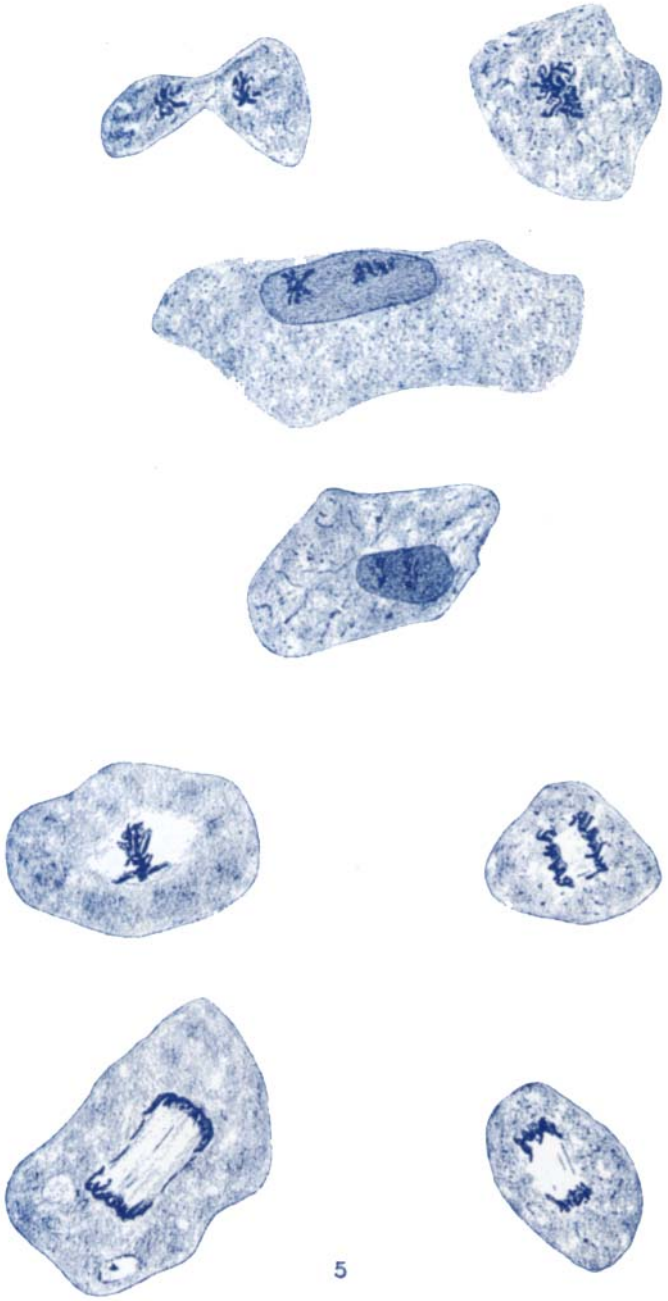

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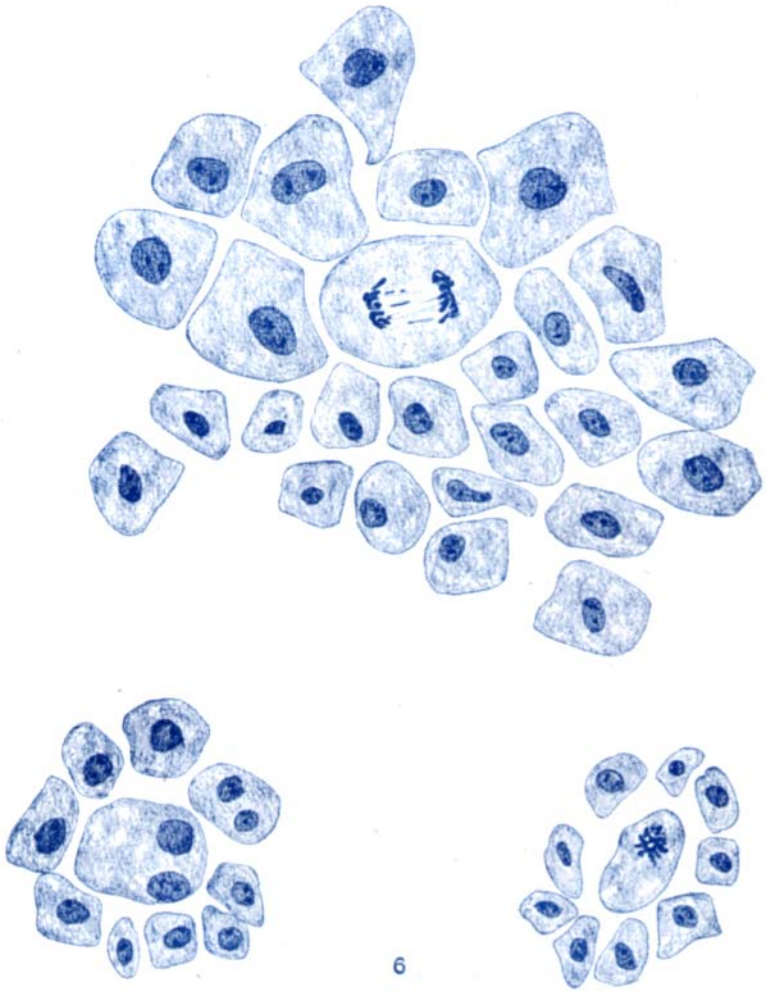

(Fischer: A strain of epithelium.)