MIXED CULTURES OF PURE STRAINS OF FIBROBLASTS AND EPITHELIAL CELLS.

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PLATES 15 TO 18.

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For years the question of dedifferentiation or transformation of tissue cells into an indifferent embryonic cell type when cultivated \textit{in vitro} has been under discussion. Champy\textsuperscript{1} cultivated kidney tissue, and found that its structure changed; indifferent tubules were formed which later lost their renal and finally their epithelial characteristics. From this and other experiments, he concluded that embryonic tissue cells cultivated \textit{in vitro} dedifferentiate sooner or later, sometimes after a few hours. Uhlenhuth\textsuperscript{2} showed that tapetum cells from the retina changed their typical epithelial character with modifications in the consistency of the culture medium. We found also that, when epithelial cells were allowed to grow embedded in the clot, their shape changed from polygonal to fusiform. Therefore, under these conditions, cultures of epithelial cells looked like cultures of fibroblasts, but under high magnification, there was no difficulty in distinguishing epithelial cells from fibroblasts. Although the epithelial cells proliferating in the clot are spindle-shaped, they have a peculiar way of growing close to each other (pavement-like), which is not true of fibroblasts. In a previous article,\textsuperscript{3} it was shown that epithelial cells in pure cultures still remained typical after more than 3 months. The morphological characteristics of the cells did not change as long as the strain was kept under constant cultural conditions; that is, on the free surface of the plasma clot under a film of embryonic tissue juice.

\textsuperscript{1} Champy, C., \textit{Bibliog. Anat.}, 1913, xxxii, 184.
\textsuperscript{2} Uhlenhuth, E., \textit{J. Exp. Med.}, 1915, xxii, 76.
\textsuperscript{3} Fischer, A., \textit{J. Exp. Med.}, 1922, xxxv, 367.
In order to settle the question of dedifferentiation, it was thought of interest to determine whether epithelial cells and fibroblasts could be distinguished from one another after they had been allowed to grow side by side in the same culture for several generations.

I.

Technique.

Fragments of the strains of fibroblasts and epithelium were cultivated side by side under identical conditions (Fig. 1). After 48 hours, a distinct difference was observed in the character of the growth of the two fragments. The epithelium grew in a compact mass, with the individual cells in close contact. The fibroblasts migrated into the culture medium and formed a network. In the fixed preparations stained with methylene blue, it was not possible to determine definitely whether any amalgamation of the two cell types took place after the growth from both fragments had united. No more striking result was obtained by the Van Gieson method. Then, fragments of a 2 month old strain of epithelium and a 10 year old strain of fibroblasts were cultivated together for several generations. After a few passages, the fibroblasts overgrew the epithelial fragment, covering it completely. The combined culture showed a peripheral growth composed of fibroblasts, and the only apparent indication of the presence of epithelium was that the central portion of the culture appeared semitransparent and homogeneous, and not as dense and opaque as a typical culture of pure fibroblasts which had not been divided for the same number of generations. At this stage, the mixed cultures were divided and subcultures made. These in turn were allowed to grow for 48 hours, and were again divided through the central portion. This procedure was continued for seven generations and then the preparations were fixed, sectioned, and stained by Van Gieson's method. The sections showed typical epithelial and connective tissues, as found in the organism. The epithelium appeared greenish yellow in contrast with the connective tissue, which appeared pink and contained many fibrillae which were stained a marked pink. No parts of the section showed an amalgamation of the two cell types. The epithelial cells were everywhere distinctly
differentiated. In many places a definite structural arrangement of the cell elements was observed (Fig. 2). The epithelial cells had grouped themselves to form tubules with distinct lumina. In several of the sections, the lumina could be seen filled with a homogeneous colloidal secretion (Fig. 3). The arrangement of the epithelial cells forming the tubules resembled the conformation found in sections of salivary glands. The individual cells which formed the tubules had their nuclei disposed close to the basal membrane.

In some parts of the section, epithelial cells could be seen penetrating the surrounding layer of fibroblasts and appearing on the free surface of the tissue fragment. In other parts, large masses of amorphous material (dead epithelial cells and secretions), surrounded by a layer of low epithelium, could be observed (Fig. 2).

II.

DISCUSSION.

These experiments show that epithelium cultivated for 2 months in vitro retained its morphological characteristics which differed decidedly from those of fibroblasts. But a still more striking fact was observed; namely, that the differential Van Gieson stain brings out the chemical difference between the two cell types when they are allowed to grow together. The epithelium was observed to have formative ability; i.e., the epithelial cells arrange themselves in winding tubules. This has already been mentioned in an earlier report of experiments in which the epithelial cells, cultivated on the free surface of the clot, grew in a single layer and were described as organizing themselves in structures which closely resembled cross-sections of glands. The experiments herein described confirm this statement, since the tubular arrangement may be followed throughout serial sections.

Champy states that no strains of cells can be cultivated for any length of time in vitro without a change occurring in their morphological characteristics. We have shown that fibroblasts and epithelial cells may be cultivated in pure cultures for long periods without dedifferentiation. Therefore, in this case it seems that Champy's

statement does not confirm the experimental data. On the other hand, he also states that epithelial cells in the presence of connective tissue cells do not dedifferentiate. This fact is fully substantiated by the present work.

The foregoing experiments have made it possible to analyze more accurately the character of the growth of epithelium in vitro. When the fragment is cultivated upon the surface of a clot, the growth is characterized by the formation of a membrane, the individual cells are polygonal, and their growth resembles a pavement epithelium. This is the typical formation obtained by surface cultivation. When the fragment is cultivated in the clot, the growth seems to depend upon the existing condition within the medium and the disposition of the embedded fragment. When the cells are allowed to invade the medium uniformly, an extensive membrane is formed, very much like that obtained in surface growth (Fig. 4), but the individual cells are spindle-shaped (Fig. 5) and not polygonal. If the condition of the coagulum prevents the uniform outgrowth of new cells from the mother fragment, then the cell invasion is characterized by the formation of branching tubules of various forms, but essentially the arrangement of the growing cells is such as to form a more or less organized structure resembling hollow tubes (Fig. 6). The growth and migration of epithelial cells seem to be much more dependent upon the mechanical conditions than those of fibroblasts.

When growth occurs in membrane formation, it is rapid and extensive. When the tubular type results, the rate of growth is markedly slower and the actual increase in mass is small, although the length of the tubular outgrowth may be extensive.

Now that it has been proved that epithelium and fibroblasts cultivated in vitro remain two different types of cells, with individual characteristics, innumerable experimental possibilities are opened. It is obvious that the study of their respective interactions under different experimental conditions will lead to interesting findings.

III.

CONCLUSIONS.

1. Strains of epithelium and fibroblasts cultivated side by side in the same medium keep their individual characteristics. When sec-
tioned and stained by the Van Gieson method, the cultures show
the epithelium stained greenish yellow and the fibroblasts and
their fibrillae pink.
2. There are no transition forms between the epithelial cells and
fibroblasts.
3. The epithelial cells belonging to an older strain are still able to
form primitive structures of winding tubules, with typical glandular
epithelium.
4. Under the conditions of the experiments, no dedifferentiation
takes place.

EXPLANATION OF PLATES.

PLATE 15.

Fig. 1. Experiment 1532-4. Culture of fibroblasts and epithelial cells cul-
vivated together, after 48 hours incubation. Stained with methylene blue. A,
fragment of a 10 year old strain of fibroblasts; B, fragment of a 2 month old
strain of epithelium. × about 20.

Fig. 2. Experiment 1387-1. Section through a mixed culture of a 10 year
old strain of fibroblasts and 2 month old strain of epithelium. The preparation
was fixed and stained by Van Gieson’s method after having undergone seven
passages in vitro. A, glandular arrangement of epithelial cells surrounded by
fibroblasts; B, network of fibroblasts and bundle of fibrillae; C, an area of degener-
ated epithelial cells surrounded by low epithelium. × 120.

PLATE 16.

Fig. 3. Experiment 1387-1. Another section through the same culture as Fig. 2.
A, glandular arrangement of epithelium; B, lumina; C, a lumen filled with secre-
tion; D, waved connective tissue fibrillae; E, characteristic position of the nucleus
in the cell as it appears in secretory epithelium. Stained by Van Gieson’s method.
× 400.

PLATE 17.

Fig. 4. Experiment 25970-2. Seventeenth passage of a pure culture of epithe-
lium growing in a membrane. Fragment cultivated embedded in the clot. Fixed
and stained with Azur II after 48 hours incubation. × about 80.

PLATE 18.

Fig. 5. Experiment 25970-2. Same preparation as in Fig. 4. The individual
cells appear spindle-shaped and flat. × about 200.

Fig. 6. Experiment 1128-2. Thirteenth passage of a pure culture of epithelium
growing embedded in the clot. The new growth appears as solid processes and
tubules. Stained with Azur II. × about 160.
(Ebeling and Fischer: Fibroblasts and epithelial cells.)
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