strains which show the highest residual growth energy cease to grow at a relatively low concentration of embryonic tissue juice.

Accordingly, with these results before us, it remains only to repeat that tissue cells cannot be defined solely according to their morphological characteristics. Their physiological properties should constitute the first claim to any definitions which are to be attempted.

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Differentiation and Proliferation in vitro.

ALBERT FISCHER AND RAYMOND C. PARKER.

From the Kaiser Wilhelm Institute für Biologie, Berlin-Dahlem, Gastabteiluna Dr. A. Fischer aus Kopenhagen.

In an earlier paper[1] the conditions were described under which undifferentiated sheets of epithelial cells became differentiated into a tissue which formed tubules. When pure strains of epithelial cells and fibroblasts were cultivated together in the same medium, it was found that the 2 cell types retained their individual characteristics indefinitely.[2] The connective tissue cells promptly surrounded the epithelial cells which arranged themselves into structures resembling glandular tissue, with distinct luminae. This same observation was made by Drew[3] in connection with skin epithelium and carcinoma cells. Drew stated that differentiation occurred only as the result of the interaction of 2 different tissues. However, one of us[1] has shown that differentiation is not necessarily dependent upon the interaction of other tissues. In order to obtain keratinization and tubule formation in cultures of pure epithelial cells, it is only necessary to refrain from cutting the culture through the center when transferring it from one medium to another. In other words, differentiation begins to take place as soon as the tissue becomes thick and in such a state that the central portion becomes badly nourished and poorly aërated. When epithelial cultures grow in membrane formation, the growth is rapid and extensive; when the tubular type of colonies appear, growth proceeds very slowly and the actual increase in mass is relatively small, although the outgrowing tubules may become of very considerable length.

[1] Fischer, A., *J. Exp. Med.*, 1924, xxxix, 585.

[2] Ebeling, A. H., and Fischer, A., *J. Exp. Med.*, 1922, xxxvi, 285.

[3] Drew, A. H., *Brit. J. Exp. Path.*, 1922, iii, 20; *Lancet*, 1923, i, 785, 833.

The experiments, here to be described, deal with factors which, it is believed, play a very considerable rôle in the mechanism of differentiation. The material used consisted of a 6 months old strain of fibroblasts derived from bone, but from which all trace of the original tissue was very early eliminated by selection of only the new outgrowth at the time of transfer. This process was repeated at frequent intervals, yet the cells never lost a very remarkable property, namely, the ability to transform themselves into typical bone cells when this was permitted under the conditions of the experiment. As has already been indicated elsewhere,[4] these fibroblasts possessed no morphological features which rendered them readily distinguishable from the common connective tissue fibroblasts isolated from the embryonic heart.

In order to study carefully the progressive stages in this process, a large series of such fibroblast cultures were isolated from the new growth and subjected to varying amounts of growth-promoting substances. From time to time representative cultures from the different series were fixed, cut in serial sections, and stained for histological examination. The findings will be reported more fully at a later date.

When the usual method of tissue cultivation was employed, *i. e.*, the combination of plasma and embryonic tissue juice, this transformation proceeded very slowly or not at all. But when the rate of multiplication of the cells was suppressed by a reduction of the growth-promoting substances in the medium, differentiation occurred. Cultures which were carried in flasks, or by the hanging-drop method on slides in pure plasma, the center of the culture being retained at each transfer, showed, after a few passages, the formation of typical bone tissue. Such a transformation was distinctly retarded in the control cultures, which were cultivated for the same length of time in equal parts of plasma and embryonic tissue juice.

These experiments have demonstrated that proliferation inhibits the normal function of the cells, namely, the building up of structures peculiar to their type. Hence, reproductive quiescence is an important factor in differentiation.

[4] Parker, R. C., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 580.