sensible loss per kilo body weight per hour. The data cited by Benedict and Root as well as their own data show about this value except that for their heavier subjects, above 70 kg., it tends to be less. At present we cannot say whether our greater loss than their heavier subjects is climatic or individual difference.

If we classify our data in reference to duration of time in bed, which is here about synonymous with duration of sleep, we find the following:

8 nights of 6.5 hrs. or less, ave. loss per hr._____36.2 gm.
16 nights of 6.55-7.5 hrs., ave. loss per hr. _____38.0 gm.
16 nights of 7.55 hrs. or more, ave. loss per hr._____41.8 gm.

The differences are consistent but they are not large and fail of statistical significance. However, they tend to verify Sanctorius' aphorism quoted above and probably indicate that with the sounder or fore part of the sleep period there is a slower loss. In the longer normal sleep the latter portion approaches more to the waking condition and has a higher rate of loss.

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Classification of "Fibroblasts" According to Their Physiological Properties.

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Years ago it was stated that epithelial cells cultivated *in vitro* dedifferentiated or returned to an indifferent cell type which could not be distinguished from fibroblasts.[1] However, the conclusions drawn at that time were mainly due to an inefficient technique which resulted in impure strains of tissue cells. Since we are now able to cultivate pure strains of tissue cells indefinitely, we know that the cells, under the conditions of the experiment, remain typical and retain their morphological characteristics throughout the entire period of cultivation. In the case of fibroblasts and epithelial cells, it has become a relatively simple matter to distinguish between the 2 cell types. One is aided not only by marked morphological differ-

[1] Champy, C., *Compt. rend. Soc. biol.*, 1912, lxxii, 987; Uhlenhuth, E., *J. Exp. Med.*, 1916, xxiv, 689.

ences in the cells themselves, but also by striking differences in the mode of growth of the cell colony.[2]

Within recent years, we have been mainly interested in studying the maintenance of special properties of tissue cells under various experimental conditions *in vitro*. We now know that cell types which are morphologically similar may possess functions which are extremely diverse. Pigment epithelium, when cultivated *in vitro*, continues to produce pigment;[3] thyroid epithelium produces colloid;[4] cancerous epithelium, when inoculated *in vivo*, produces cancer.[5] These findings show clearly that cell properties are maintained under the conditions of cultivation *in vitro*.

The experiments, which are to be reported here, show that various mesenchyme cells, which would all be designated morphologically as "fibroblasts," possess properties and characteristics which are decidedly unlike. Up to the present time, the fibroblasts which have been most intensely studied *in vitro* have been derived from the embryonic chick heart. The purpose of the present experiments was to study one of the more elementary properties of living tissues, namely, their inherent growth potencies. In this connection, heart fibroblasts were compared with other fibroblast-like cells isolated from various tissues of the organism. Four cell strains were isolated simultaneously from the same embryo. The first strain of cells was isolated from the periost of the frontal bone of the skull, according to the method described by Dolschansky.[6] These will be referred to as osteoblasts. The second strain was derived from the perichondrium of the sphenoid. These cells will be referred to as chondrioblasts. The third strain consisted of fibroblasts from the muscle of the leg; the fourth, fibroblasts from the heart. Carrel's 17-year-old fibroblasts were also used for the purpose of comparison.

We know that the activity of a tissue *in vitro* at a given instant is a function of its activity at the preceding instant and of the concentration in the pericellular fluid of the substances which increase or decrease cell activity.[7] Accordingly, the experiments demanded that the different strains be cultivated under absolutely identical conditions from the moment of isolation. This was rigidly carried

[2] Fischer, A., *J. Exp. Med.*, 1922, xxxv, 367.

[3] Ebeling, A. H., *Compt. rend. Soc. biol.*, 1924, xc, 562.

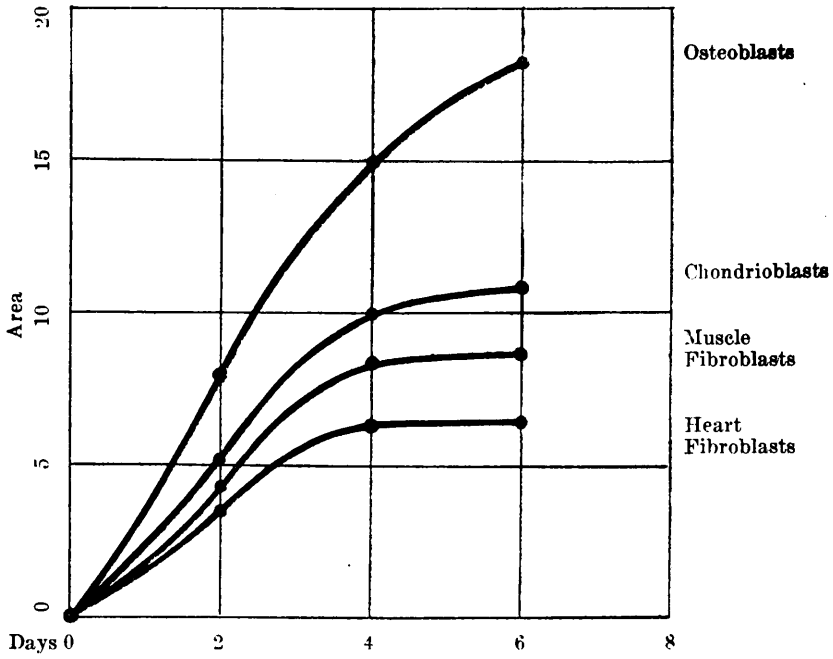
[4] Ebeling, A. H., *Compt. rend. Soc. biol.*, 1924, xc, 1383.

[5] Fischer, A., Demuth, F., Laser, H., und Meyer, H., *Münch. med. Wochenschr.*, 1928, lxxv, 651.

[6] Dolschansky, L., *Z. f. Zellforschung u. mikr. Anatomie*, 1929, viii, 789.

[7] Carrel, A., *Physiol. Rev.*, 1924, iv, 1.

FIG. 1. Residual growth energy of the four strains of fibroblasts.



out. On different occasions, only the zones of new growth were retained in order to equalize the density of the tissue fragments and to eliminate thoroughly all traces of the original explants. It was found that the tissues differed vastly in their rate of proliferation as well as in their response to increasing concentrations of embryonic tissue juice. The comparative experiments were made in Carrel flasks and the rate of growth was measured by the usual method. Reference will be made here to a few typical experiments which well illustrate the general findings. From Fig. 1 it can be seen that the 4 different strains of fibroblasts exhibit different residual growth energies[8] when cultivated in Tyrode solution alone. That for the osteoblasts is highest; next in order come the chondrioblasts, muscle fibroblasts, and last of all, the heart fibroblasts. Carrel and Ebeling have clearly shown that the rate of growth of the heart fibroblasts is a function of the concentration of embryonic tissue juice in the medium.[9] This fact has been substantiated for the heart fibroblasts, and for other types of fibroblasts up to certain concentrations of the growth promoting substances. It appears to be clear that those

[8] Carrel, A., *J. Exp. Med.*, 1923, xxxviii, 521.

[9] Carrel, A., and Ebeling, A. H., *J. Exp. Med.*, 1921, xxxiv, 317.

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.

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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.