

so that apparently the appearances described may be produced not only by mixtures of sporulating and non-sporulating strains but also by mixtures of rapidly and slowly sporulating strains.

The appearances described are similar to those described by Andrevont and Simon¹ and by Pesch². A more complete description and discussion of our observations and those of the authors cited will appear in a subsequent publication.

3089

Chemical nature of some substances required for the growth of fibroblasts and epithelial cells

ALEXIS CARREL and LILLIAN E. BAKER. (Introduced by Michael Heidelberger).

[*From the Laboratories of The Rockefeller Institute for Medical Research, New York City.*]

Pure strains of fibroblasts or of epithelial cells increase in mass in an unlimited manner, when they are cultivated in plasma and embryonic tissue juice. For 14 years, colonies of a strain of fibroblasts have doubled in size every 48 hours in such a medium. Pavement and thyroid epithelium also have been found to manufacture unlimited amounts of protoplasm from the constituents of embryonic juice. Neither epithelial cells nor fibroblasts multiply in serum proteins, egg albumin, crystallized egg albumin, amino acids from embryonic juice, or artificial mixtures of amino acids for a longer time than in Tyrode solution. So far, embryonic juice is the only material which has been found to maintain epithelial cells and fibroblasts in a condition of true cultivation.

Investigation of the chemical nature of the nutritive materials in the embryo juice has led to the conclusion that the nitrogenous substance utilized by the tissues is the protein itself. The amino acids and other ultra-filtrable and dialyzable constituents slightly stimulated the migration and multiplication of the cells, but failed to produce an increase in the mass of the tissues. Since the protein of the embryo juice is utilized by the cells, it seems evident

¹ Andrevont, H., and Simon, C. E., *Amer. J. Hyg.*, 1924, iv, 386.

² Pesch, K. L., *Centralbl. f. Bakt.*, Abt. I, Orig., xciii, 525.

that it must be hydrolyzed and some intermediate product absorbed. Complete digestion by trypsin and pepsin produced only toxic substances.

However, some of the higher cleavage products formed by the partial hydrolysis of protein have been found to produce the same effect as the protein of the embryo juice, causing continuous multiplication of cells and increase in the mass of tissue for long periods of time. In fact, some preparations of these protein hydrolytic products have given a larger increase in the mass of the tissue than has ever been obtained by embryo juice.

A most significant finding is that these growth-promoting proteolytic products have been obtained from foreign proteins as well as from embryo tissue. Egg white, commercial blood fibrin, and rabbit brain give excellent nutrient material on hydrolysis. In fact, the products of fibrin half digested by pepsin have given the best results thus far obtained. Witte's peptone is also capable of causing the multiplication of cells, resulting in an increase in the mass of tissue. Therefore, it is probable that no specific substance or hormone is required for the multiplication of cells, but only the proper nutrient materials.

Fractionation of these proteolytic products has been begun and has shown that the metaprotein has some action, but that proteose is more active. This proteose was obtained from Witte's peptone by first removing the protein, metaprotein, and any other products precipitated in 2.5 per cent trichloroacetic acid, and then removing the peptones and smaller degradation products by precipitating the proteoses with sodium sulfate at 33° C., and reprecipitating four times and dialyzing. This purified proteose solution has caused continued growth of tissue *in vitro* and an increase in the mass of the tissue which is twice as great as that produced by embryonic juice. Tissues cultivated in digested fibrin have become four times as large as their control in embryo juice.

At last, the chemical nature of the nitrogenous substances which are used specifically by tissue cells in the process of multiplication has been discovered. The synthesis of protoplasm by these cells, when fed on proteoses, leads one to suppose that the marvelous effect of embryonic juice on tissue growth is merely due to a special condition of its proteins, which renders possible their splitting into proteoses by the action of the fibroblasts and epithelial cells.