Notes on the finer structure are desirable; also notes on the color, which might vary according to the chemicals used.

After the study of recent pollen grains, it would be advisable to search samples from the surface of the peat for pollen grains. The pollen grains found among the branches of living *Sphagnum* and in the moss cover of stumps and fallen trees give a picture of the composition of the contemporaneous pollen grains. That would give a key to the conclusions which can be drawn from fossil pollen in general, but, in this respect, too much care can not be exercised. We know, for instance, that pollen grains can be carried by wind for very long distances, so that coniferous pollen might be encountered in the peats of Greenland; and, further, that the pollen grains of some trees might be under-represented in the pollen-spectra, owing to their being distributed at a time when the lakes and the peat surfaces are still frozen, or from other causes are not as fit for catching and preserving the pollen grains as at a later season. Because the delicate *Populus* pollen grains may not be preserved in peat, it is understandable, too, that a virgin Cordilleran coniferous forest would produce somewhat the same pollen-spectra in the mountain bogs as do some of the poplar forests, with scattered conifers, in the muskegs of the Great Western Plains. I do not mention this to discourage any one wishing to take up pollen-statistics. Its renown as a good and helpful paleontological research method can not be jeopardized, as shown by its success in Europe. I only mention it because, in my opinion, a thorough study of the recent and sub-recent pollenflora of American bogs would be more valuable as a start for pollen-statistical investigations in America than the presumably rather hazardous task of identifying a multitude of pollen types from old deposits with material often much decayed and altered.

Further information on pollen-statistics can be obtained from the papers listed in "Literature on Pollen-statistics published before 1927" (Geol. Fören. Förh., 49, 196–211, Stockholm, 1927) and "Literature on Pollen-statistics published during the years 1927–1929" (ibid., 52, 191–213, 1930).

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STRUCTURAL AND FUNCTIONAL VARIATIONS OF FIBROBLASTS IN PURE CULTURES

A MEDIUM has already been described[1] for the long-continued cultivation of mesenchyme cells under conditions which allow of very limited cell proliferation. Instead of the cells being fed upon the growth-promoting substances contained in embryonic tissue juice, they are treated with adult blood plasma. Cultures which are so nourished grow very slowly and can be kept in good condition over a much longer period of time than cultures which are allowed to proliferate at their maximum rate.

The experiments here reported have given additional information concerning the properties of cells as manifested under conditions of slow growth. The material consisted of various pure strains of mesenchyme cells which were isolated simultaneously from an embryo chick. These strains were derived from heart muscle, skeletal muscle, the perichondrium of cartilage and the periost of bone, respectively. Although these cell types exhibit striking differences in their nutritional properties,[2] it has not yet been possible to distinguish them morphologically. Until used for the experiments, which were made in flasks, the strains were carried by the hanging drop method on media favorable for the maximum proliferation of the various cell types. Each series of experiments was made at the same time from strains of the same age. Although regularly washed and treated with blood plasma, a number of cultures which have been allowed to remain in the same flasks for as long as one hundred days without being disturbed have continued to show appreciable growth over the entire period. In the case of those cell strains which become early adjusted to the plasma medium, growth becomes progressively more and more active from passage to passage. Sufficient heparin is added to the plasma to prevent its coagulation during each period of treatment. In the concentration used, appropriate experiments have shown, however, that the heparin has no appreciable effect upon the rate of growth of fibroblasts. When the cultures are subdivided and transferred, the new clots are allowed to coagulate spontaneously without the customary addition of tissue juices, and the same manner of treatment is resumed. Hence, we can definitely state that these cells are able to live and multiply at the expense of the food substances contained in the plasma alone.

It is undoubtedly true that the process of adaptation to the plasma treatment begins from the moment that the fibroblasts are transferred from an environment of ample food to one which is deficient in readily available food substances But while certain cell colonies are able to adjust themselves to the new environmental conditions without very pronounced structural changes in the cells themselves, it does not follow that this is always the case, even among sister

[1] A. Fischer and R. C. Parker, *Arch. f. exper. Zellforschung,* 8, 325, 1929.

[2] R. C. Parker, *Arch. f. exper. Zellforschung,* 8, 340, 1929.

cultures originating from the same strain. To illustrate: after the first series of experiments had been carried in the flasks under this treatment for about twelve days, it was noticed that the cells of one of the cultures belonging to a strain of fibroblasts derived from muscle had very suddenly given rise to a broad band of macrophages. A few days later a culture of the same age, but belonging to a strain of heart fibroblasts, behaved similarly. Less than two weeks later a third culture showed the same phenomena. This third culture, which had been treated for twenty-eight days in the flask when the transformation occurred, had been made from a strain of fibroblasts originating from bone periost, a strain which had been carried for twelve passages before the experiment was made. It was therefore quite obvious that the phenomenon was not limited to any one cell type. It is interesting to note that Carrel and Ebeling,[3] Fischer[4] and Ephrussi and Hughes[5] have reported the occasional occurrence *in vitro* of similar transformations, although the factors responsible for the changes have never been clearly defined.

After these and many similar observations had been made, new experiments were set up in an endeavor to duplicate as closely as possible every step in the treatment of these cultures in the hope that the changes might recur. And since this proved to be the case, we had a better opportunity for studying the predisposing conditions. Since, also, but a limited number of the cultures comprising the various experiments showed the phenomena, it was possible to make a comparative study of the general condition and rate of growth of cultures which had transformed and of those which had not. It was found that those cultures which had not transformed fell into two groups, namely, cultures which responded very favorably to the plasma treatment, as evidenced by the condition of the cells and their rate of proliferation, and those which could not adapt themselves to the new medium and very early succumbed when deprived of the abundant food substances which they had received before being placed under the conditions of the experiments. When transformation did occur, it seemed to take place at some critical period in the life of a culture in which the degeneration process was already quite evident, but was advancing at a relatively gradual rate.

These cells have been referred to as macrophages because they appeared identical with macrophages both in form and behavior. They were quite independent, very active, and showed no tendency to form a tissue. They also possessed the typical undulating membrane. Their origin was very easily ascertained. They were invariably budded off from the fibroblast-like cells at the periphery of a culture which showed unmistakable signs of degeneration. The cells from which the macrophages were derived were always very heavily granulated and distended. Several macrophages were usually pinched off from a single fibroblast, until finally nothing was left of it but a small remnant packed with the globules and granules of the original cell. When the transformation process had once begun, it continued with great rapidity. The macrophages wandered out into the medium at a uniform rate from the entire periphery, and, inasmuch as plasma is the optimal medium for their multiplication, they eventually covered an area which was, in some cases, twelve times as great as that finally covered by the colony of fibroblasts from which they were derived. The identity of the macrophages was further borne out by certain reactions, typical of blood and tissue macrophages, to alterations in the chemical constitution of the medium.

It seems reasonable to conclude that the macrophage and the fibroblast represent functional variations of the same cell type. The extent to which a cell changes its form depends, however, upon its physiological condition at any one moment and upon the chemical and physico-chemical properties of the medium. In order to determine the nature of such properties as may be responsible for these changes, it now becomes necessary to study the effect upon the cells of various constituents of the medium employed.

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[3] A. Carrel and A. H. Ebeling, *J. Exp. Med.*, 44, 261, 1926.

[4] A. Fischer, *Arch. f. exper. Zellforschung*, 3, 345, 1926.

[5] B. Ephrussi and Y. Hughes, *C. R. de la Soc. de biol.*, 105, 697, 1930.

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