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Development of Non-granular Leucocytes (Lymphocytes and Monocytes) into Polyblasts (Macrophages) and Fibroblasts in Vitro.*

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The question whether the non-granular leucocytes of the blood of mammals are mature, specifically differentiated cells, or cells endowed with possibilities of progressive development is still subject to discussion. The prospective potencies of these elements are especially manifest in inflammation.

Maximow[1] has claimed, since 1902, that the polyblasts or mononuclear exudate cells of inflamed tissue arise in part from local fixed cells, the resting wandering cells or the histiocytes of the tissue, but for the major part represent lymphocytes and monocytes, which have migrated from the blood vessels and have undergone rapid hypertrophy in the tissue. This double origin of the polyblasts is quite natural, for a study of the embryonic histogenesis of the connective tissue and blood reveals a close relationship between the histiocytes and the non-granular leucocytes.[2] In later stages of inflammation Maximow found the further transformation of polyblasts into fibroblasts. Thus, as the polyblasts arise in part from lymphocytes, he advocated the possibility of a transformation of lymphocytes and monocytes into fibroblasts.

This idea of a progressive transformation of non-granular blood leucocytes into polyblasts and finally into fibroblasts has met strong resistance. According to the dominant opinion, the polyblasts develop from the cells of the walls of the blood vessels (endothelium, adventitial cells). The monocytes and lymphocytes are usually considered as specifically differentiated elements which are unable to produce any other cell types.

The method of tissue culture has had a decisive influence on the problem under consideration. Several experimenters have shown that the non-granular blood leucocytes can transform themselves into macrophages (polyblasts) and even into "fibroblast-like" cells *in vitro* (Awrorow and Timofejewsky,[3] Carrel and Ebeling,[4] Maximow,[5] Lewis,[6] Fischer[7]). However, the problem is not completely solved as yet. Most of the investigators using the tissue culture

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method, with the exception of Maximow[5] and Timofejewsky and Benewolenskaja,[8] believe that the lymphocytes are incapable of progressive development, and that the monocytes are genetically independent from the lymphocytes. They accordingly discriminate sharply between these two cell types. The developmental possibilities of the monocytes are admitted, whereas the fate of the lymphocytes *in vitro* remains doubtful (Lewis[6]). Moreover the development of regular connective tissue from leucocytes also has not as yet been observed, only "fibroblast-like" cells were found.

The use of heparin enables us easily to apply tissue culture experiments to animals, in which the plasma clots very rapidly, such as guinea pigs, rats, etc. Guinea pigs seem to be especially favorable for culturing leucocytes. For the direct demonstration of the cellular transformations, in addition to sections of fixed material, the supravital neutral red staining was used.

The progressive changes of the lymphocytes in cultures of the buffy coat of guinea pig blood begin almost at once after explantation. Many of them remain unchanged and later gradually degenerate. But the major part transforms in the course of 6 to 8 hours into typical polyblasts. The nucleus becomes clearer and kidney shaped, the protoplasm increases in quantity and accumulates on one side of the nucleus. Whereas in the blood the lymphocytes show very few small neutral red vacuoles around the cytocentrum, *in vitro* they rapidly develop a large rosette of small and large neutral red vacuoles. The cells display intense ameboid movements with formation of hyaline membrane-like pseudopodia. During the second day the cells increase further in size and accumulate fat droplets. In the meantime the monocytes present in the culture also hypertrophy and accumulate an increasing quantity of neutral red vacuoles and fat. After 2 to 3 days monocytes and lymphocytes indiscriminately have transformed themselves into typical large phagocytic polyblasts. Thus, direct observation proves beyond doubt the rapid development of lymphocytes into polyblasts (macrophages).

A large part of the macrophages of lymphocytic and monocytic origin, during the third day, especially at the periphery of the explant, begin to stretch out and to develop long, spear shaped, nonmotile processes, while the large nucleus acquires a more regular oval shape. At first, however, the condition of these "fibroblast-like" cells must be in a peculiar unstable equilibrium. As soon as they are focused under an immersion lens with a sufficiently strong light, they at once rapidly contract, round off, and resume their former ameboid wandering with highly motile membrane-like pseudo-

podia. However, after a sufficient length of time (6 days or more) the changes seem to become irreversible. Typical fibroblasts appear and in numerous cultures, especially those growing in bottles, instead of slides, large colonies of typical fibroblasts develop, which cannot be distinguished from cultures of fibroblasts, obtained from regular connective tissue.

Thus, the possibility of the transformation of a small lymphocyte into a macrophage (polyblast) and further into a fibroblast seems to be demonstrated.

This is a preliminary report.

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[3] Awrorow, P., and Timofejewsky, A., *Virch. Arch.*, 1914, ccxvi, 184.

[4] Carrel, A., and Ebeling, H., *J. Exp. Med.*, 1922, xxxvi, 365.

[5] Maximow, A., *Klin. Wochenschr.*, 1925, iv, 1486.

[6] Lewis, M., *Am. J. Path.*, 1925, i, 91.

[7] Fischer, A., *Compt. rend. soc. biol.*, 1925, xciii, 109.

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Experimental Obstruction of the Mesonephric Ducts.

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In a previous paper[1] the writer showed that destruction of the growing ends of the Wolffian ducts in young chick embryos resulted in the failure of these ducts to establish connection with the cloaca. Under these circumstances, the Wolffian bodies became hydronephrotic, the cloaca failed to differentiate a urodaeal sinus, the allantois remained rudimentary, and the chicks died not later than the end of the sixth day from either lack of oxygen or a possible retention of urine. Since up to that time the allantoic fluid of normal embryos had never been analyzed by modern microchemical methods, the latter possibility could not at first be determined. But investigations begun in 1924[1] have shown that the end product of nitrogen metabolism in chick embryos is uric acid; that measurable amounts of it appear in the allantoic fluid as early as the fifth day of incubation; that it is eliminated in rapidly increasing amounts up to at least the 15th day[2]; and that during all this time, from the 5th