Missouri Branch

St. Louis University School of Medicine, St. Louis, Mo., May 26, 1926.

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Rosette and Granule-Hyaline Phagocytes in Tissue Cultures Stained Supravitally with Neutral Red.

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The rosette and the diffuse granule types of phagocytes were observed by Sabin, Doan and Cunningham[1] in peritoneal exudates stained supravitally with neutral red. The latter type may contain few dye granules or none whatever,[2, 3] and is therefore called the diffuse granule-hyaline type. There is no proof that either of these phagocytes is related to the peroxydase-reacting monocyte of Naegeli.[4] In the human blood there is some evidence that the Naegeli monocyte is neither the rosette nor the diffuse granule-hyaline cell.[3] In the human, the epithelioid cells of the tubercle of lymph glands contain the largest and most characteristic rosettes seen, but these cells in smears do not react to the peroxydase test that colors intensely the Naegeli monocytes in the blood smears of the same individual.[6] The final conclusion of Cunningham, Sabin and Doan[5] is that the rosette type arises from the reticulum, and that the diffuse granule cell comes from endothelium. These investigators did not find the rosette cell in the lymph nodes. In my experiments[2, 3] the rosette cell was found to be abundant in the lymph nodes where it was identified as the reticulo-endothelial cell. In tissue cultures of rabbit lymph nodes the rosette cell is almost the sole

phagocyte present. On the other hand, the phagocytes of the tissue cultures of rabbit liver are almost exclusively of the diffuse granule-hyaline type.[6] On the basis of these findings, considered together with the other data set forth in the papers cited, the conclusions reached were that the rosette cell arose from the reticulo-endothelium of lymphoid tissue and that the diffuse granule-hyaline phagocyte arose from the vascular endothelium.

Tissue cultures of rabbit spleen were made in order to determine the occurrence there of the two types of nonoxydase-reacting phagocytes. The methods used have been described elsewhere.[6] Upon supravital staining on the fourth to the seventh day the explanted bit of tissue often separates from the remainder of the plasma clot, and onto the glass beneath the phagocytes are spread out where they can be photographed to advantage. To preserve the dye in the cells the preparations are fixed for one hour and Zenker-formol solution, washed quickly, bottled dry, allowed to dry for one hour and mounted in balsam after clearing in xylol. Most of the phagocytes are of the rosette type. Extremely long cytoplasmic processes which approach reticulum fibers in size often connect the cells. In their reaction to neutral red and in structure they are like the reticulo-endothelial cells of the rabbit lymph node cultures. The occurrence in the spleen cultures of so many rosette cells that have all the characters of those present in lymph node cultures leads one to think that there is present in the spleen fixed tissue of the same type as the reticulo-endothelium of the lymph nodes.

The diffuse granule-hyaline cells are present in all cultures and small groups of them connected by cytoplasmic processes may be found. After placing the cultures in the neutral red solution, dye granules are often seen first in a focus near the nucleus, but later they appear scattered about elsewhere in the cytoplasm. In their reaction to neutral red there is much similarity between fibroblasts and diffuse granule-hyaline phagocytes. Therefore, the intravenous injection of carbon suspension some time before removing the spleen for the cultures marks the phagocytes and facilitates the accurate differentiation of the diffuse granule-hyaline cells and fibroblasts. The diffuse granule-hyaline phagocytes are like those of the rabbit liver cultures.

CONCLUSIONS.

1. In tissue cultures of rabbit spleen there are present the two varieties of mononuclear phagocytes which were demonstrated by Sabin, Doan and Cunningham in material obtained directly from the spleen of the rabbit under anesthesia. Both cells grow as reticular tissue with cytoplasmic processes which connect the individual phagocytes.

2. Since the rosette variety of phagocyte is constantly present in the tissue cultures of rabbit spleen in such large numbers, lymphoid reticulo-endothelium of the type found in lymph nodes is thought to be present in the normal spleen.

[1] Sabin, F. R., Doan, C. A., and Cunningham, R. S., PROC. SOC. EXP. BIOL. AND MED., 1924, xxi, 330.

[2] McJunkin, F. A., *Am. J. Path.*, 1925, i, 305.

[3] McJunkin, F. A., *Arch. Int. Med.*, 1925, xxxvi, 799.

[4] Naegeli, O., *Blutkrankheiten und Blutdiagnostic*, Berlin, 1923.

[5] Cunningham, R. S., Sabin, F. R., and Doan, C. A., *Contrib. to Embryol.*, 1925, lxxxiv, 227.

[6] McJunkin, F. A., Paper presented at meeting of Assoc. of Path. and Bact., Albany, N. Y., April 3, 1926. Complete report of this work will appear in *Arch. für Exp. Zellforschung.*

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Reaction Produced by Lipoid Solvents in Animals Fed Diets Deficient and High in Vitamin A.

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Lipoid solvents introduced into the subcutaneous tissue of rats fed a diet deficient in vitamin A remain well encapsulated by a zone of fixed tissue cells. This zone of tissue undergoes hyalinization after a period of thirty days and the mass of lipoid solvent remains encapsulated and quiescent within this zone.[1] The livers removed from these animals present a normal appearance grossly and microscopically.