Illinois Branch.

(WITH THE BIOLOGICAL CLUB.)

University of Chicago, March 1, 1927.

3467

Transformation of Lymphocytes of Thoracic Duct into Polyblasts (Macrophages) in Tissue Culture.*

WILLIAM BLOOM.

From the Department of Anatomy, University of Chicago.

The ability of the lymphocytes of the blood stream to develop into either myeloid or phagocytic cells is denied by most investigators. In a long series of experiments Maximow[1] showed that the lymphocytes and monocytes of the blood wander into the tissues in inflammation and transform there in a very short time into exudate mononuclear cells or polyblasts, as he called them. A portion of the latter he also derived from his resting wandering cells or histiocytes. These, as he showed, have a close embryogenetic relationship to the lymphocytes.

Several investigators have studied the development of lymphocytes and lymphoid tissue in tissue culture. Their results have not been uniform or decisive because it was very difficult to follow the fate of the various kinds of cells present in the explanted tissues.

At the suggestion, and with the aid of Dr. A. Maximow, I cultivated the lymph of the thoracic duct of the rabbit. This fluid in the rabbit contains only lymphocytes, or at most an occasional monocyte. (Simpson,[2] Thorne and Evans,[3] Kindwall.[4]) Lymph was pipetted aseptically from the cervical portion of the thoracic duct of adult rabbits (anesthetized with ether) which had been starved for 24 hours. The lymph was allowed to clot; it was then minced in Ringers solution and explanted in heparin plasma with various tissue extracts and tissues. This report gives the results obtained in cultivating lymph with embryonic extract.

* This work has been conducted under a grant from the Douglas Smith Foundation for Medical Research of the University of Chicago.

Except for a few scattered monocytoid cells and erythrocytes, the lymph used in these experiments, as seen in spravital, dry and wet smears, contained only lymphocytes.

After the cultures were studied in the living condition, both with and without supravital staining with neutral red, they were fixed in Zenker-formol, imbedded in celloidin, cut in serial sections, and stained with hematoxylin-eosin-azure II.

Immediately after explantation the cultures contain a compact fibrin clot filled with large numbers of small, medium sized and occasional large lymphocytes with here and there an erythrocyte. After the cultures have become warm in the incubator, the lymphocytes begin to migrate from the clot. After 7 or 8 hours a distinct change is to be seen in the cells within the clot. Many of them have become distinctly larger and contain many vacuoles which stain a brilliant red with neutral red. These vacuoles are usually arranged in typical rosettes about the indentation of the nucleus. There are numerous transition forms between these cells and the lymphocytes. After 20 hours the lymph clots contain very great numbers of the large cells. These are usually 3 or 4 times the diameter of the original small lymphocytes. The large cells have, as far as one can see in the clot, movable, dull, transparent, membrane-like pseudopodia, and, a large amount of cytoplasm which contains a variable number of bright lipoid drops. As the cultures grow older, the number of these cells increases. Mitotic figures are exceedingly rare. When lithium carmine is added to the cultures the large cells store the dye in large amounts in granular form. After three days there appear a few long, spindle shaped cells.

Shortly after explanation many of the small, medium sized and large lymphocytes become round and degenerate within the clot. Most of the cells which have migrated from the explanted lymph clot also die in the course of the first two days.

The fixed and stained sections of the cultures give a much clearer idea of what has taken place within the explants than has been obtained by the study of the living cultures.

Several hours after explantation the great majority of the lymphocytes begin to show marked changes. They are now much larger than in those cultures fixed immediately after explantation. The relative percentage of medium sized lymphocytes has increased greatly. Mitotic figures are entirely absent at this stage. Concomitantly with the marked increase in size of the cells there appears a distinct loss of basophilia of the cytoplasm. The nucleus of many of the cells becomes much larger and seems more vesicular,

in that the coarse chromatin in particles have divided into many smaller ones. The nucleus becomes decidedly bean-shaped because of a one-sided indentation. The nucleolus of the lymphocytes undergoes no change. The small and medium sized lymphocytes of the thoracic duct usually do not contain a demonstrable attraction sphere; occasionally one can see a small acidophilic area opposite the indentation of the nucleus. In the above described hypertrophy of the lymphocytes in the cultures the sphere becomes easily visible, it increases in size and may in a short time become as large as the nucleus. The latter then is excentrically located and seems relatively much smaller than before.

In some of the lymphocytes the described nuclear changes may be somewhat delayed. In these the cytoplasm increases greatly in size while the nucleus remains small and retains its original trachychromatism. In such cells the nucleus-plasma relation shifts markedly in favor of the cytoplasm. The latter may lose its basophilia almost entirely; only the outer edge of the cell body may stain blue. Inside this blue area vacuoles may appear and thin membrane-like pseudopodia may extend from the edge of the cell. The differences between these hypertrophied lymphocytes and the normal cells of the same lymph before and shortly after explantation are particularly striking when studied with the aid of a comparison ocular.

Twenty hours after explantation, the explanted bits of lymph clots contain the debris of many dead lymphocytes and fairly large numbers of actively ameboid, small and medium sized lymphocytes. But the dominant feature of the cultures is the presence of great numbers of large ameboid cells which cannot be differentiated from the exudate mononuclear cells (polyblasts) which are typical for the field of inflammation.

This hypertrophy of the lymphocytes has not taken place in all of our series of cultures of lymph. Our best results have been obtained from those rabbits in which the lymph was water clear and seemed free from fat.

The phagocytopoietic ability of the small and medium sized lymphocytes of the thoracic duct and, implicitly, of the blood stream of the rabbit is clearly demonstrated in these experiments.

This is a preliminary report.

---

[1] Maximow, A., *Beitr. z. path. Anat. allg. Pathol.*, 1902, Suppl. V; 1903, xxxiv, 153; 1904, xxxv, 43; 1905, xxxviii, 301; 1906, xxxix, 333; 1907, xli, 122. *Arch. f. mikrosk. Anat.*, 1909, lxxiii, 444.

[2] Simpson, M., *J. Med. Res.*, 1922, xliii, 77.

[3] Thorne, G., and Evans, H., *Anat. Rec.*, 1922, xxiii, 42.

[4] Kindwall, J., *Johns Hopkins Hosp. Bull.*, 1927, xi, 39.