GROWTH AND PROLIFERATION OF THE ENDOTHELIUM OF THE BONE MARROW SINUSOIDS IN TISSUE CULTURES

M. G. Shcherbakova

UDC 612.419:612.6]-085.23

The endothelium of the bone marrow sinusoids has been likened by many investigators functionally and genetically to reticular tissue. This is not in agreement with the conception of the endothelium as an independent tissue system [6-10]. In N. G. Khlopin's histogenetic classification of tissues, the reticular tissue and the endothelium cannot be placed in a common group, and this means that the reticulo-endothelial cells (a term widely used in pathology and hematology) cannot exist.

During cultivation of bone marrow for the purpose of studying hemopoiesis, previous investigators regarded the appearance of stroma cells in the zone of growth as growth of fibroblasts or of fibroblast-like elements. Only a few investigators have described the outgrowth of capillaries from the grafted tissue [15].

The object of the present investigation was to study the nature of the cells lining the sinusoids of the bone marrow by means of the tissue culture method.

EXPERIMENTAL METHOD AND RESULTS

Bone marrow was taken from 46 adult rabbits. Seeding took place into Carrel's flasks, and the solid phase consisted of dilute cock's plasma while the liquid phase contained Hanks's solution, medium No. 199, and human group AM serum. The sections of the cultures and total preparations were treated by various histological methods.

Stroma cells began to migrate into the zone of growth after the 2nd day. Usually they were large, widely spread cells, possessing processes and oval, lightly stained nuclei (Fig. 1); their cytoplasm was feebly basophilic and foamy. They were located within the fibrin at various levels from the surface, as was clearly seen in vertical sections through the cultures. From the 2nd to the 6th day the number of these cells in the zone of growth increased, and they formed a well-defined network. They evidently developed from the undifferentiated cells of the connective-tissue septa, for they grew most actively where a large blood vessel approached the edge of the central fragment. Here also could be seen solitary fat cells from the peripheral portion of the fragment which had lost their fat. Most investigators call such cells fibroblast-like.

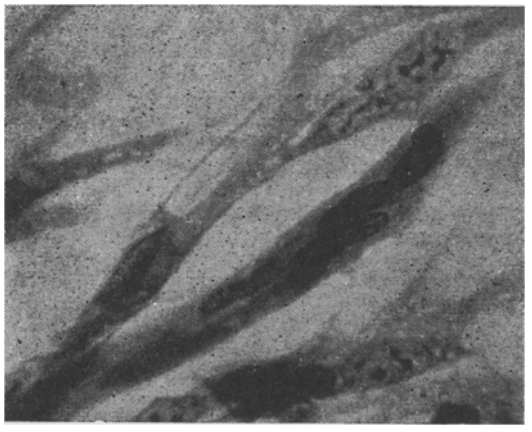

Fig. 1. Three-day culture of rabbit's bone marrow. End of a vascular bud growing from a divided arteriole, numerous nuclei with constriction rings. Total preparation; fixation with Carnoy's fluid, stained with azure II-eosin. Objective 60, ocular 10.

On the 2nd or 3rd day in some cultures the rapid outgrowing of vascular buds could be seen from the capillaries of the bone marrow or, more often, from the arterioles, with one or two layers of smooth-muscle cells. The vascular buds consisted of endothelial cells with a lumen between them, sometimes containing solitary erythrocytes; such a bud always ended in a small pool of cytoplasm. The nuclei in the growing bud were situated in a closely packed group, they were greatly elongated or curved, and they possessed several deep fissures (see Fig. 1). The staining of the nuclei and cytoplasm of the endothelial cells differed sharply from that of the surrounding fibroblast-

Laboratory of Experimental Morphology, Institute of Oncology, Academy of Medical Sciences of the USSR, Moscow [the research was directed by Active Member of the Academy of Medical Sciences of the USSR N. G. Khlopin (deceased)]. (Presented by Active Member of the Academy of Medical Sciences of the USSR A. I. Serebrov). Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 62, No. 7, pp. 89-93, July, 1966. Original article submitted October 3, 1964.

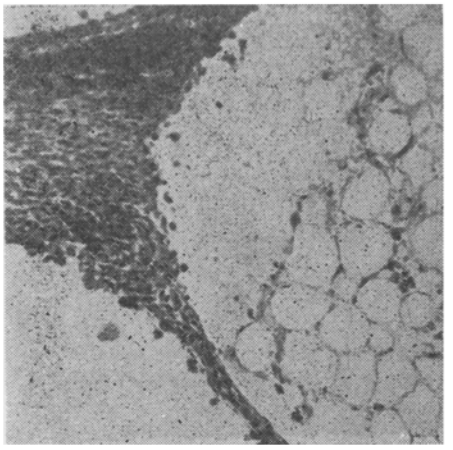

Fig. 2. Horizontal section through an 11-day culture of bone marrow. Spread of a sheet of endothelium from the surface of a fragment to the zone of growth over the fibrin. Fixation with Zenker-formol, stained with azure II-eosin. Objective 8, ocular 10.

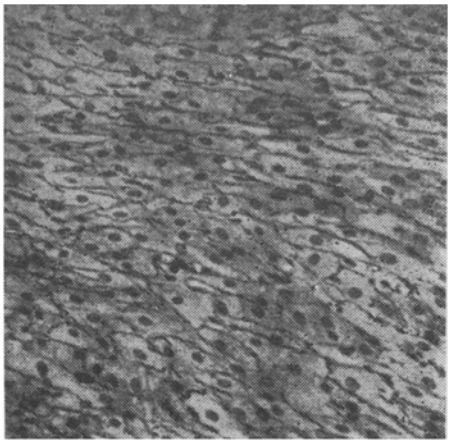

Fig. 3. Culture of bone marrow (31 days); 14 days after the last passage with excision. Growth of tightly packed endothelial cells. Total preparation, impregnation with silver, fixation with Carnoy's fluid, stained with azure II-eosin. Objective 8, ocular 10.

like elements. The vascular buds could be seen until the 5th day, after which they merged with the bands or sheets of endothelium growing from the sinusoids.

In the sections through the central fragment the endothelium of the sinusoids was seen particularly clearly in the 1- and 2-day cultures, when the sinusoids inside the fragment were packed with erythrocytes and normoblasts and the endothelium contained a large amount of hemosiderin, as a result of which the thinnest areas of the cytoplasm of the elongated cell could be seen. In these preparations the large collection of sinusoids passing between each pair of fat cells was easily demonstrated.

The endothelium of the sinusoids divided during seeding contained little hemosiderin. In the 2- and 3-day cultures the cell nuclei had become hyperchromic, larger in size, and with increased basophilia of their cytoplasm. In the 4- and up to 8-day cultures they filled the spaces between the fat cells, although no mitoses were visible in them. By the 7th-9th day the surface of the fragment was covered by 1 or 2 layers of polygonal or elongated cells, which then spread extremely rapidly(by the 11th-14th days)by growing over the fibrin and fibroblast-like elements as far as the edges of the mica sheet in the form of a single-layered membrane of closely packed cells (Figs. 2 and 3). The zone of growth at this time was exactly the same in appearance as that during cultivation of pieces of the posterior vena cava [14].

After excision and transplantation of the central fragment into a new flask the endothelium continued to grow as a sheet of tightly packed cells, and after a few passages it preserved its characteristic appearance (Fig. 3).

The difference between the cells of the endothelial sheet and the fibroblast-like elements was demonstrated clearly by the formation of liquefaction cavities. As the fibrin began to liquefy, the connective tissue cells lying under the sheet of endothelium retracted their processes and became round. The endothelial membrane then ruptured and, without losing its complexity, shrank and moved towards the edges of the developing cavity. The connective-tissue cells remained in the liquefaction cavity in the form of rounded polyblasts, which subsequently spread directly over the mica, forming wide, fan-shaped processes. These cells also behave differently toward ink or fluorochrome in the liquid nutrient medium. The connective-tissue cells ingested the ink, became rounded, and lost all connection with one another, whereas the endothelial cells contained the ink in the form of tiny particles, uniformly distributed throughout their cytoplasm, and in these circumstances the cells retained their complexity. The results of the experiments with fluorochromes will be discussed in a special paper.

It may be concluded from data in the literature and the results of the present investigation that the view, according to which the endothelium of the bone marrow sinusoids is a less highly differentiated part of the lining of the blood vessels, resembling most of all the cambium of the mesenchymal derivatives or the reticular tissue, is incorrect because, despite the differentiation inherent in all the cultivated tissues, it retained the typical complexity

of endothelium. This was revealed still more demonstratively during cultivation of embryonic tissue, in which blood vessels were present but no mesenchyme had yet appeared. The endothelium of these vessels, similar in function to the endothelium of the bone marrow sinusoids, grew as membranes of tightly packed cells, readily distinguishable from growth of the mesenchyme.

The endothelium of the bone marrow sinusoids in these cultures showed all the signs of a determinate tissue, in the same way as the endothelium of the large vessels [11-14, 15], the heart valves [3-5], and of hemangiomas of the skin [2]. Underestimation of the specific properties of the endothelium during cultivation of various tissues and tumors has often led to misunderstandings, some of which are analyzed in N. L. Kamenskaya's paper [2].

The results of the study of sections of the central fragment show that with the disappearance of its hematogenic cells, the only cells remaining between the fat cells were those which Pease [16], who reconstructed the sinusoids from electron micrographs, calls reticulo-endothelial. However, their behavior in the cultures was clearly distinguishable from the behavior of the relatively undifferentiated reticular cells accompanying the small vessels and capillaries [15].

Consequently, the cells situated between the fat cells, and appearing in the sections to possess processes, must be regarded as endothelial, whereas the reticular elements accompany the blood vessels and capillaries. During the first 2-3 days of cultivation the sinusoids of the intermediate zone of the central fragment were packed with maturing blood cells, but they continued to hold blood which did not overflow into the surrounding spaces. This fact conflicts with the views of those authors who consider that the circulation in the bone marrow is open. It may be assumed (this hypothesis has been put forward by N. G. Khlopin) that some sinusoids are periodically isolated from the blood flow, and their endothelium may then acquire a more or less reticular structure. This hypothesis does not, however, exclude the epithelial origin of the tissue, as has been shown by the study of the thymus [1].

SUMMARY

The bone marrow of the femur of adult rabbits was cultivated in Carrel's flasks with passage within a period of more than two months. The endothelium of the sinusoids, as distinct from the reticular and fibroblast-like elements of the connective tissue, proliferated by means of the membranes of closely-arranged cells similarly to the endothelium of larger vessels, retaining a characteristic appearance during the entire period of cultivation. The term "reticulo-endothelial cells" which is incorrect in the author's view is discussed on the basis of the author's own data and analysis of the literature data, since the reticular and endothelial cells show different predetermined properties in the cultures and fail to transform into one another. The endothelium of the capillaries and arterioles during the early days of cultivation grows from the central piece as vascular buds, coalescing later with the membranes of sinusoid endothelium.

LITERATURE CITED

1. Sh. D. Galustyan, The Structure of the Thymus in the Light of Experimental Analysis [in Russian], Moscow (1949).
2. N. L. Kamenskaya, Vopr. Onkol., No. 11 (1964), p. 54.
3. M. P. Ptokhov, Doklady Akad. Nauk SSSR, Vol. 116, No. 3 (1957), p. 501.
4. M. P. Ptokhov, Proceedings of the 6th All-Union Congress of Anatomists, Histologists, and Embryologists [in Russian], Vol. 1, Khar'kov (1961), p. 652.
5. M. P. Ptokhov, Vopr. Onkol., No. 10 (1962), p. 42.
6. N. G. Khlopin, The General Biological and Experimental Basis of Histology [in Russian], Leningrad (1946).
7. N. G. Khlopin, In: Problems in the Etiology and Pathogenesis of Tumors [in Russian], Moscow (1957), p. 21.
8. N. G. Khlopin, Arkh. Anat., Gistol., i Émbriol., No. 1 (1958), p. 13.
9. N. G. Khlopin, Arkh. Anat., Gistol., i Émbriol., No. 7 (1961), p. 3.
10. N. G. Khlopin, In: Problems in General Zoology and Medical Parasitology [in Russian], Moscow (1962), p. 278.
11. N. G. Khlopin and N. M. Chistova, Doklady Akad. Nauk SSSR, Vol. 114, No. 2 (1957), p. 425.
12. N. G. Khlopin and N. M. Chistova, Doklady Akad. Nauk SSSR, Vol. 119, No. 4 (1958), p. 803.
13. N. G. Khlopin and N. M. Chistova, Doklady Akad. Nauk SSSR, Vol. 122, No. 3 (1958), p. 508.
14. N. G. Khlopin and M. G. Shcherbakov, Tsitologiya, No. 6 (1961), p. 644.
15. R. Altschul, Endothelium: Its Development, Morphology, Function, and Pathology, New York (1954).
16. D. C. Pease, Blood, Vol. 11 (1956), p. 501.
17. L. Weiss, Bull. Johns Hopk. Hos., Vol. 108 (1961), p. 171.
18. L. Zamboni and D. C. Pease, J. Ultrastruct. Res., Vol. 5 (1961), p. 65.