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Abstract

A single withdrawal of blood (about 0.6 ml) from a splenectomized mouse induced extramedullary hemopoiesis in the liver. Twenty days after splenectomy, blood was taken from the retroorbital sinus. Hemopoietic foci in the liver increased in number daily reaching maximum value 6 days after blood withdrawal, then decreased gradually to the initial level with recovery of the hematocrit value and disappearance of reticulocytosis 25 days after blood withdrawal. Hemopoietic foci were pure erythrocytic, granulocytic, megakaryocytic or unclassified, but not mixed. Small unclassified cell foci appeared first, increased in number, followed by the development of erythrocytic, granulocytic and megakaryocytic foci. This suggests that small unclassified cell foci grow to erythrocytic and large granulocytic ones. Most of the liver hemopoietic foci were in the intralobular area. Some were in the portal area; none of these were megakaryocytic. Electron microscopic observation revealed that lymphoid cells having distinct nucleoli migrate into Disse's space through the sinusoidal walls. There they proliferate by cell division to form large foci in the perisinusoidal area. The morphologic characteristics of the lymphoid cell are discussed.

KEYWORDS: extramedullary hemopoiesis, liver, stem cell, extravascular site, adult mouse

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A STUDY ON THE MECHANISM OF THE DEVELOPMENT OF EXTRAMEDULLARY HEMOPOIESIS IN ADULT MOUSE LIVER

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Abstract. A single withdrawal of blood (about 0.6 ml) from a splenectomized mouse induced extramedullary hemopoiesis in the liver. Twenty days after splenectomy, blood was taken from the retroorbital sinus. Hemopoietic foci in the liver increased in number daily reaching maximum value 6 days after blood withdrawal, then decreased gradually to the initial level with recovery of the hematocrit value and disappearance of reticulocytosis 25 days after blood withdrawal. Hemopoietic foci were pure erythrocytic, granulocytic, megakaryocytic or unclassified, but not mixed. Small unclassified cell foci appeared first, increased in number, followed by the development of erythrocytic, granulocytic and megakaryocytic foci. This suggests that small unclassified cell foci grow to erythrocytic and large granulocytic ones. Most of the liver hemopoietic foci were in the intralobular area. Some were in the portal area; none of these were megakaryocytic. Electron microscopic observation revealed that lymphoid cells having distinct nucleoli migrate into Disse's space through the sinusoidal walls. There they proliferate by cell division to form large foci in the perisinusoidal area. The morphologic characteristics of the lymphoid cell are discussed.

Key words : extramedullary hemopoiesis, liver, stem cell, extravascular site, adult mouse.

Extramedullary hemopoietic foci are frequently encountered in the spleen, liver, lymph node, adrenal gland and kidney of patients with hematologic disorders. These hemopoietic foci may be erythrocytic, granulocytic, megakaryocytic or mixed (1-4).

Adult mice and rats generally have no, or only poor, hemopoietic foci in the liver, but foci appear there under a variety of experimental conditions (5-14); for example, injection of methylcellulose in splenectomized mice (5), phenylhydrazine intoxication (6, 12), partial hepatectomy (9), bone damage by ⁸⁹Sr (10, 11) and dimethylaminoazobenzen (DAB) feeding (13). Different hemopoietic foci appeared in different conditions; for example, predominate by erythrocytic foci in the centrilobular area after phenylhydrazine injection (6), erythrocytic and granulocytic foci after methylcellulose injection in splenectomized mice (5), and erythrocytic foci in the area of pathologic cell growth in DAB feeding (13). Each of these is hemopoiesis in damaged or distorted liver tissue; there-

fore, it is difficult to clarify interactions between hemopoietic cells and host tissue.

I found that withdrawal of blood from splenectomized mice induces active hemopoiesis in the liver. This provides a convenient tool for observation of the development of hemopoietic foci in relation to liver tissue cells. In this paper, the development of extramedullary hemopoiesis in the liver is presented morphologically.

MATERIALS AND METHODS

Eighty adult, female C₃H/He mice were used. They were provided by the experimental animal center of Okayama University Medical School.

The animals were divided into 3 groups. The first group consisted of 50 animals which received total splenectomy followed by one withdrawal of 0.6 ml of blood 20 days later. The second group consisted of 25 animals which were splenectomized but which did not have any blood withdrawn. The third group consisted of 5 animals and served as untreated controls.

After blood was taken, the animals in the first group were sacrificed at certain day intervals by bleeding to death (5 animals at each time). Hematocrit values, reticulocyte counts, white blood cell counts and hemograms were examined by routine methods. Femoral bone marrow was taken, fixed with formalin, embedded in paraffin, sectioned and stained with H.E. by routine methods for light microscopy. The livers were perfused with 5 ml of Millonig solution through the portal vein, then fixed by reperfusion with 5 ml of 2 % glutaraldehyde in Millonig solution. After perfusion with glutaraldehyde, small pieces of liver tissue were taken and refixed with 2 % glutaraldehyde at 37 °C for 30 min. All tissues were postfixed with OsO₄ at 4 °C for 1.5 h, dehydrated through an ethanol series, and embedded in Epon 812. Semithin sections of about 0.2 μ were prepared and stained with toluidine blue for light microscopy. Thin sections were stained with uranyl acetate and lead nitrate for electron microscopy. A Porter type I ultramicrotome and an Electron Microscope HU-11A, Hitachi, Japan, were used in these procedures.

The animals in the second group were sacrificed 2, 4, 6, 15 and 20 days after splenectomy (five animals each time). Blood and bone marrow were examined as in the first group. Liver tissues were fixed with formalin, embedded in paraffin, sectioned and stained by H.E. for light microscopy as for bone marrow tissue. These samples were not examined by electron microscopy.

Control animals were sacrificed without any treatment. Blood, bone marrow and liver were examined as for the second group.

RESULTS

Adult healthy C₃H/He mice had no, or only poor, hemopoietic foci in the liver. The active hemopoiesis was found solely in bone marrow and spleen.

Splenectomy hardly induced any plethoric condition. The splenectomized animals showed a normal hematological picture, though a few small hemopoietic foci appeared in the liver 6 days after splenectomy. These foci decreased in number gradually and most of them had disappeared by 15 days, though some hemopoietic foci were seen even 20 days after splenectomy. They were mainly

granulocytic. Under such conditions, blood was drawn from animals in the first group. Hematocrit values had decreased to 29% (mean) by 2 days after blood withdrawal, but later increased gradually reaching 40% at 6 days and had returned to the normal range by 10 days. Reticulocyte counts rose to 28% at 4 days but had decreased to the original level by 25 days (Fig. 1). White blood cell counts had slightly increased 2 days after blood depletion, 11,000/mm³, but had recovered to normal by 4 days (Fig. 2). Monocytes and lymphocytes in circulating blood were slightly higher in number at 2, 3, 4 days and "lymphoid cells" were present in the peripheral blood on these days (Fig. 2, Photo 1. a).

After splenectomy and blood withdrawal, bone marrow showed a picture of activated hemopoiesis without any pathologic changes.

In the liver, only a few granulocytic foci were found before blood withdrawal, but many foci appeared after blood loss and were accompanied by reticulocytosis in the peripheral blood.

By 2 days after blood depletion, a number of small hemopoietic foci composed of 2 to 4 cells had appeared in the liver of splenectomized animals. Later, the number of hemopoietic foci increased daily and large foci (10-19, 20-cells) formed. The number of hemopoietic foci reached a maximum at 6 days, decreased gradually thereafter, and returned to the original level 25 days after

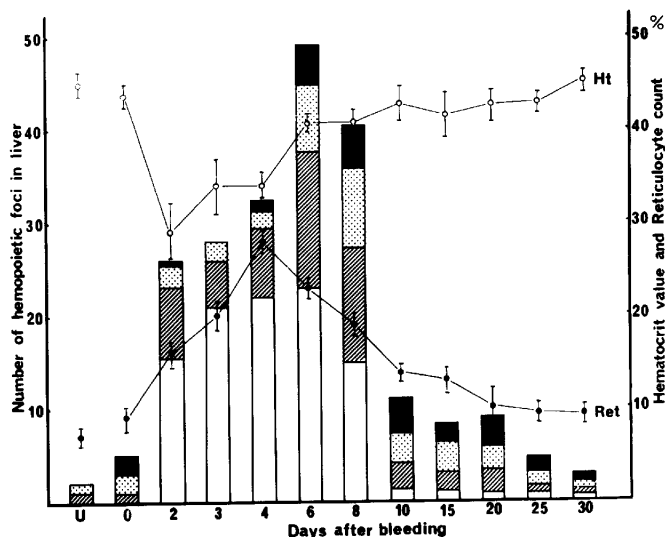


Fig. 1. Daily changes in number of hemopoietic foci in liver, hematocrit value and reticulocyte count in circulating blood after withdrawal of blood from splenectomized mice. Number of hemopoietic foci in 100 sight fields ($\times 400$) was recorded. Each value shows the mean of 5 animals. Columns show the number of hemopoietic foci in liver; filled columns, foci composed of more than 20 cells, dotted, 10-19 cells, hatched, 5-9 cells and open, less than 4 cells. Filled circles: reticulocyte count. Open circles: hematocrit value. U: untreated control. O: values 20 days after splenectomy just before blood withdrawal.

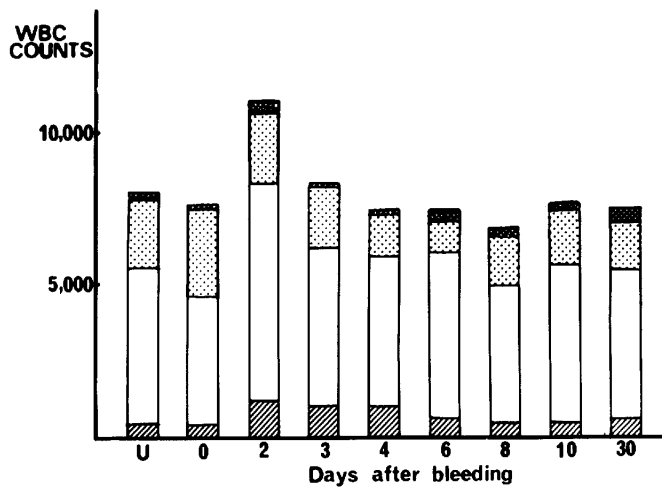


Fig. 2. White blood cell counts and the classification of circulating blood before and after blood withdrawal. Filled column: basophil. Crossed one: eosinophil. Dotted one: neutrophil. Open one: lymphocyte. Hatched one: monocyte. U and O: see the legend of Fig. 1.

blood depletion (Fig. 1). A cell classification of the hemopoietic foci made at 6 days showed that most of the small foci were composed of unclassified cells of immature blastic nature. Most of the large hemopoietic foci were composed of granulocytic cells. Erythrocytic foci also appeared but were medium in size, being composed of 5 to 9 cells (Table 1). Thus, with lapse of time, the small sized hemopoietic foci composed of unclassified cells (Photo 1. b) developed into large sized ones with differentiation of the immature cells forming erythrocytic (Photo 1. c) and granulocytic foci (Photo 1. d). Erythrocytic foci had a few of macrophages and granulocytic foci had some monocytes. Megakaryocytic foci were seen most often as isolated cells (Photo 1. e).

Most hemopoietic foci were found in the intralobular area (92%), and some in the portal area (8%). Both erythrocytic and granulocytic foci were found

TABLE 1. RELATIONSHIP BETWEEN CELL NUMBER AND CELL CLASSIFICATION OF HEMOPOIETIC FOCI FOUND IN THE LIVER OF SPLENECTOMIZED MICE 6 DAYS AFTER THE WITHDRAWAL OF BLOOD.

cells one focus	Number percent of blood cells in each focus				
	Unclassified	Erythrocytic	Granulocytic	Megakaryocytic	Total
1- 4	33	8	0	5	46
5- 9	11	16	8	0	35
10-19	0	3	9	0	12
20-	0	0	7	0	7

Each numeral shows the number in 100 foci counted.

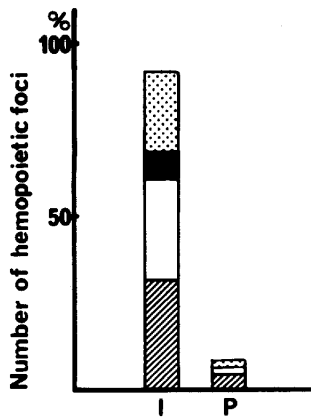


Fig. 3. Classification of hemopoietic foci in liver of splenectomized mice 6 days after blood drawing. I: foci in intralobular area. P: foci in portal area. Dotted column: unclassified cell foci. Filled column: megakaryocytic foci. Open one: granulocytic foci. Hatched one: erythrocytic foci.

in the intralobular and portal areas, but megakaryocytic foci were found only in the intralobular area (Fig. 3).

Optic microscope observation of H.E. stained sections and toluidine blue stained sections failed to give any precise information about the exact localization of the hemopoietic cells in the intralobular area of liver, though they appeared to be situated in association with fat storing cells in Disse's spaces (Photo 1. f).

Electron microscope observation of samples from the early stages after blood withdrawal revealed some lymphoid cells adhering to the sinusoidal wall (Photo 2. a). By observing a number of sections, cells passing through the fenestration of endothelial cells on the way to Disse's space (Photo 2. b) and others situated in Disse's space (Photo 2. c) were found. They were the same kind of cell morphologically. In large hemopoietic foci found in sections from samples of later stages after blood withdrawal, there were many differentiated cells with some mitotic figures. They were situated in enlarged Disse's spaces in direct contact with liver parenchymal cells and separated from sinusoidal spaces by sinus endothelial cells. The picture shows clearly that liver hemopoiesis occurs in Disse's spaces (Photo 2. d). No hemopoietic cell foci were found in the sinusoidal spaces. Fat storing cells were usually found near hemopoietic cells. The surface of the hemopoietic focus facing to the sinusoid was covered by a single layer of endothelial cells (Photo 2. e).

DISCUSSION

Generally, adult mouse liver has no hemopoietic activity; but under some circumstances, it develops extramedullary hemopoiesis, for example, after splenectomy or more actively after blood loss in splenectomized animals. This latter condition gives good material for the morphologic observation of the development of hemopoietic foci, because the back ground structure is simple and easy

to analyse. Actually, blood loss of 0.6 ml once was enough to give rise to many hemopoietic foci in the liver of splenectomized animals, probably to compensate for insufficient bone marrow hemopoiesis after splenectomy.

Many of the hemopoietic foci developed intralobularly as pure foci of erythrocytic, granulocytic, megakaryocytic or unclassified cells. Mixed cellular foci were not seen. Lymphocytic foci, if they were present, could not be distinguished from undifferentiated cell foci morphologically.

Small "lymphoid cells" penetrated into Disse's space through the fenestration of endothelial cells, and proliferated and differentiated there to form granulocytic and erythrocytic foci. The findings indicate that the stem cell in circulating blood settled first on endothelial paving cells, penetrated the pavement to reach Disse's space, then formed large hemopoietic cell foci.

The stem cells that settled on, or penetrated, endothelial cells were small lymphoid cells with a distinct nucleolus. It is unclear whether the cells are CFU-S, CFU-C, CFU-E or CFU-M. They had a lymphocytic profile, the chromatin mass being densely flocculated rather than finely dissociated as demonstrated by van Bekkum and associated (15). The picture is similar to that of the transitional cell of Yoffey (16) or the monocytoïd cell of Everett (17). The flocculated chromatin probably loosens in the succeeding blast formation for cell division.

Thus, developing hemopoietic foci are always found in Disse's space but not in the sinusoidal space. When they develop to large sized foci, they seem to get space for growth by passing beside parenchymal cells, but not by repenetrating into the sinusoidal spaces. Lining endothelial cells can always be seen separating foci from sinusoids. Among the foci there were usually some fat storing cells (Ito's cell) (18) suggesting their role in the development of hemopoietic foci. Some macrophage-like cells were also observed but they were few in number. Foci were directly attached to liver parenchymal cells. The liver cells seem to be involved in hemopoietic cell proliferation but no specific morphologic changes were seen in the neighboring liver cells. Many investigations have been reported (5-14) and some of them stress focus formation in sinusoidal spaces. But most observations were made on hemopoiesis in damaged liver tissue and lacked electron microscopic observation. This leads to misunderstanding because of non-visible endothelial cells between foci and sinusoidal spaces. Pleomacher and van Soest (6) studies the liver hemopoiesis in phenylhydrazine intoxication with the electron microscope. They observed foci formation in the sinusoids in association with macrophages. However, phenylhydrazine damages cell membrane (19, 20) and may destroy the sinusoidal endothelial cells. Thus, liver hemopoiesis in the adult animal occurs in the extravascular space as in embryonic liver hemopoiesis (21, 22, 23).

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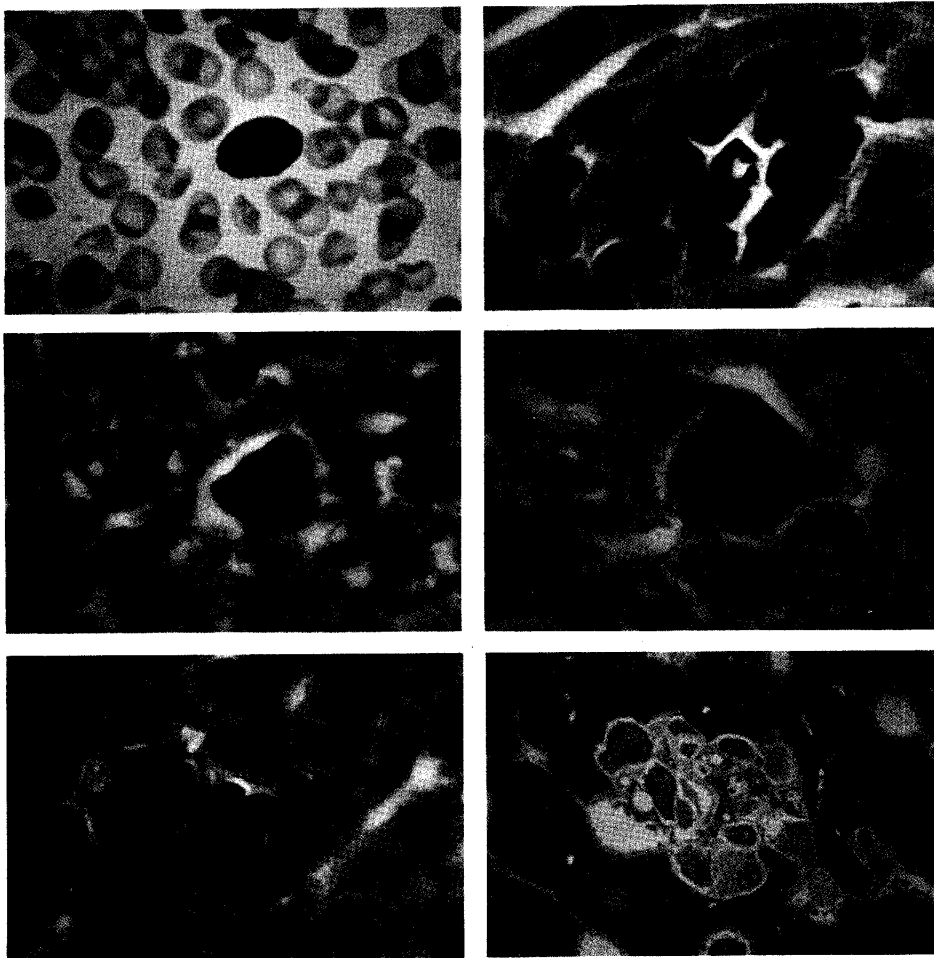


Photo 1. Light microscope pictures of a blood smear stained with Giemsa, H.E. stained paraffin sections and toluidine blue stained-Epon section. Method: see text.

- a: Lymphoid cell having nucleoli with deeply stained cytoplasm found in peripheral blood 3 days after withdrawing blood from, splenectomized mouse, Giemsa stain ($\times 1,000$).
- b: An unclassified cell focus found in the liver of a splenectomized mouse 4 days after drawing blood. H.E. stain ($\times 1,000$).
- c: Erythrocytic focus found in the liver of a splenectomized mouse 6 days after drawing blood. H.E. stain ($\times 1,000$).
- d: Granulocytic focus found in the liver of the same animal as in Photo 1. c. H.E. stain ($\times 940$).
- e: Megakaryocytic focus in the liver of the same animal as in Photo 1. c. H.E. stain ($\times 950$).
- f: Hemopoietic focus found in the liver of the same animal as in Photo 1. c. Toluidine blue stain ($\times 930$).

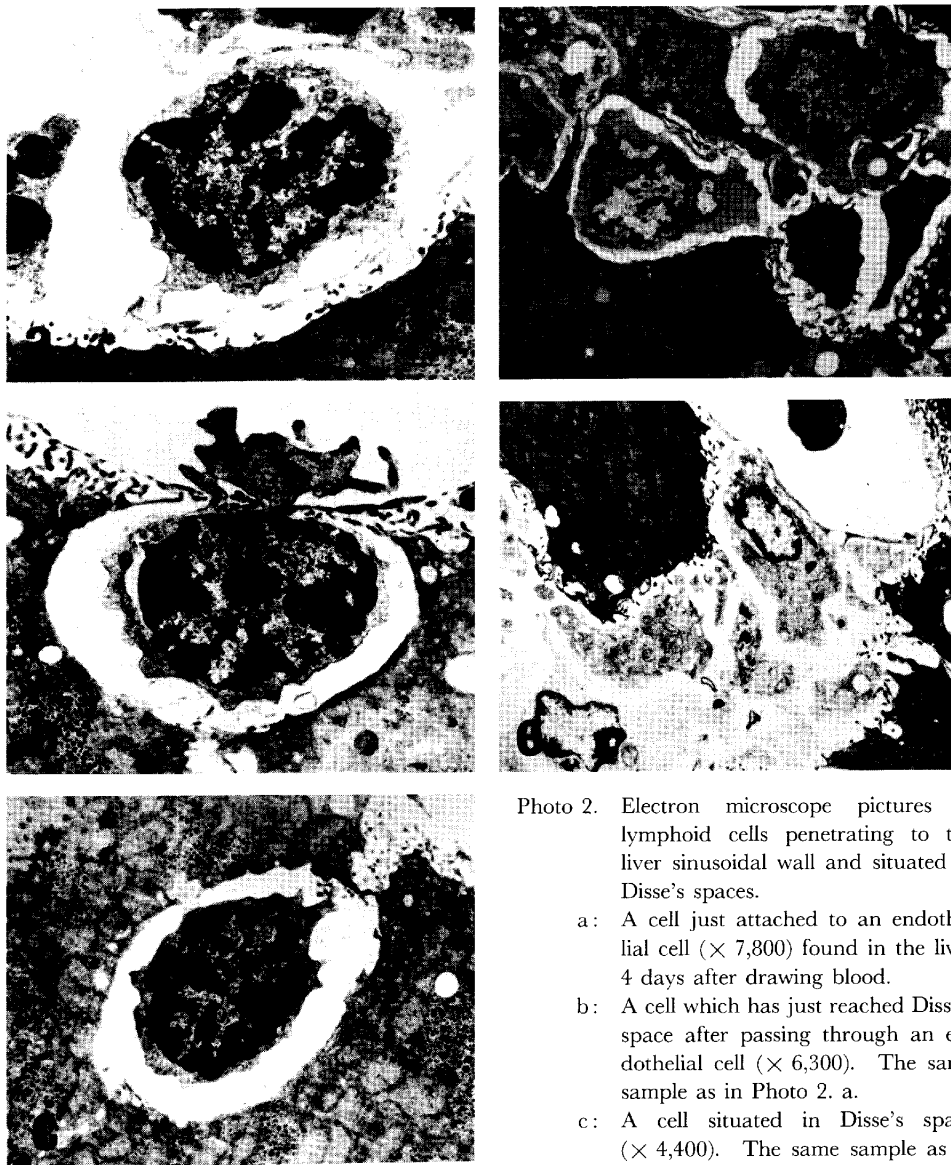


Photo 2. Electron microscope pictures of lymphoid cells penetrating to the liver sinusoidal wall and situated in Disse's spaces.

- a: A cell just attached to an endothelial cell ($\times 7,800$) found in the liver 4 days after drawing blood.
- b: A cell which has just reached Disse's space after passing through an endothelial cell ($\times 6,300$). The same sample as in Photo 2. a.
- c: A cell situated in Disse's space ($\times 4,400$). The same sample as in Photo 2. a.
- d: The cells in a granulocytic focus. Picture shows the foci developing in Disse's space directly associated with a liver parenchymal cell. The cytoplasm of Ito's cell can be seen ($\times 3,200$). Six days after blood withdrawal.
- e: A granulocytic focus developing in Disse's space being separated from the sinusoidal space by the thin cytoplasm of endothelial cells ($\times 3,200$). The same sample as in Photo 2. d.