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Studies on reticuloendothelial system and hematopoiesis. II. A study on the morphology and function of erythroblastic islet

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Abstract

1. For the purpose to clarify the role of reticuloendothelial cells in the center of erythroblastic islet, the medullary and extramedullary erythropoietic foci in convalescence of phenylhydrazine arternia were observed by light and electron microscopies, and the mode of development of anemia in rabbit having blocked RES. 2. Light microscopic observations revealed the stimulated formation of erythroblastic islet in phenylhydrazine anemia. Electron microscopic observations revealed the rhopheocytotic vesicles in the membrane of erythroblast, some of which contained ferritin particles. 3. Repeated India ink injections induced anemia with RES damage by being heavily laden with soot particles. Color index of these animals was not reduced to the state of hypochromic anemia. Anemia is due to the damage of erythroidcell reproduction, but not due to the disturbed hemoglobin metabolism by the inhibited iron uptake. 4. From the results obtained the author concludes that the role of reticuloendothelial cell in islet is not to transfer ferritin to erythroblast but the reproduction of erythroid cells.

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STUDIES ON RETICULOENDOTHELIAL SYSTEM AND HEMATOPOIESIS

II. A STUDY ON THE MORPHOLOGY AND FUNCTION OF ERYTHROBLASTIC ISLET

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In the previous paper[1], the author demonstrated that the disintegration of reticuloendothelial system (RES) resulted in the release of bone-marrow cells into the circulating blood and the subsequent suppression of the function of reticuloendothelial (RE) cells inhibited the recovery of hematopoiesis. The results showed that hematopoiesis was supported by the healthy function of RE cells. This is true in the case of extramedullary hematopoiesis in liver, spleen and lymph nodes as well as in the case of bone-marrow hematopoiesis. Concerning the role of RES in hematopoiesis, BESSIS[2,3,4] mentioned that the role of RE cells in erythropoiesis was to supply iron to erythroblasts in the form of ferritin. Of course, other nutritional substances may be given to erythroblasts by rhopheocytosis as well as iron. But it is not definitely proven that the essential role of RES in erythropoiesis is to supply the nutritional substances. For the purpose to clarify the role of RES in erythropoiesis, the author observed first the islet formation in anemic rabbit by light and electron microscopes and then hemoglobin formation in anemia induced by RES damage. In this paper, it is reported that the essential function of RES in hematopoiesis is to give some information to the stem cell for the differentiation of erythroid cells, but not to supply iron to erythroblast.

MATERIALS AND METHODS

Normal seven male rabbits weighing 2.5—3.0kg were used. Five of them received the injections of 2.5% HCl-phenylhydrazine, 1.5cc subcutaneously daily for four times respectively. Of these animals, daily changes of red cell number, hemoglobin content and the percentage of nucleated cell species in the circulating blood were investigated. One to two days after the maximum level of anemia, the rabbits were killed by head contusion. Bone marrow, liver and spleen were obtained. Cut surface of each organ was imprinted on glass slides, fixed with methanol and then stained with Giemsa solution. Tissues were fixed with 10%

formol in 0.25 M sucrose solution, or 6% glutaraldehyde in 0.1 M phosphate buffer (pH, 7.4) for light microscopy. These materials were dehydrated, embedded in paraffin, sectioned and stained with hematoxylin-eosin by routine method and some of them by Berlin blue reaction. For electron microscopy, small tissue blocks about 0.5 mm in size obtained from tissues fixed with the glutaraldehyde solution for thirty minutes were refixed with 1% osmium tetraoxide solution[5], or 3% $KMnO_4$ solution in veronal buffer (pH, 7.2—7.4)[6] for thirty minutes to one hour. They were dehydrated, embedded in Epon[7], sectioned in thickness of 400—500 Å by ultramicrotome (Porter-Blum type) and counter-stained with 5% uranyl acetate in 70% ethanol.

Another two rabbits received the intravenous injection of India ink, (Fueki-Bokujū), 6 cc mixed 20 cc saline solution per animal, daily for nine to ten weeks. Red cell count and hemoglobin level of the circulating blood were observed at certain intervals. At the termination of experiment they were sacrificed by head contusion, and bone marrow, liver and spleen were fixed, sectioned and stained with hematoxylin-eosin by the routine method for light microscopy.

OBSERVATIONS AND RESULTS

By the repeated injection of phenylhydrazine, red cell count decreased day by day and after four injections it reached the level less than one third of the original. Reticulocytes increased occupying 60—70% of whole red cells and considerable numbers of orthochromatic and polychromatic erythroblasts appeared in the circulating blood (Fig. 1).

Light microscopic observations on each of tissues revealed erythroid marrow, and extramedullary erythropoietic foci in liver and spleen. In bone marrow, well developed erythropoietic foci surrounded by sinusoid were found. They increased both in size and number. Each of foci was composed of one or two reticulum cells surrounded by erythroblasts in various differentiation stages. The reticulum cells were swollen by phagocytizing hemosiderin which is stained blue by Perl's reacton (Figs. 3, 4, 5, 12). The finding to be stressed here, is that the younger erythroblasts, large in size, are found in the central area of islet, in a close contact with the reticulum cell, and the rather differentiated ones are situated in the peripheral area of islet just near the sinusoid which is filled with mature red cells. On the imprinted preparation of bone marrow, reticulum cells appeared frequently being surrounded by several to many erythroblasts in various maturation stages. Spleen, which was moderately swollen and dark reddish, contained a large number of erythroblasts. They were found in the sinusoids of red pulp, forming a mass or masses and in some marked groups a reticulum cell was found, showing the formation of erythroblastic islet (Figs.

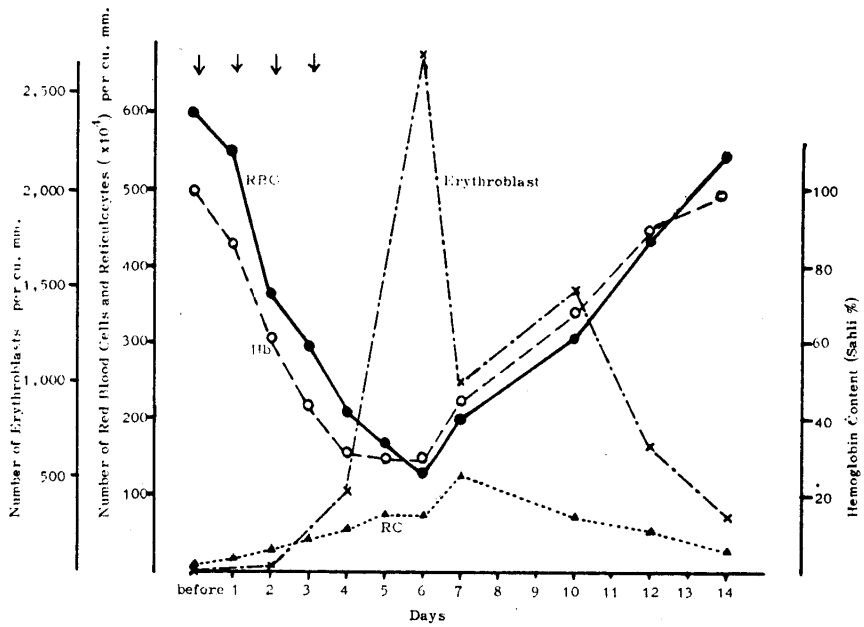


Fig. 1 Changes in number of red blood cells (RBC), reticulocytes (RC) and erythroblasts and in hemoglobin content (Hb) in the circulating blood of phenylhydrazine-induced anemic rabbit for two weeks. Arrows (↓) show injection of 2.5% HCl-phenylhydrazine 1.5 cc subcutaneously.

9, 10, 11, 13). In the area surrounding lymph follicles and blood vessels, the massed colonies of myeloid cells were found. On the imprinted preparation erythroid islets were observed as in the case of bone marrow. Liver, which looked normal macroscopically, showed some sinusoids which were extended and contained groups of erythroblasts surrounding Kupffer cells (Figs. 6, 7, 14). This finding was distinctly observed on the imprinted preparation (Fig. 8). There were no signs of parenchymal injury, but Kupffer cells contained hemosiderin.

Electron microscopic picture showed clearly the relation of reticulum cells and erythroblasts in erythroblastic islet. Almost the identical pictures were observed in bone marrow, liver and spleen. The cell membrane of reticulum cell in a close contact with that of erythrobasts. Observing precisely, almost all erythroblasts seemed to have contact surface with reticulum cell. Among the cellular spaces of erythroblasts there exists an elongated cytoplasm of the reticulum cell and both cytoplasmic membranes made close contact. Most of the nuclei of the reticulum cell appeared round or oval in shape and chromatin network was relatively coarse. Their abundant cytoplasm contained a variety of substances, i. e. erythrocyte fragment, dense masses in lysosomes, hemosiderin

and ferritin, and ferritin molecules scattered diffusely in cytoplasm. Erythroblasts appear to be round having round nuclei, and according to the advance of their differentiation stage, they reduce cell size and nuclear size as well, and chromatin network of the nucleus becomes denser with the increase in electron density of their cytoplasm. As BESSIS pointed, small engulfment of cytoplasmic membrane of erythroblast, rhopheocytotic vesicles, can clearly be seen. They contain cytoplasmic contents of reticulum cell and sometimes with ferritin molecules. The wall of rhopheocytotic vesicles appears electron dense and thickened. The picture of the section fixed with $KMnO_4$, where the membrane systems are distinct, shows clearly ferritin particles in the cytoplasm of erythroblasts to be all inside the vesicular membrane system, which seemed to have developed from rhopheocytotic vesicles (Figs. 15—24).

Observations on anemic rabbits induced by the repeated intravenous injections of India ink for a long period of time, nine to ten weeks developed severe anemia. Red cell count reached half or less of the original level and hemoglobin contents were also reduced markedly. But the color index which means hemoglobin content per cell, remained around 1 or higher (Fig. 2), showing

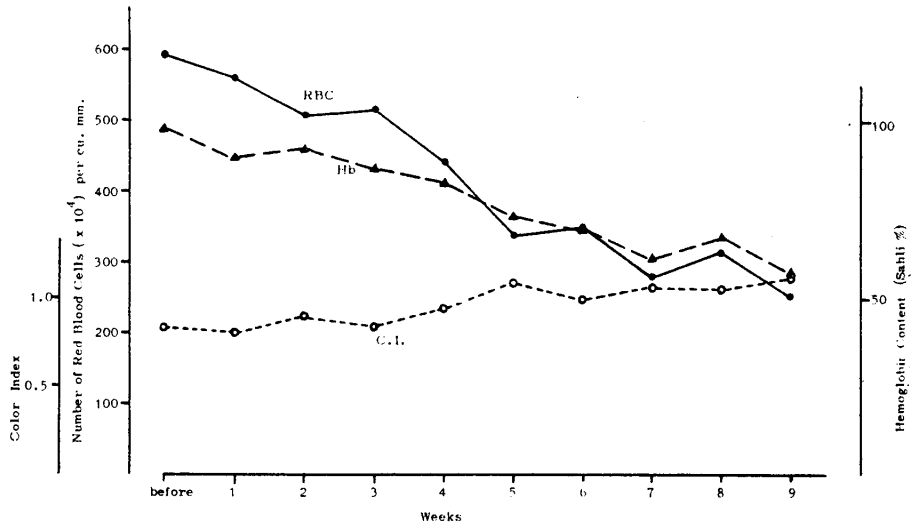


Fig. 2 Changes in number of red blood cells (RBC) and in hemoglobin content (Hb) and color index (C. I.) in the circulating blood of the two anemic rabbits induced by the repeated intravenous injections of India ink for a long time.

that there is not any disturbance in hemoglobin formation. Microscopic observations of bone marrow, liver and spleen showed faded erythropoiesis with RE cells heavily loaded with India ink particles (Figs. 25, 26, 27).

DISCUSSION

For a long time, it has been recognized that in the hematopoietic focus, especially erythropoietic one in bone marrow, there is a unit of erythropoiesis, in which a group of erythroblasts surrounding a reticulum cell called "erythroblastic islet". By the use of electron microscope, BESSIS and coworkers[2,3,4] stressed that in erythroblastic islet erythroblasts took up iron from reticulum cell, demonstrating that ferritin molecules were transferred directly from reticulum cell to erythroblasts by the rhopheocytotic mechanism. Thus a new process in iron metabolism was proposed against the past opinions that iron as the type of transferrin in serum was transferred to erythrons[8,9,10,11]. But it is still problematical whether or not this way of iron uptake is the main pathway of iron to erythroblast, and another question is that the iron supply to erythroid cell would be the only one function of the RE cell situated in the islet. The observations on convalescent stage of phenylhydrazine anemia, in which the formation of erythroblastic islet is stimulated, may be advantageous for the analysis of iron uptake mechanism of erythroblast. And the experiment of RES blockade, where the functions of RES are paralysed, may give some informations for the present question.

Electron microscopic observations proved that ferritin molecules are transferred from reticulum cells to erythroblasts by the rhopheocytotic mechanism of BESSIS. By phenylhydrazine injection reticulum cells phagocytize injured red cells very actively and they contain much of ferritin. But rhopheocytotic vesicles found in normal animal rarely contain ferritin. Reticulocytes synthesize hemoglobin[12,13] and take up iron[8,9,14] without the aid of reticulum cells. Therefore, there seems to be a way of iron uptake for erythroblast other than by rhopheocytosis. Repeated India ink injections result in the blocking of RE-cell function and in severe anemia finally. In this case the color index remained almost unchanged, nearly 1 or higher. This fact means that RES damage induces a suppressed erythroid cell proliferation but not hemoglobin formation, that is, the damaged function of RES has no effect on the iron metabolism of erythroid cells. Increase in color index in final severe stage of anemia will mean the denucleation in an early differentiation stage of erythroblast as precisely studied by SENO and collaborators[15]. All these facts clearly show that the ferritin molecules found in the rhopheocytotic vesicle are nothing but an incidental phenomenon, not showing any important pathway of iron transfer from RES to erythroblast.

An important thing is the suppression of erythroid proliferation and this seems to be closely correlated to the main function of reticulum cell in erythroblastic islet. It is obvious that erythroid proliferation is supported by the increase in proerythrblasts, which will be the first step of erythroid-cell differentiation from

the stem cell. In tissue culture of bone-marrow cells, the young erythroblast can mature to more differentiated stage, but the proliferation of proerythroblast does never occur[16,17,18,19,20]. This fact also shows that the structural configuration of erythroblastic islet is important for the proliferation of proerythroblast and the RE cell may pull the trigger for proliferation of stem cells and their differentiation to proerythroblast. The cellular arrangement in the islet supports this view. As pointed out in the foregoing, young undifferentiated erythroblasts or stem cells are found in close contact with reticulum cell in the central part of islet and more matured cells appear in the outskirt area of islet being isolated from the reticulum cell.

SUMMARY

1. For the purpose to clarify the role of reticuloendothelial cells in the center of erythroblastic islet, the medullary and extramedullary erythropoietic foci in convalescence of phenylhydrazine anemia were observed by light and electron microscopies, and the mode of development of anemia in rabbit having blocked RES.

2. Light microscopic observations revealed the stimulated formation of erythroblastic islet in phenylhydrazine anemia. Electron microscopic observations revealed the rhopheocytotic vesicles in the membrane of erythroblast, some of which contained ferritin particles.

3. Repeated India ink injections induced anemia with RES damage by being heavily laden with soot particles. Color index of these animals was not reduced to the state of hypochromic anemia. Anemia is due to the damage of erythroid-cell reproduction, but not due to the disturbed hemoglobin metabolism by the inhibited iron uptake.

4. From the results obtained the author concludes that the role of reticuloendothelial cell in islet is not to transfer ferritin to erythroblast but the reproduction of erythroid cells.

REFERENCES

1. MATSUOKA, K.: Studies on reticuloendothelial system and hematopoiesis. I. Studies of extramdullary hematopoiesis. *Acta Med. Okayama* 19, 107, 1965
2. BESSIS, M. and BRETON-GORIUS, J.: Ferritin and ferruginous micelles in normal erythroblasts and hypochromic hypersideremic anemias. *Blood* 14, 423, 1959
3. BESSIS, M. and BRETON-GORIUS, J.: Iron metabolism in the bone marrow as seen by electron microscopy; A critical review. *Blood* 19, 635, 1962
4. BESSIS, M.: The erythroblastic islet. The Ultrastructure of Cells. Sandoz Monographs.
5. SABATINI, D. D., BENSCH, K. and BARRNETT, R. J.: Cytochemistry and electron microscopy. The preservation of cellular ultrastructure and enzymatic activity by aldehyde fixation. *J. Cell Biol* 17, 19, 1963
6. LUFT, J. H.: Permanganate — A new fixative for electron microscopy. *J. Biophys. Biochem.*

Cytol. 2, 799, 1956

7. MARUYAMA, K.: Behavior of membrane system in the cell during cell divisions in microsporogenesis in *Tradescantia paludosa. Members of the Colledge of Science, University of Kyoto.* Series B, 30, 9, 1963
8. ALLEN, D. W. and JANDLE, J. H.: Kinetics of intracellular iron in rabbit reticulocytes. *Blood* 15, 71, 1960
9. JANDLE, J. H. and KATZE, J. H.: The plasma-to-cell cycle of transferrin. *J. Clin. Invest.* 42, 314, 1963
10. POLLYCOVE, M. and MORTIMER, R.: The quantitative determination of iron kinetics and hemoglobin synthesis in human subjects. *J. Clin. Invest.* 40, 753, 1961
11. POLLYCOVE, M. and MAQSOOD, M.: Existence of an erythropoietic labile iron pool in animals. *Nature* 164, 152, 1962
12. SENO, S.: The structure and the function of reticulocyte. *Acta haematologica Japonica* 21, 351, 1958
13. KRUH, J. and BORSOOK, H.: Hemoglobin synthesis in rabbit reticulocytes *in vitro. J. Biol. Chem.* 220, 905, 1956
14. AWAI, M.: unpublished communication.
15. SENO, S., MIYAHARA, M., ASAKURA, H., OCHI, O., MATSUOKA, K. and TOYAMA, T.: Macrocytosis resulting from early denucleation of erythroid precursors. *Blood* 24 582, 1964
16. ASTALDI, G. and TOLENTINO, P.: Studies *in vitro* on the maturation of erythroblasts in normal and pathological conditions. *J. Clin. Path.* 2, 217, 1949
17. ASTALDI, G.: Differentiation, proliferation and maturation of haematopoietic cells studied in tissue culture. *Ciba Foundation Symposium on Haematopoiesis.* p. 99. Churchill, London, 1960
18. LAJTHA, L. G.: Culture of human bone marrow *in vitro.* The reversibility between normoblastic and megaloblastic series of cells. *J. Clin Path.* 5, 67, 1952
19. BERMAN, L., STULBERG, C. S. and RUDDLE, F. H.: Long-term tissue culture of human bone marrow. I. Report of isolation of a strain of cells resembling epithelial cells from bone marrow of a patient with carcinoma of the lung. *Blood* 10, 896, 1955
20. SHIBATA, T.: Studies on erythroipoesis. I. A study on cell size of erythroid cells from anemic animal. *Acta Med. Okayama* 18, 119, 1964

EXPLANATIONS

Fig. 3 Section of bone marrow, showing very active erythropoiesis in the anemic rabbit induced by phenylhydrazine injection. ×130

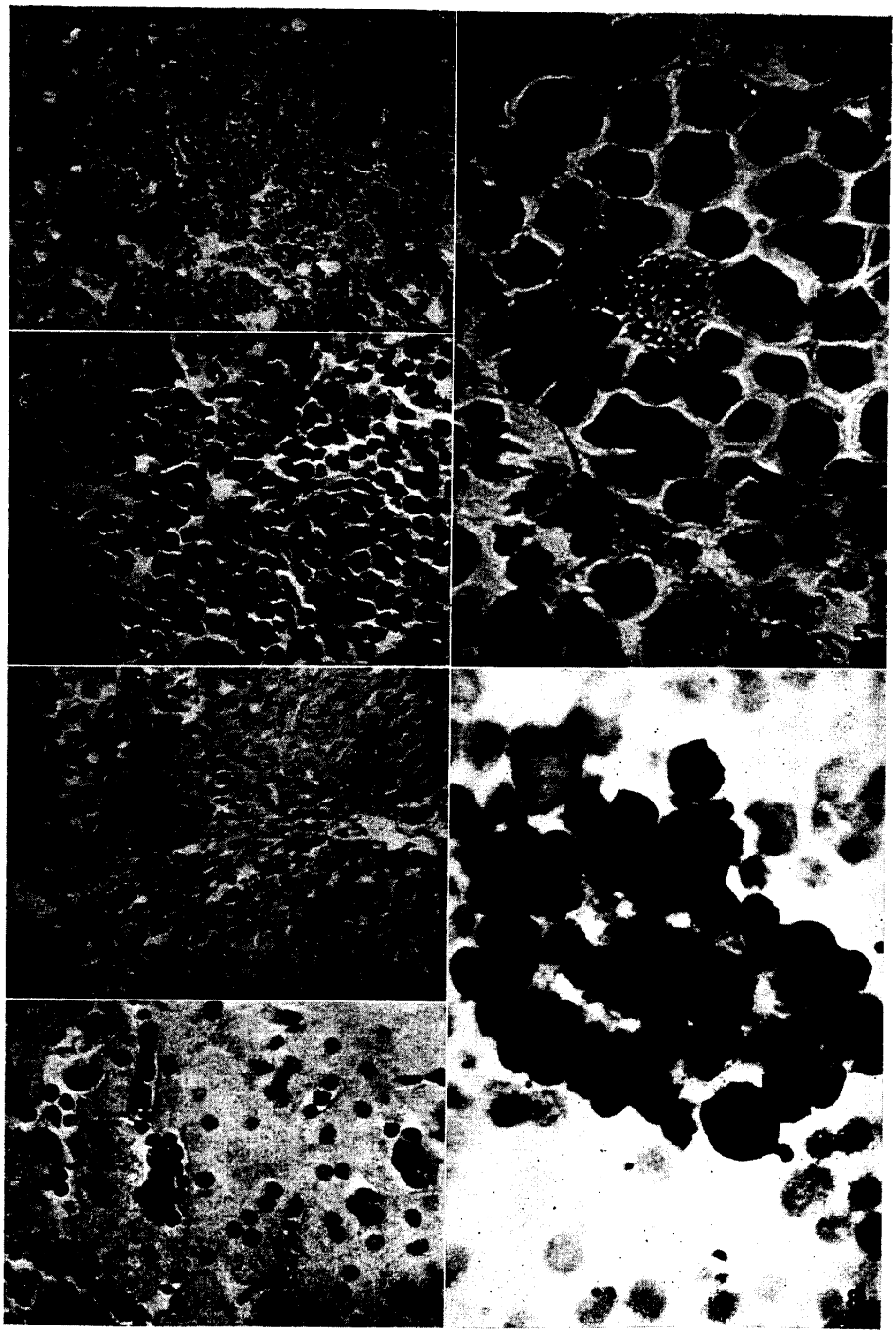
Fig. 4 Higher magnification of the above, distinctly illustrating erythroblastic islets. ×400

Fig. 5 The still higher magnification of Fig. 3, showing the detail of erythroblastic islet. In the center of the islet there exist a reticulum cell and a relatively large sized erythroblast, suggestive of younger one being in contact with it. In peripheral area, small sized erythroblasts, suggestive of being more mature, are observed. ×1,300

Fig. 6 Section through the liver of the same material as the above, showing extramedullary erythropoiesis. Parenchymal cells are almost normal. ×120

Fig. 7 Higher magnification of Fig. 6, illustrating erythroblastic islets. ×400

Fig. 8 The imprinted preparation of the same liver, showing the islet separately. In the center of the picture there is a Kupffer cell containing hemosiderin and other substances, and many erythroblasts are massively in contact with it. ×1,000



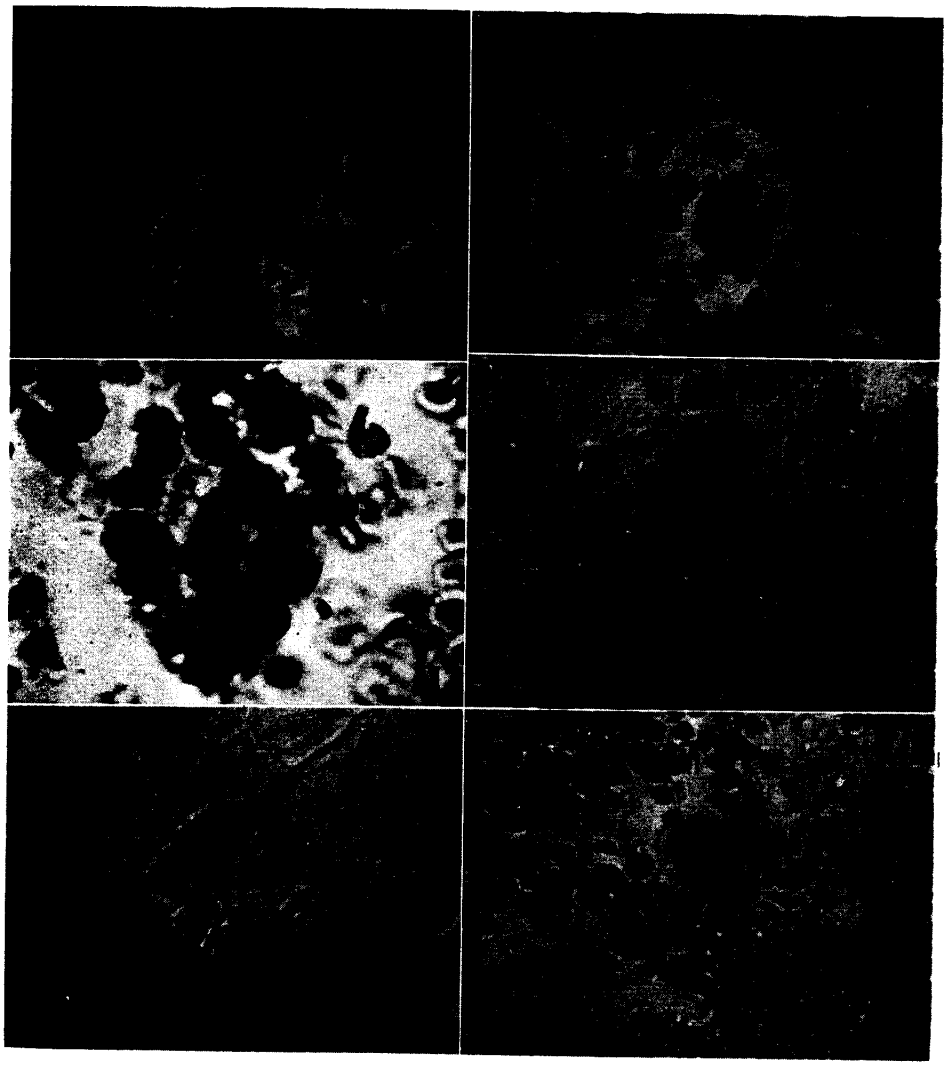


Fig. 9 Section through the spleen of the same material, showing many erythroblasts in the sinusoids of red pulp. ×120

Fig. 10 Higher magnification of Fig. 9, illustrating the erythroblastic islet in the sinusoid. ×470

Fig. 11 The still higher magnification of the islet of the spleen, showing the identical picture of liver and bone marrow. ×1,300

Figs. 12, 13, 14 Berlin blue reaction of the sections through bone marrow, liver and spleen, showing the reticulum cell in the islet positively stained. ×600

Figs. 15-24 Electron microscopic pictures.

Fig. 15 Bone marrow of anemic rabbit induced by phenylydrazine. In the center of the picture reticulum cell is observed. ×6,500

Fig. 16 Erythroblastic islet in bone marrow of the same material. Reticulum cell (RES) phagocytizes electron dense materials. Erythroblasts are in a close contact with the former. ×7,500

Fig. 17 Erythroblastic islet in spleen of the same animal. Completely identical picture is observed in bone marrow as well. ×6,500

Fig. 18 Higher magnification illustrating the contact of reticulum cell (RES) with erythroblast (Erb). Pinocytotic vesicles (p) are seen in the cytoplasmic surface of erythroblast and reticulum cell. n: nucleus, m: mitochondria of the erythroblast. ×30,000

Fig. 19 Pinocytotic or rhopheocytotic vesicles (p) of the erythroblast are illustrated. ×50,000

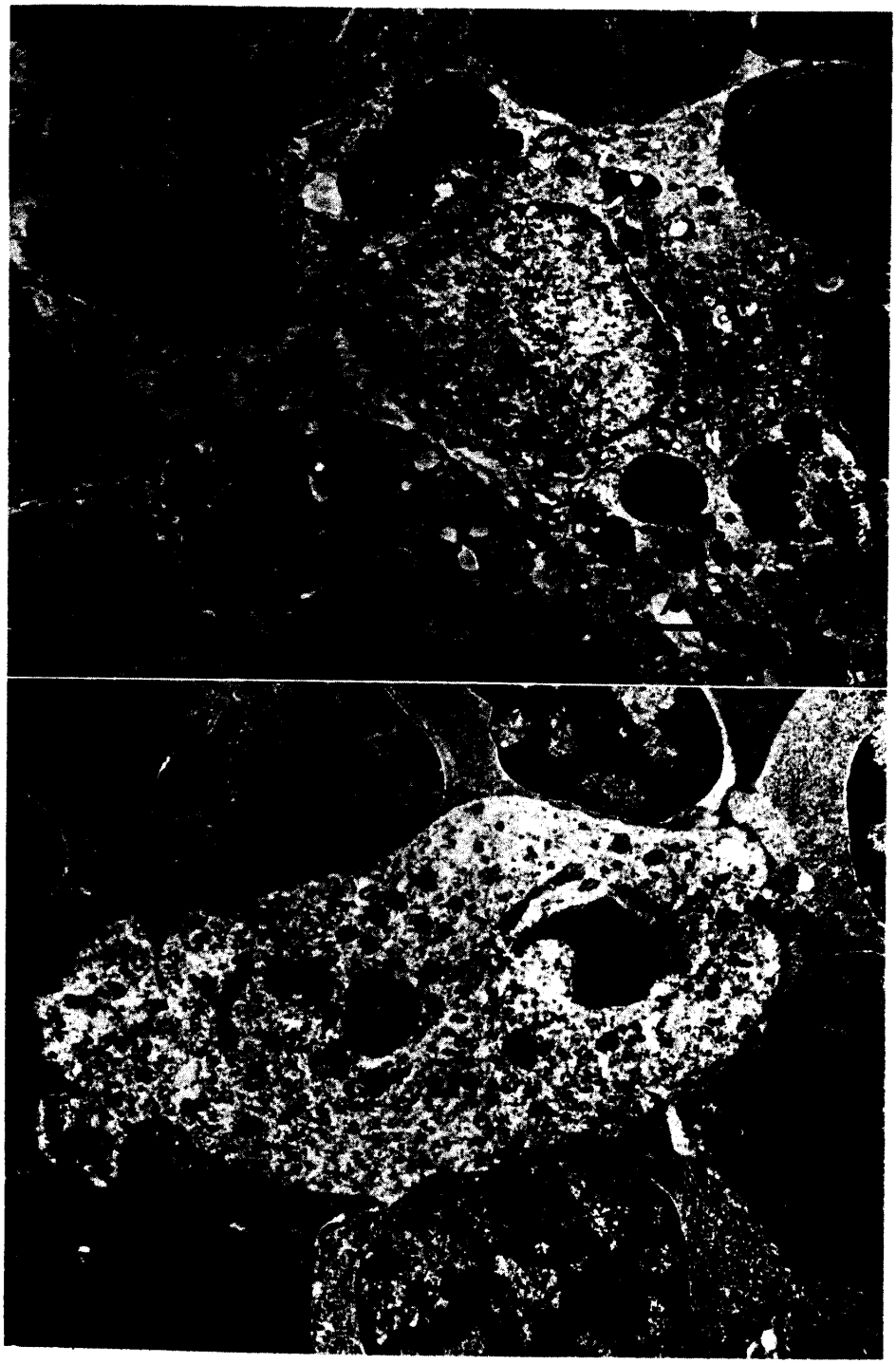
Fig. 20 Higher magnification of rhopheocytotic vesicles (p), showing ferritin molecules (f) in the vesicle and intercellular space. ×115,000

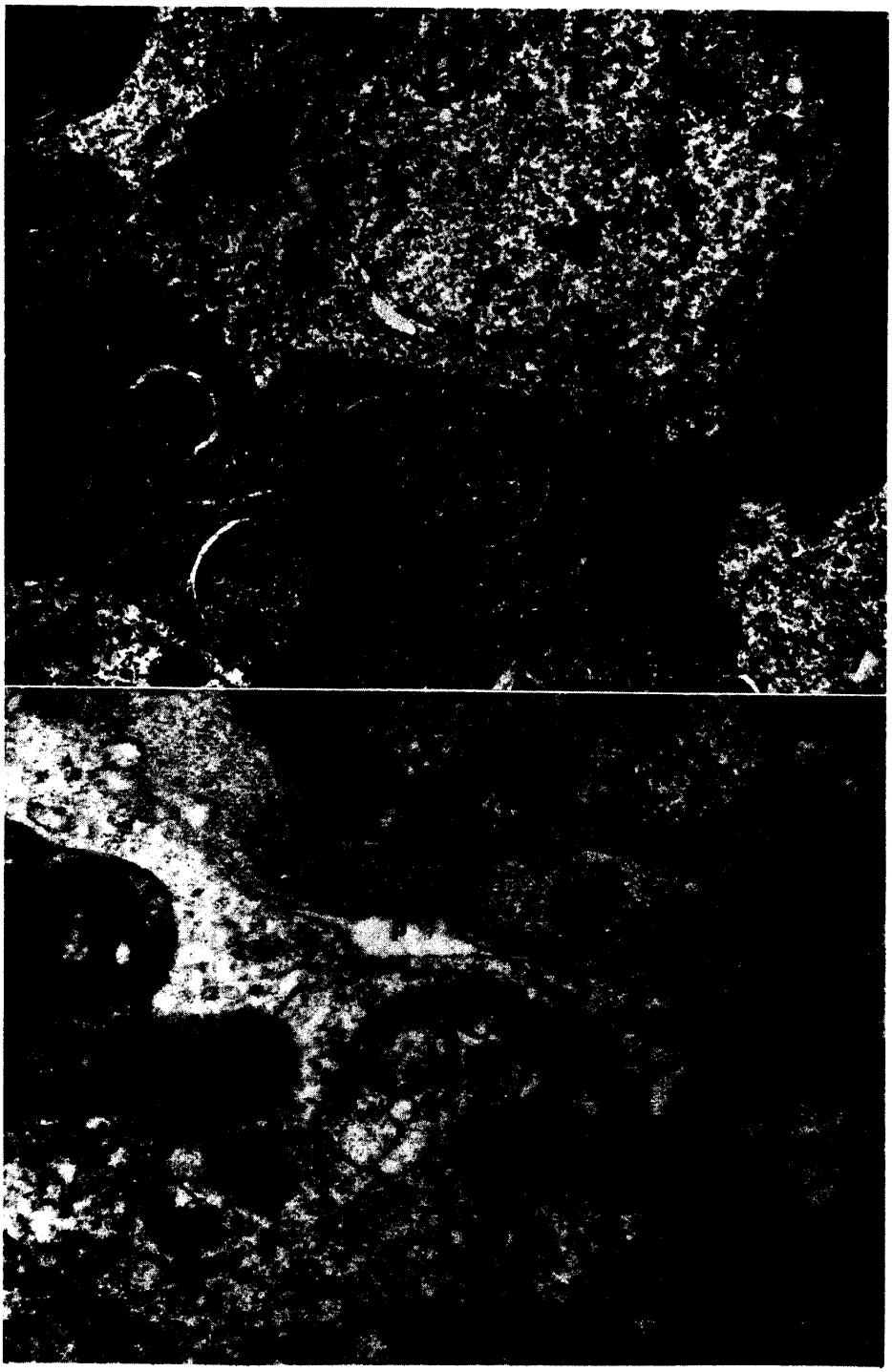
Fig. 21 Erythroblast in the islet of spleen, showing many aggregated ferritin particles in the cytoplasm. ×30,000

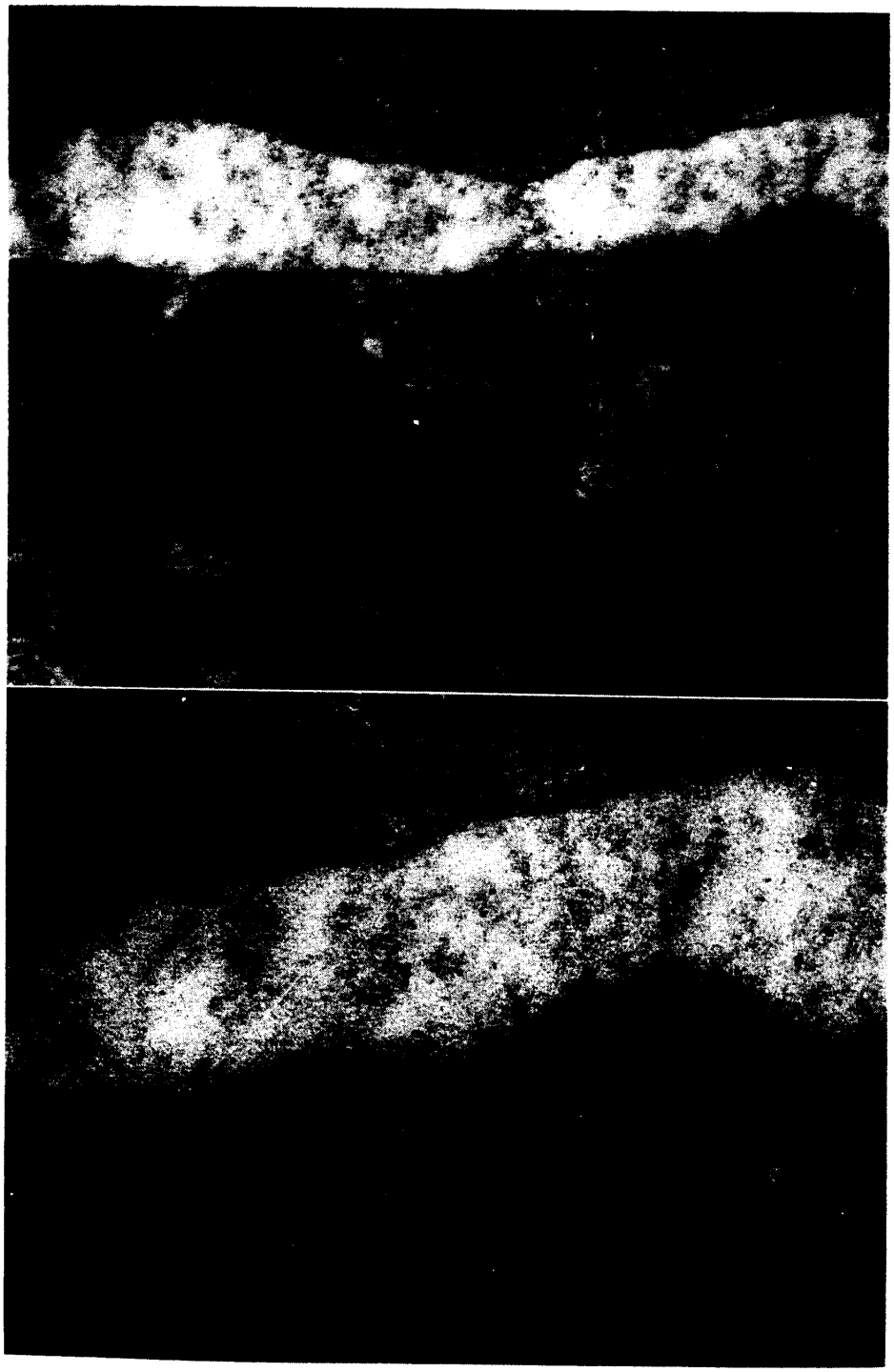
Fig. 22 Higher magnification of the above, illustrating ferritin particles (f) containing vesicular structure, so-called siderosome in the cytoplasm. ×115,000

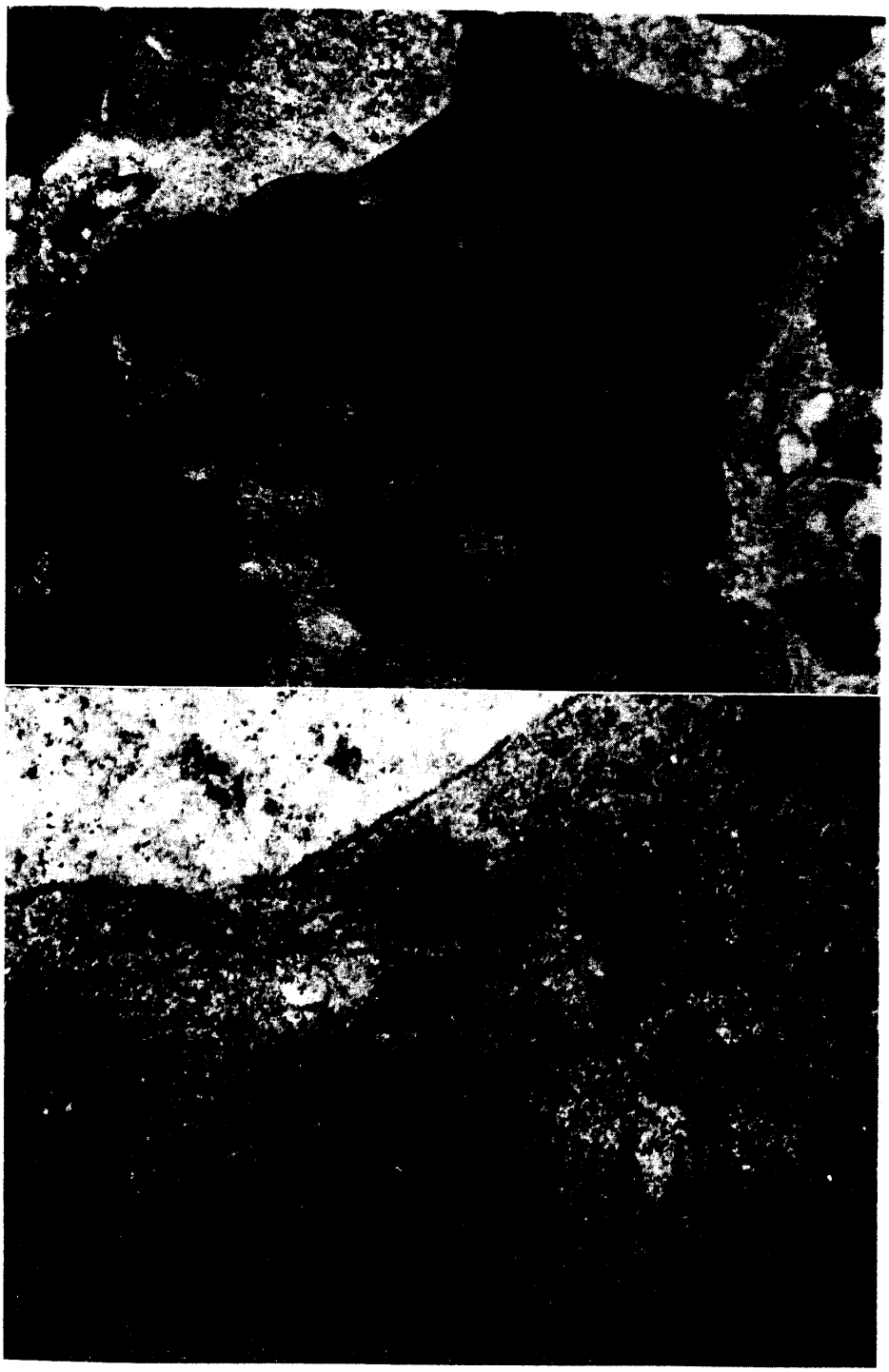
Fig. 23 Section of the islet in bone marrow fixed with $KMnO_4$, showing relation between reticulum cell (RES) and erythroblasts (Erb) and ferritin particles (f) in the membrane system of the cytoplasm of erythroblast. n: nucleus, m: mitochondria. ×55,000

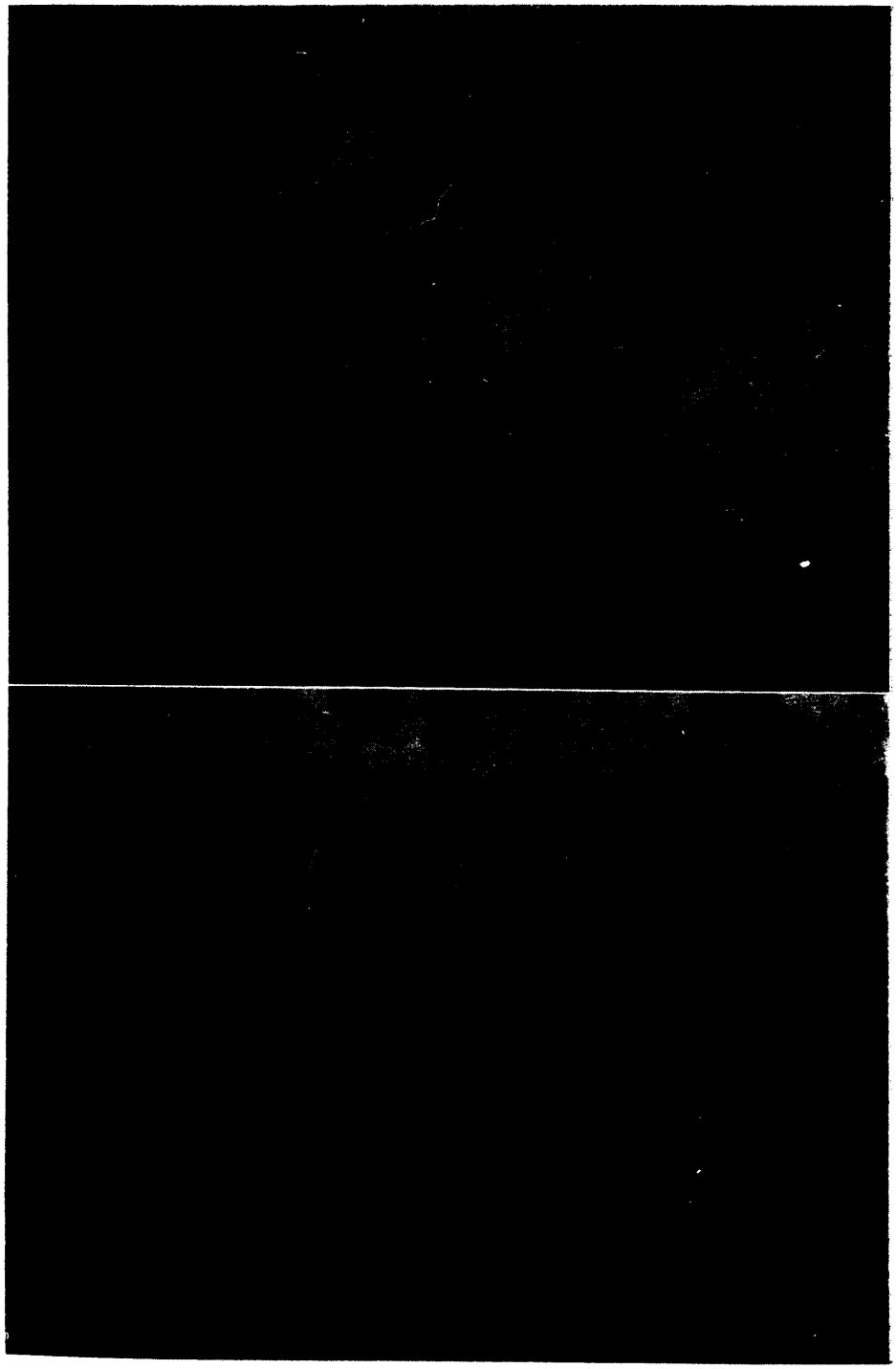
Fig. 24 Another section of the same material as in Fig. 23. ×35,000











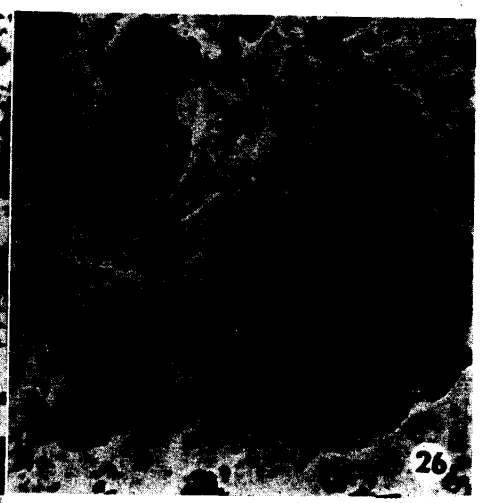


Fig. 25 Section through bone marrow of RES-blocked rabbit, showing RE cells heavily laden with India ink particles. Hematopoietic structure is severely destroyed. ×150

Fig. 26 Section through liver of the same animal as the above. Kupffer cells are predominantly swollen by phagocytizing many carbon particles. ×470

Fig. 27 Section through spleen of the same animal. Reticulum cells show an identical picture as bone marrow and spleen. In sinusoid of red pulp, some bone-marrow cells are dispersed. ×470