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ULTRASTRUCTURE OF BONE MARROW OF RATS AFTER SEVERE HEMODILUTION WITH STARCH OR MODIFIED HEMOGLOBIN

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Abstract

The ultrastructure of bone marrow of rats was studied 24 h after exchange-transfusion with solutions of starch or modified hemoglobin to a hematocrit of 10-15. Blood smears of the transfused rats had 17-20% reticulocytes as compared to 5-6% for sham operated controls. In the transfused rats marrow macrophages had numerous heterolysosomes apparently containing the starch or hemoglobin from the transfused solutions. Endothelial cells and reticular cells also possessed a few heterolysosomes thought to contain starch or hemoglobin. Reticular cells of the transfused rats contained numerous glycogen particles scattered throughout the cytoplasm or arranged in large masses. Synthesis of glycogen may indicate a metabolic change in reticular cells in response to tissue hypoxia induced by the exchange-transfusion procedure.

KEY WORDS: Bone marrow, ultrastructure, stromal cells, reticular cells, glycogen, heterolysosomes.

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Introduction

Several investigators have presented evidence that the stromal cells of bone marrow form the microenvironment necessary for hematopoiesis (see Dexter, 1982; Bentley, 1982). The stromal cells, usually described as consisting of reticular cells and macrophages (Weiss, 1976), have numerous, long processes that extend among the developing blood cells (Weiss and Chen, 1975; Weiss, 1976). Some of the reticular cells, referred to as adventitial cells, lie adjacent to the sinusoids where they form an incomplete cover upon the endothelium. During states of increased blood cell delivery to the sinusoids, the extent of the adventitial cell cover is reduced (Weiss, 1965, 1970; Chamberlain et al., 1975a; Leblond et al., 1975), suggesting that the adventitial cells help regulate the migration of blood cells across the sinusoidal wall. Dark branching stromal cells have recently been described in association with developing blood cells during heightened eosinophilopoiesis (Sakai et al., 1981) and erythropoiesis (Brookoff et al., 1982). These dark cells, probably arising from reticular cells, appear to be a modification of the stroma in response to intense hematopoietic activity. Since the stromal cells are so intimately involved in hematopoiesis, changes in these cells during altered hematopoietic states are of considerable interest.

During experiments to determine the effects of hemodilution on liver oxygen supply and ultrastructure, it became apparent that an investigation of the marrow would also be of interest. Since changes in the liver indicated insufficient oxygen supply, it was felt this model could be useful for studying short-term responses, if any, of marrow stromal cells to diminished oxygen availability. This study was undertaken to determine possible ultrastructural changes in cells of the bone marrow of rats within 24 hours, following replacement of blood with isotonic 6% hydroxyethylstarch or with a 10-12% solution of modified hemoglobin.

Materials and Methods

Male Sprague-Dawley rats weighing 170 to 280 g were anesthetized and cannulated in a carotid artery. Using a stepwise procedure, blood was withdrawn in 2 ml volumes and replaced with similar amounts of either 10-12% pyridoxalated polymerized hemoglobin (DeVenuto and Zegna, 1983) or with isotonic 6% hydroxyethylstarch (Hespan, American Critical Care) until the hematocrit was 10-12 for the hemoglobin and 14-15 for the starch transfused rats. Controls were anesthetized and cannulated but not hemodiluted. Each group consisted of three rats. Following the surgical procedure, rats were placed in warmed cages with food and water and left to recover. After 24 h animals were again anesthetized, the hematocrit was determined (microsample), blood smears were made, the thoracic aorta was cannulated, the inferior vena cava cut, and the vascular system was flushed with isotonic Krebs' buffer containing one unit of heparin per 100 ml. When the vascular bed cleared, 100 ml of fixative (2% glutaraldehyde in 0.1 M phosphate) was infused into the aorta. Pieces of femoral marrow were removed, cut into blocks, fixed an additional hour in 4% glutaraldehyde in 0.1 M phosphate, rinsed overnight in buffer, osmicated, and embedded in Polybed. Since we had previously noted that liver macrophages of these animals contained phagocytized starch or hemoglobin, blocks of liver were similarly processed to compare marrow and liver macrophages. Blood smears were stained with new methylene blue (Brecher, 1949) for reticulocytes. Thick sections were stained with azure II. Thin sections were stained with uranyl acetate and lead citrate and examined in a Philips EM 200 electron microscope operating at 60 kV.

Results

Reticulocyte counts were 5-6% in controls, 18-20% in starch transfused rats, and 17-19% in hemoglobin transfused rats. The hematocrit was 17.5 ± 0.9 for the starch transfused rats and 15.0 ± 0.9 for the hemoglobin transfused rats. Light microscopic examination of marrow sections stained with azure II showed an increased number of heterolysosomes in marrow macrophages of the transfused rats as compared to the controls, but otherwise the marrow of the transfused rats appeared normal.

Ultrastructural study of the bone marrow of the transfused rats showed several features consistent with the reticulocytosis noted in the blood smears. Marrow sinusoids were largely empty as a result of vascular perfusion, but among the few remaining cells many reticulocytes were noted. Reticulocytes were numerous in the extravascular space near the sinusoids and several were encountered migrating through pores in endothelial cells.

Macrophages within the marrow cords were scattered about in a fashion similar to that of controls, but they contained more heterolysosomes. In the starch transfused rats some heterolysosomes contained fragments of erythroblast nuclei or other cellular debris similar to that of controls, but others were largely filled with electron lucent material. Electron dense material when present was typically located at the periphery of these structures (fig. 1). The dense material at the periphery of the heterolysosomes appeared to be of lysosomal origin, because lysosomes were occasionally noted fusing with them (arrow, fig. 1). Similar heterolysosomes were observed in Kupffer cells of the liver of these rats (fig. 3A), but heterolysosomes having this unusual structure were not seen in marrow macrophages of controls. In the hemoglobin transfused rats (fig. 2), some heterolysosomes of marrow macrophages were also similar to those of controls, but others more closely resembled the heterolysosomes of Kupffer cells of these animals (fig. 3B). In the transfused rats, particularly those transfused with starch, the cytoplasm of marrow macrophages was often packed with heterolysosomes, but the macrophages still had numerous processes extending among the developing blood cells. Also, no concentration of macrophages along the sinusoidal wall was observed.

Reticular cells of marrow from the tranfused rats were distributed throughout the marrow as in controls; some had long processes extending among the developing blood cells, others had extensions that partially covered the abluminal surface of endothelial cells. Many of the reticular cells, however, contained cytoplasmic inclusions not present in reticular cells of controls. The inclusions were of two types: heterolysosomes similar to those of the macrophages and electron dense granules measuring about 40 nm in diameter. The size, shape and electron density of the granules indicated they were glycogen. Within the marrow cords, glycogen was present in reticular cells of both starch (fig. 4) and hemoglobin (fig. 5) transfused rats. The adventitial reticular cells often contained particularly large amounts of glycogen (figs. 6, 7). The glycogen was scattered about in the cytoplasm as single particles, small rosettes, or in large masses, and seemed to have no constant relationship to endoplasmic reticulum or other cytoplasmic organelles. Within the large masses, the glycogen particles sometimes formed scroll-like configurations (arrow, fig. 6). Frequently, vacuoles were observed near or within the glycogen masses (figs. 4, 5, 6).

Other elements of the marrow, such as granulocytic cells, eythroid cells, and macrophages, did not show accumulations of glycogen. Particles of glycogen were occasionally observed in various cells of the transfused rats, but they consisted of only a few scattered particles and resembled those sometimes seen in marrow cells of control animals. The reticular cells were the only

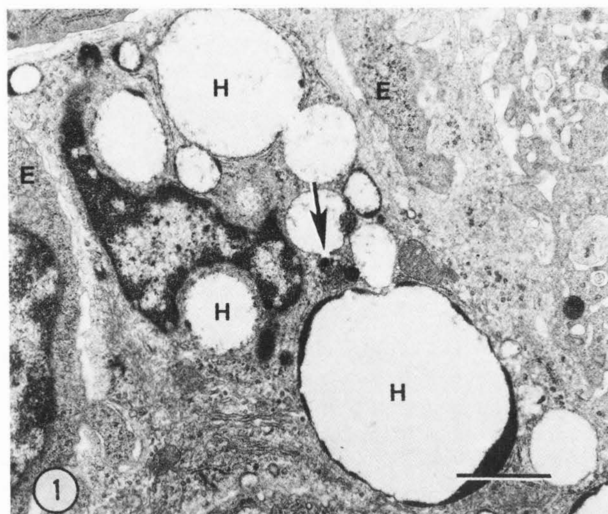

Figure 1. A macrophage from a starch transfused rat. Erythroblasts (E) and heterolysosomes (H) of the macrophage are labeled. Note fusion of a lysosome with a heterolysosome (arrow). Bar = 1 μm.

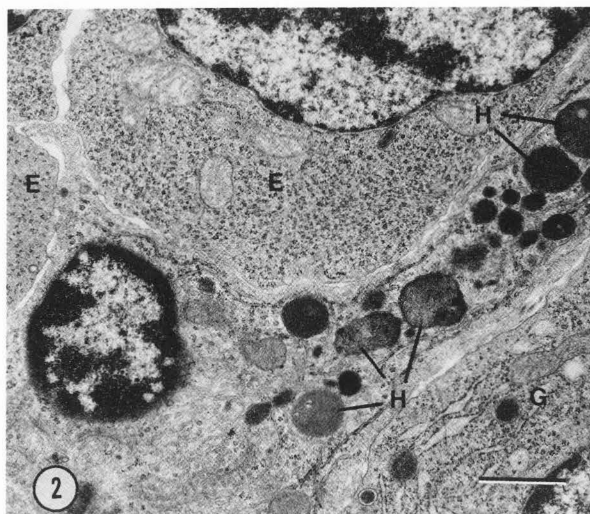

Figure 2. A macrophage from a hemoglobin transfused rat. Erythroblasts (E), a granulocyte (G) and heterolysosomes (H) of the macrophage are labeled. Bar = 1 μm.

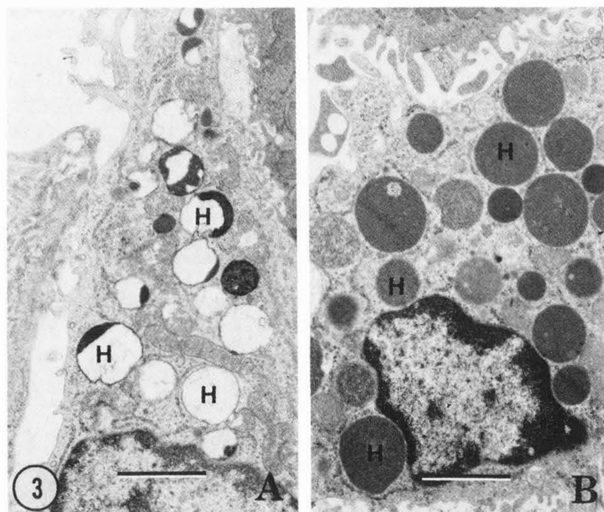

Figure 3. Kupffer cells from starch (A) and hemoglobin (B) transfused rats. Heterolysosomes (H) are labeled. Bar = 1 μm.

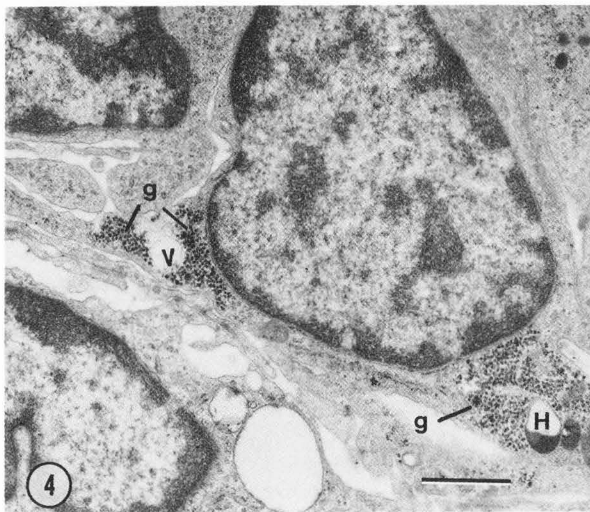

Figure 4. A reticular cell from marrow of a starch transfused rat containing glycogen particles (g), a heterolysosome (H) and a vacuole (V). Bar = 1 μm.

cells containing large numbers or masses of glycogen particles.

A few heterolysosomes were present in reticular cells of the transfused rats. In the starch transfused rats (fig. 4), the heterolysosomes were similar to those of macrophages described above, and thus appeared to contain starch. The identity of the heterolysosomal material was less certain in reticular cells of the hemoglobin transfused rats (fig. 7), but it did resemble the apparent hemoglobin of heterolysosomes seen in liver and marrow macrophages of these animals.

In the transfused rats the endothelial cells also possessed a few heterolysosomes (fig. 6), apparently containing hemoglobin or starch. The endothelial cells had numerous coated pits and coated vesicles (fig. 7) along their luminal surface, but starch or hemoglobin was not identified in these structures. Glycogen was occasionally present in endothelial cells of the transfused rats, but it consisted of only a few particles.

Discussion

In this study blood smears from the transfused rats had an increased number of reticulocytes as compared to controls. The

electron microscopic studies showed numerous reticulocytes near sinusoids, migrating across the sinusoidal wall, and in the lumina of sinusoids. These observations indicated that the marrow was releasing large numbers of reticulocytes. Indeed, the reticulocytosis observed at 24 h was intense compared to values given by other investigators. For example, Mel et al. (1977) reported 10% reticulocytes in blood of rats three days after removal of blood; Tavassoli (1977) reported 4-5% reticulocytes in the blood of rabbits 24 h after phlebotomy; and Chamberlain et al. (1975b) recorded 13% reticulocytes at 41 h following administration of erythropoietin. In the present study the marked reticulocytosis (17-20%) was likely due to the low hematocrit (10-15) and anemia. The latter condition was induced most rapidly in the starch transfused rats, while it developed more slowly in the hemoglobin transfused group. The reticulocytosis is viewed as related to tissue hypoxia. Support for this comes from measurements of oxygen tension in the liver of these animals showing severe hypoxia (Rink and Campbell, submitted). It therefore seemed likely that other tissues including the bone marrow were hypoxic. The reticulocytosis noted here would thus appear to be related to tissue hypoxia.

The histochemical studies of Wetzel et al. (1967), Weston and Bainton (1979), and Beckstead and Bainton (1980) have demonstrated lysosomal enzymes in marrow macrophages. Also, marrow macrophages typically contain phagocytized material much of which appears to be cellular debris (Berman, 1967). It is not surprising then that marrow macrophages appeared to contain the starch or hemoglobin used in exchange-transfusion. In the starch transfused rats some of the heterolysosomes had an unusual ultrastructure and resembled those of Kupffer cells of these animals. It was therefore apparent that marrow macrophages of these rats had engulfed large amounts of starch. In the hemoglobin transfused rats, the heterolysosomes of marrow macrophages were more like those of controls, but many resembled those of Kupffer cells. It seemed likely that many of these heterolysosomes contained hemoglobin from the solution used in transfusion. During intense phagocytic activity marrow macrophages might be expected to lose their normal associations with the developing blood cells, but examination of the macrophages in this study showed that they did not.

The Kupffer cells of the liver were in direct contact with the substances transfused into the blood of these rats, so numerous heterolysosomes containing hemoglobin or starch were anticipated. Since nearly all marrow macrophages lie in the extravascular space, substances contained in the blood would have to move into the extravascular space to reach the macrophages. Therefore, it was somewhat surprising to find such a large number of heterolysosomes, apparently containing starch or hemoglobin, in macrophages of the marrow. This observation indicated that the starch and hemoglobin were transported rapidly across the endothelium, but how this transport occurred was not apparent.

Several investigators (Bankston and DeBruyn, 1974; DeBruyn et al, 1975, 1985; Soda and Tavassoli, 1984) have shown that coated pits and vesicles of marrow endothelial cells are involved in uptake of substances from the sinusoidal lumina, but whether this uptake is an integral part of transport across the endothelium is controversial. The studies of Bankston and DeBruyn (1974) indicate that transendothelial transport of carbon particles occurs through diaphragms of endothelial fenestrae, but Soda and Tavassoli (1984) believe coated vesicles are associated with transendothelial transport of iron-transferrin complex by marrow endothelial cells. In the present study, coated vesicles and pits were presumably involved in uptake of hemoglobin and starch, but these substances were not identified in these pits or vesicles. The starch is poorly visualized and little of the transfused hemoglobin remained in the blood after 24 h (Rink and Campbell, submitted). Starch or hemoglobin did appear to be present in heterolysosomes of the endothelial cells, indicating that the endothelial cells were enzymatically degrading some of the starch or hemoglobin. This observation is consistent with findings of DeBruyn et al. (1975) and Weston and Bainton (1979) demonstrating lysosomal enzymes in endothelial cells of the marrow.

Probably the most interesting observation of this study was the accumulation of many glycogen particles in reticular cells of the transfused rats. Glycogen was observed in reticular cells of the marrow cords and in adventitial reticular cells. Glycogen appeared to be synthesized by the reticular cells for several reasons. To begin with, no glycogen was present in the solutions used for transfusion, thus eliminating the possibility that the reticular cells contained exogenous glycogen. Secondly, no evidence was observed for phagocytosis of glycogen by reticular cells. Thirdly, the ultrastructure of the large glycogen masses closely resembled that of cells that are synthesizing glycogen (see Robinson et al., 1982; Rikihisa, 1984). Finally, the vacuoles associated with the glycogen masses seen here appeared similar to those observed in other glycogen synthesizing cells (see Eyal-Giladi et al., 1979). Glycogen was not observed in all reticular cells examined, but it is uncertain whether this was due to the plane of section, or whether some reticular cells were not synthesizing glycogen.

Glycogen synthesis has been studied extensively in liver where it occurs in close association with the smooth endoplasmic reticulum (reviewed by Cardell, 1977). In cell types other than liver, glycogen synthesis may be associated with the endoplasmic reticulum and intermediate filaments (Rybicka, 1981), or may occur free in the cytoplasm unrelated to the endoplasmic reticulum

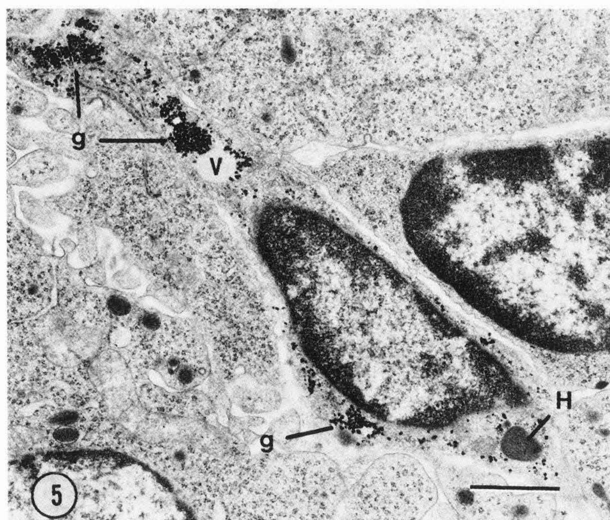

Figure 5. A reticular cell from the bone marrow of a hemoglobin transfused rat containing glycogen particles (g), a vacuole (V), and a heterolysosome (H). Bar = 1 μm.

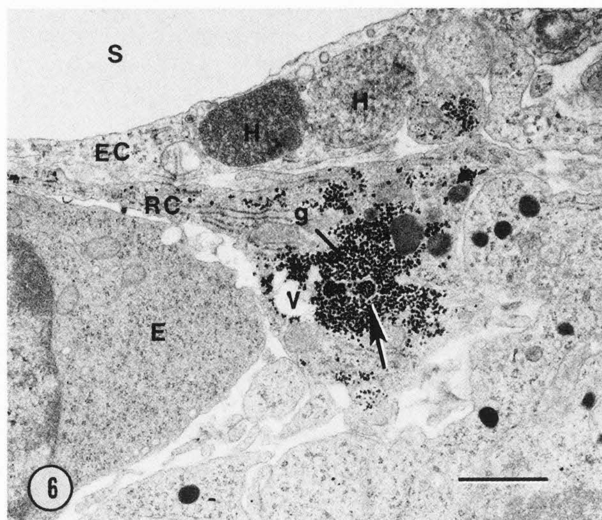

Figure 6. An adventitial reticular cell (RC) from the marrow of a hemoglobin transfused rat containing a large mass of glycogen particles (g). Within the glycogen mass, some glycogen particles form a scroll-like figure (arrow). A vacuole (V) lies near the glycogen mass. An erythroblast (E), an endothelial cell (EC) and the lumen of a sinusoid (S) are labeled. The endothelial cell contains two heterolysosomes (H). Bar = 1 μm.

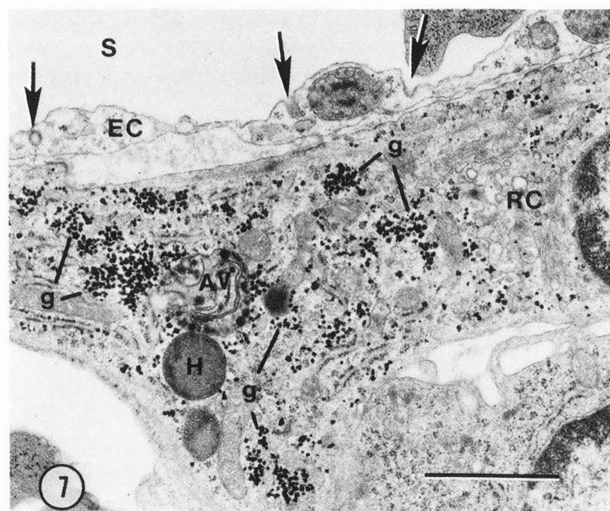

Figure 7. An adventitial reticular cell (RC) from marrow of a hemoglobin transfused rat containing glycogen granules (g), a heterolysosome (H) and an autophagic vacuole (AV). The endothelial cell (EC) of a sinusoid (S) has several coated pits and vesicles (arrows). Bar = 1 μm.

(Takeuchi, 1983). In the present study smooth endoplasmic reticulum was observed in reticular cells but the glycogen had no apparent association with this organelle.

Glycogen is not commonly seen in reticular cells; to the authors' knowledge glycogen has not been previously reported in reticular cells, and none was observed in reticular cells of controls. Why reticular cells of this study synthesized large amounts of glycogen is an interesting question that may be answered in part by consideration of glycogen synthesis by other cells. Large masses of newly synthesized glycogen are observed in neutrophils elicited into the peritoneal cavity (Robinson et al., 1982; Rikihisa, 1984). Neutrophils in many tissues, including the bone marrow, synthesize large amounts of glycogen following administration of endotoxin (Chen and Bryant, 1984). According to Monos et al. (1984), splenic lymphocytes stimulated by mitogen synthesize large quantities of glycogen. Monocytes activated by attachment to a substratum have an increased amount of glycogen compared to unattached monocytes (Lazdins et al., 1980). In all these reports cells are synthesizing and storing glycogen in response to altered environmental or physiologic conditions. These conditions may alter the metabolism of these cells. In the present study it seems reasonable that accumulation of glycogen is a result of metabolic changes in the reticular cells. As pointed out by Monos et al. (1984) certain cells probably store glycogen for energy needed for future functions. Synthesis of glycogen by reticular cells reported here would appear to be triggered by hypoxia, but how synthesis and storage of glycogen by these cells is related to their functions in hematopoiesis is unknown.

Heterolysosomes observed in reticular cells of the present study appeared to contain tranfused starch or hemoglobin. This observation was of interest because only

occasionally have lysosomal enzymes been reported in these cells (Burgio et al., 1984). However, the small number of heterolysosomes in these cells indicated that they have limited phagocytic activity.

Acknowledgments

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References

Bankston PW, DeBruyn PPH. (1974) The permeability to carbon of the sinusoidal lining cells of the embryonic rat liver and rat bone marrow. Amer. J. Anat., 141:281-290.

Beckstead JH, Bainton DF. (1980) Enzyme histochemistry on bone marrow biopsies: reactions useful in the differential diagnosis of leukemia and lymphoma applied to 2-micron plastic sections. Blood, 55:386-394.

Bentley SA. (1982) Bone marrow connective tissue and the haemopoietic microenvironment. Br. J. Haematol., 50:1-6.

Berman I. (1967) The ultrastructure of erythroblastic islands and reticular cells in mouse bone marrow. J. Ultrastr. Res., 17: 291-313.

Brecher G. (1949). New methylene blue as a reticulocyte stain. Amer. J. Clin. Pathol., 19:895-896.

Brookoff D, Maggio-Price L, Bernstein S, Weiss L. (1982) Erythropoiesis in ha/ha and sph/sph mice, mutants which produce spectrin-deficient erythrocytes. Blood, 59:646-651.

Burgio VL, Magrini U, Ciardelli L, Pezzoni G. (1984). An enzyme-histochemical approach to the study of the human bone-marrow stroma. Acta Haematol., 71:73-80.

Cardell Jr. RR. (1977) Smooth endoplasmic reticulum in rat hepatocytes during glycogen deposition and depletion. Intern. Rev. Cytol., 48:221-279.

Chamberlain JK, Leblond PF, Weed RI. (1975a) Reduction of adventitial cell cover: an early direct effect of erythropoietin on bone marrow ultrastructure. Blood Cells, 1:655-674.

Chamberlain JK, Weiss L, Weed RI. (1975b) Bone marrow sinus cell packing: a determinant of cell release. Blood,46:91-102.

Chen LT, Bryant MW. (1984) Increased glycogen particles in neutrophils in endotoxin-treated rats. Circ. Shock, 13:295-305.

DeBruyn PPH, Michelson S, Bankston PW. (1985) In-vivo endocytosis by bristle-coated pits and intracellular transport of endogenous albumin in the endothelium of the sinuses of liver and bone marrow. Cell Tissue Res. 240:1-7.

DeBruyn PPH, Michelson S, Becker RP. (1975) Endocytosis, transfer tubules and lysosomal activity in myeloid sinusoidal endothelium. J. Ultrastr. Res., 53:133-151.

DeVenuto F, Zegna A. (1983) Preparation and evaluation of pyridoxalated-polymerized human hemoglobin. J. Surg. Res., 34:205-212.

Dexter TM (1982) Stromal cell associated haemopoiesis. J. Cell. Physiol., Suppl. 1: 87-94.

Eyal-Giladi H, Raveh D, Feinstein N, Friedlander M. (1979) Glycogen metabolism in the prelaid chick embryo. J. Morphol., 161: 23-38.

Lazdins JK, Koech DK, Karnovsky ML. (1980) Oxidation of glucose by mouse peritoneal macrophages: a comparison of suspensions and monolayers. J. Cell. Physiol., 105:191-196.

Leblond PF, Chamberlain JK, Weed RI. (1975) Scanning electron microscopy of erythropoietin-stimulated bone marrow. Blood Cells,1:639-654.

Mel HC, Prenant M, Mohandas M. (1977) Reticulocyte motility and form: studies on maturation and classification. Blood, 49: 1001-1009.

Monos D, Gray I, Cooper HL. (1984) Glycogen regulation in LPS-stimulated murine splenocytes Exp. Cell Res., 151:306-313.

Rikihisa Y. (1984) Glycogen autophagosomes in polymorphonuclear leukocytes induced by Rickettsiae. Anat. Rec., 208:319-327.

Robinson JM, Karnovsky ML, Karnovsky MJ. (1982) Glycogen accumulation in polymorphonuclear leukocytes, and other intracellular alterations that occur during inflammation. J. Cell Biol., 95:933-942.

Rybicka K. (1981) Binding of glycosomes to endoplasmic reticulum and to intermediate filaments in cardiac conduction fibers. J. Histochem. Cytochem., 29:553-560.

Sakai N, Johnstone C, Weiss L. (1981) Bone marrow cells associated with heightened eosinophilopoiesis: an electron microscopic

study of murine bone marrow stimulated by ascaris suum. Amer. J. Anat., 161: 11-32.

Soda R, Tavassoli M. (1984) Transendothelial transport (transcytosis) of iron-transferrin complex in the bone marrow. J. Ultrastr. Res., 88:18-29.

Takeuchi T. (1983) Cellular synthesis of macromolecular glycogen and its deposition. J. Histochem. Cytochem., 31:146-149.

Tavassoli M. (1977) Adaptation of marrow sinus wall to fluctuation in the rate of cell delivery: studies in rabbits after blood-letting. Br. J. Haematol., 35:25-32.

Weiss L. (1965) The structure of bone marrow. Functional interrelationships of vascular and hematopoietic compartments in experimental hemolytic anemia. An electron microscopic study. J. Morphol., 117:467-538.

Weiss L. (1970) Transmural cellular passage in vascular sinuses of rat bone marrow. Blood, 36:189-208.

Weiss L. (1976) The hematopoietic microenvironment of the bone marrow: an ultrastructural study of the stroma in rats. Anat. Rec., 186:161-184.

Weiss L, Chen LT. (1975) The organization of hematopoietic cords and vascular sinuses in bone marrow. Blood Cells, 1:617-638.

Weston H, Bainton DF. (1979) Association of alkaline-phosphatase-positive reticulum cells in bone marrow with granulocytic precursors. J. Exp. Med., 150:919-937.

Wetzel BK, Spicer SS, Horn RG. (1967) Fine structural localization of acid and alkaline phosphatases in cells of rabbit blood and bone marrow. J. Histochem. Cytochem., 15:311-334.

Discussion with Reviewers

J.K. Chamberlain: Do the authors think the marked reticulocytosis observed in the hemodiluted hypoxic animals could at least in part be related to enhanced reticulocyte release secondary to reduced blood viscosity and increased marrow blood flow?

Authors: Several factors likely contributed to the marked reticulocytosis seen in the present study. These rats were severely hemodiluted and the liver was hypoxic. Both hypoxia (Turner et al., 1967, Br. J. Haematol., 13:942-948) and blood removal (Tavassoli, 1977, see References) result in an increased rate of release of reticulocytes. Also, as the studies of Chamberlain et al. (1975b, see References) have shown, hypertransfusion inhibits reticulocyte release, but acute lowering of the hematocrit in these erythropoietin stimulated mice, resulted in a reticulocytosis. In the present study, increased marrow blood flow could have been important, as well as the other factors mentioned above.

P.W. Bankston: What are the morphological distinctions between adventitial cells, reticular cells and marrow macrophages? Do the accumulations of glycogen in adventitial cells and reticular cells, but apparently not macrophages, confirm the basic similarities and differences between these cell types?

Authors: We usually have no difficulty distinguishing macrophages and reticular cells. The large number of heterolysosomes in macrophages, especially in the transfused rats of this study, make them easy to identify. Reticular cells have few heterolysosomes, even in the present study. There are no obvious ultrastructural differences, except for location, between adventitial cells and reticular cells of the marrow cords. The present study shows a similar response of adventitial cells and other reticular cells (i.e. accumulation of glycogen), suggesting a similar physiological role of these cells in glucose metabolism of the bone marrow.

P.W. Bankston: What is the endocytic pathway for the hemoglobin and starch taken up by the stromal cells? Is there any indication of endocytosis by coated pits in the stromal cell types?

Authors: Coated pits and vesicles are commonly observed in reticular cells and macrophages, so we presume they are involved in uptake of starch and hemoglobin. However, since neither of these molecules is easily identified in electron micrographs, we were unable to determine whether coated pits were involved in the endocytic pathway of starch or hemoglobin.