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Splenic erythropoiesis in rats under hypoxic and post-hypoxic conditions

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Summary. In rats chronic hypoxia causes extramedullary haematopoiesis mainly localized in the spleen. It is not known how splenic erythropoiesis develops and how it regresses after termination of hypoxia. In this study the spleen of rats exposed to chronic hypoxia was studied by light and electron microscopy; the findings were compared to relevant peripheral blood values.

Splenic erythropoiesis begins almost immediately after exposure to hypoxia and reaches its maximum after 2–4 weeks. It occurs mainly in the splenic cords drawing upon local erythroblasts and is accompanied by an increase in splenic weight as well as a decrease in splenic iron stores.

After termination of hypoxia marked phagocytosis by splenic cord macrophages diminishes the number of erythroblasts and of erythrocytes with a concomitant increase in splenic iron stores. Thus, splenic erythropoiesis appears to be inhibited as part of a rebound phenomenon and returned to normal by phagocytosis of erythroid cells within 4 weeks after cessation of the hypoxic stimulus.

Key words: Erythrophagocytosis – Erythropoiesis – Hypoxia – Splenic red pulp

Introduction

Under conditions of chronic hypoxia erythropoiesis in rats and other small mammals is stimulated rapidly (Pepelko 1970) and erythrocytosis develops (Yoffrey et al. 1966, Alippi et al. 1983). Haemoglobinaemia (Ou and Smith 1978) and an increased excretion of bilirubin (Ou 1980) during hypoxia, in combination with a normal erythrocyte life span (Fryers and Berlin 1952)

* Dedicated to Professor K. Lennert on the occasion of his 65th birthday

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point to an ineffective erythropoiesis (Ou et al. 1980). In rats, extramedullary haematopoiesis induced by hypoxia is predominantly localized in the spleen (Yoffrey et al. 1966; Streeter et al. 1975; Ou and Smith 1978; Ou et al. 1980). It is not clear, however, how this extramedullary hematopoiesis develops and how it regresses after termination of the hypoxic stimulus. Therefore, systematic investigations of the spleen before, during and after stimulation of erythropoiesis by chronic hypoxia were conducted.

Material and methods

24 young adult (50 days old) and 18 adult (120 days old) specific pathogen free (spf) Wistar rats were used. The animals were kept in clear plastic cages with free access to water and laboratory chow (Hope Farms, The Netherlands). At the start of the experiment the rats were placed in an altitude chamber that was permanently evacuated by a vacuum pump (GTS 2, Leybold, Hanau, FRG). The total barometric pressure inside the chamber was regulated by an air-inlet-valve that was calibrated daily. The chamber pressure was 543 torr, corresponding to an altitude of 6,000 m. The animals stayed in the chamber for the duration of the experiment; it was opened daily for 15 min in order to provide the animals with fresh water, food and straw.

The animals were exposed to hypoxia for a maximum of four weeks; each week three of them were sacrificed by decapitation after ether anesthesia. After four weeks the rest of the exposed animals were returned to a normal pressure environment; each week for an additional four weeks another three of them were studied. Three animals of each group served as controls.

Before decapitation an abdominal incision was made and blood from the aorta was drawn into a heparinized syringe. The concentration of whole blood haemoglobin was determined by spectrophotometric methods, while haematocrit values were measured by a capillary tube technique and erythrocytes were counted electronically. Spleen and liver were then removed, weighed and processed immediately.

Light microscopy. Paraffin sections from the material fixed in 4% buffered formaldehyde were stained with haematoxylin-eosin, Giemsa and Prussian Blue stains. In addition Naphthol-AS-D-Chloroacetate-Esterase Reaction was performed on deparaffinized sections (Leder 1964) Semithin sections from araldite blocks for electron microscopy were stained with toluidine blue and used for light microscopy.

Electron microscopy. Small portions of the spleen and liver were fixed in 3% glutaraldehyde in 0,1 m cacodylate buffer at pH 7,4 for 1 h at 4° C, postfixed in 1% buffered OsO_4 for 1 h at room temperature, dehydrated in graded alcohol and embedded in araldite (Serva, Heidelberg, FRG). The ultrathin sections were cut with a Reichert ultramicrotome, stained with uranyl acetate and lead citrate and were examined with a Siemens Elmiskop 101.

Erythropoiesis and haemosiderin deposits in the spleen were semiquantitatively graded from − to 4+. The highest grading corresponds to the most markedly increased erythropoiesis viz. haemosiderosis that could be observed. Figures 1 and 2 illustrate the grading system used for erythropoiesis an for haemosiderosis in the spleen.

Results

Erythrocyte counts in peripheral blood, spleen/body weight ratios and semiquantitative results obtained for splenic erythropoiesis and iron stores are given in Figs. 3 and 4.

The mean values of erythrocyte counts, haemoglobin and haematocrit were obtained from both young and adult rats. During hypoxia all those

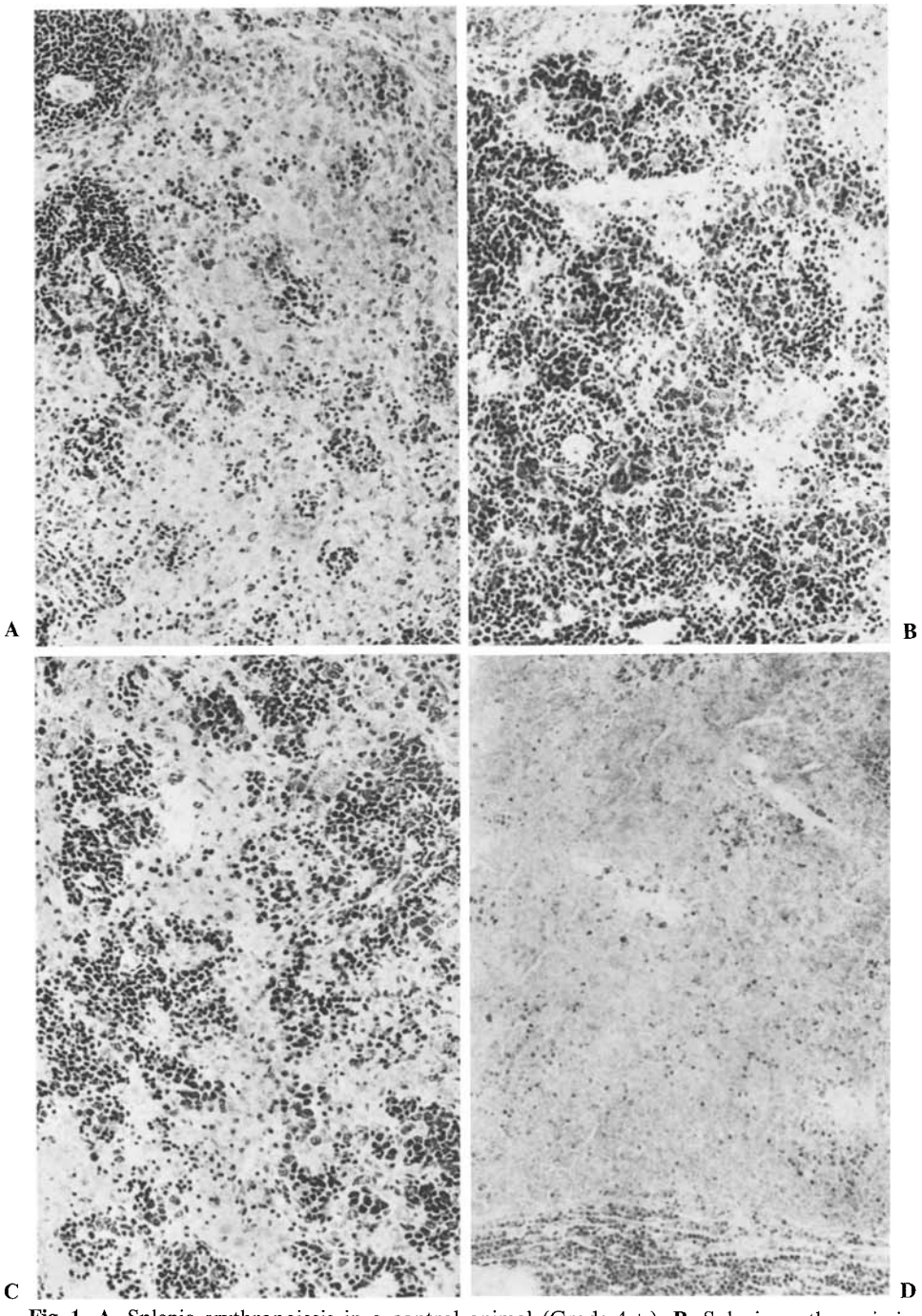

Fig. 1. A. Splenic erythropoiesis in a control animal (Grade 1+). **B.** Splenic erythropoiesis after 4 weeks of hypoxia (Grade 4+). **C.** Splenic erythropoiesis 1 week after return to sea-level pressure (Grade 3+). **D.** Splenic erythropoiesis 2 weeks after return to sea-level pressure (Grade −). Giemsa, × 100

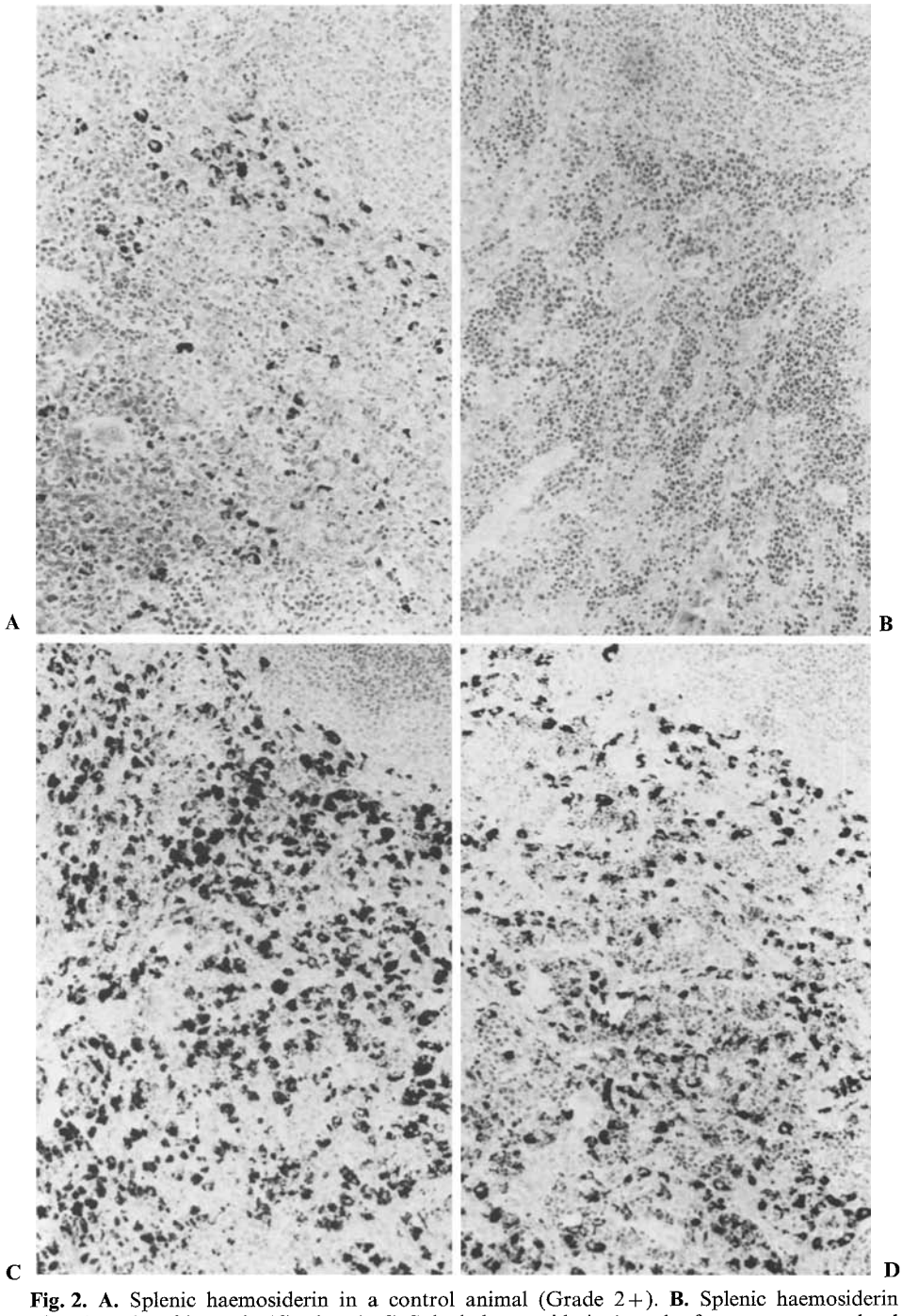

Fig. 2. A. Splenic haemosiderin in a control animal (Grade 2+). **B.** Splenic haemosiderin after 4 weeks of hypoxia (Grade −). **C.** Splenic haemosiderin 1 week after return to sea-level pressure (Grade 4+). **D.** Splenic haemosiderin 2 weeks after return to sea-level pressure (Grade 3+). Prussian Blue, × 100

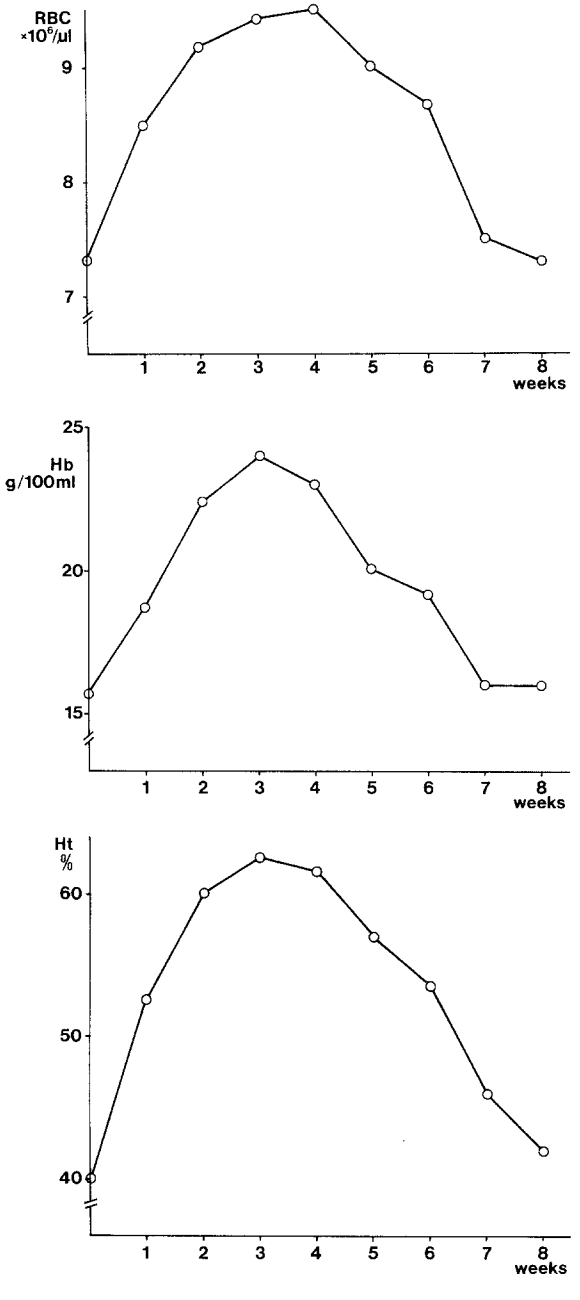

Fig. 3. Selected blood measurements during exposure to hypoxia (weeks 1 to 4) and after return to sea-level pressure (weeks 5–8). These values were obtained in young adult rats only

variables increased significantly. They gradually decreased and returned to the values obtained in controls within four weeks of return to a normal pressure environment. The mean values for spleen/body weight ratios paralleled the extent of splenic erythropoiesis. After one week of exposure to hypoxia splenic erythropoiesis was markedly increased and was maintained

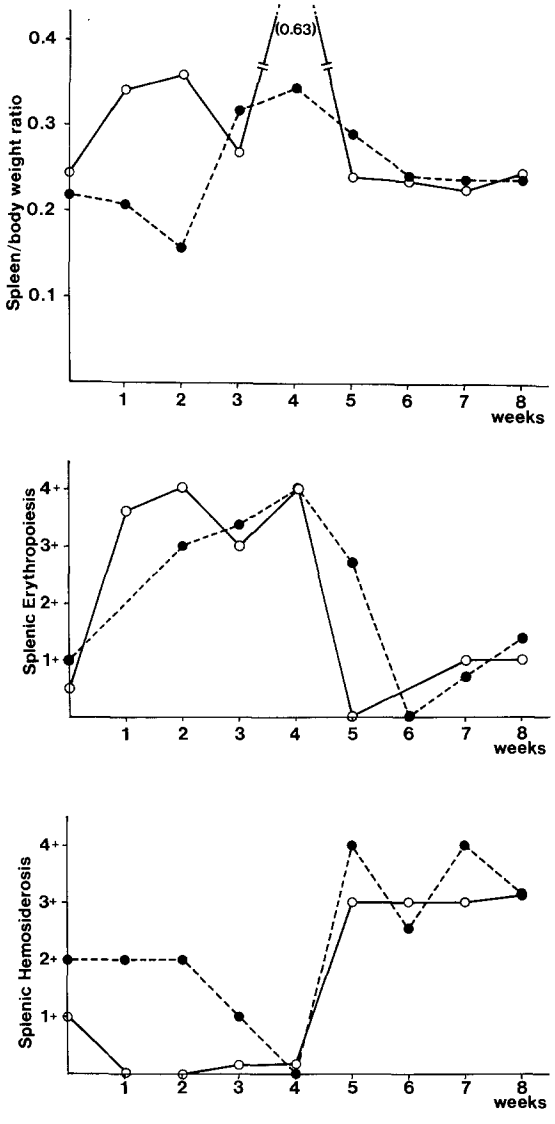

Fig. 4. Changes in the rat spleen during exposure to hypoxia (weeks 1 to 4) and after return to sea-level pressure (weeks 5 to 8). *Top:* Spleen weight/body weight ratio. *Middle:* Splenic erythropoiesis. *Bottom:* Splenic haemosiderosis. ● denotes adult rats, while ○ denotes young adult rats

at that level for the duration of hypoxia, while iron stores gradually decreased. After removal of the hypoxic stimulus iron stores in the spleen increased while erythropoiesis was markedly diminished within two weeks.

Microscopically, neither young or adult rats exhibited haematopoiesis in the liver. After three weeks of hypoxia the liver appeared congested, and three weeks after termination of hypoxia small amounts of iron could be observed in Kupffer cells.

In the spleen, however, various changes during and after hypoxia could be observed. In controls small nests of erythroblasts in different stages of maturation were occasionally found in the splenic cords but not in the splenic sinuses (Fig. 1A). In the vicinity of these erythropoietic nests small

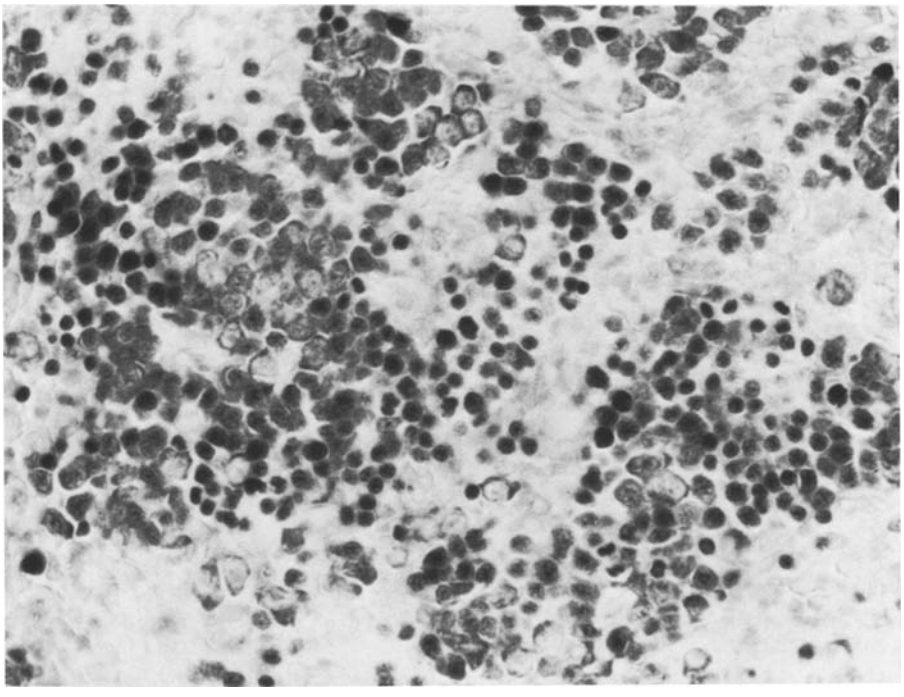

Fig. 5. Dilated splenic cords with proerythroblasts and young polychromatic erythroblasts (Third week of hypoxia). Giemsa, ×450

numbers of macrophages containing nucleated erythroid cells in addition to haemosiderin were seen. Haemosiderin-containing macrophages were commonly found in the red pulp, particularly in the splenic cords (Fig. 2A). In the white pulp and the marginal zone, however, only a few macrophages containing haemosiderin were present. Megakaryocytes were occasionally found in the splenic cords; a few foci of granulopoiesis were also present in the splenic cords. Throughout the experiment, no significant changes with respect to the quantity of granulopoiesis and thrombopoiesis in the spleen were evident.

After one week of exposure to hypoxia, erythrocyte counts, haemoglobin values and haematocrit in the peripheral blood were significantly elevated (Fig. 3). Histologically the spleen exhibited dilated splenic cords where a markedly increased number of erythroblasts could be observed (Fig. 1B). There was a predominance of early erythroid cells and a differentiation from proerythroblasts to young polychromatic erythroblasts occured (Fig. 5). Haemosiderin-containing macrophages were mainly seen in the regions of the cords adjacent to the sinuses and in the sinuses.

Under conditions of hypoxia the splenic erythropoiesis remained markedly increased while iron stores in the spleen were gradually diminished.

One week after the animals had been returned to sea-level pressure, a rapid decrease in the number of erythroblasts was accompanied by a steep increase of haemosiderin-containing macrophages in the red pulp,

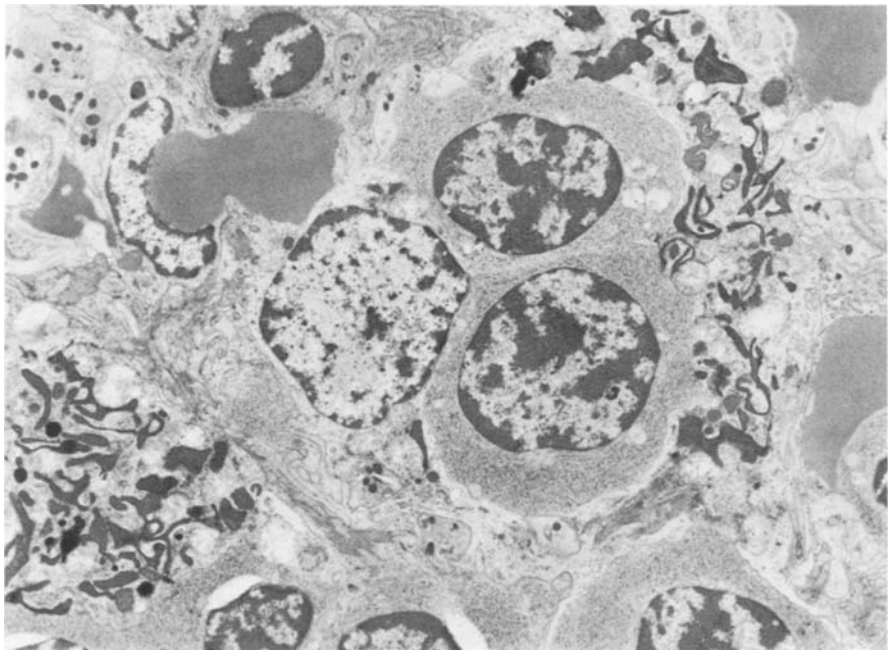

Fig. 6. Phagocytosis of two erythroblasts by a splenic cord macrophage. Erythrocyte degradation products appear in the macrophage cytoplasm. × 5,000

especially in the splenic cords (Fig. 2C). In both young and adult rats the macrophages, particularly in regions where clusters of erythroblasts were present, carried out phagocytosis of nucleated erythroid cells and contained degradation products of erythrocytes in their cytoplasm (Fig. 6).

Two weeks after the animals had been returned to sea-level pressure nests of erythropoiesis and the macrophages containing nucleated erythroid cells disappeared, although haemosiderin-containing macrophages were still present in the red pulp (Fig. 2D).

After the third week of normal pressure small-sized nests of erythropoiesis comparable in size and quantity to those observed in controls occured again in the splenic cords. They were accompanied by an abundant number of macrophages containing haemosiderin and/or phagocytosed erythroid cells. In this period of time the spleen exhibited a degree of erythrophagocytosis that exceeded the level observed in controls. Four weeks after return to a normal pressure environment spleen morphology in animals subjected to hypoxia did not differ significantly from the appearance in controls.

Discussion

In adult human beings the bone marrow plays the most important role as an erythropoietic organ both under normal conditions and under conditions of hypoxia (Merino and Reynaferje 1949). In rats, however, the spleen

satisfies an increased demand for erythrocytes in response to a hypoxic stimulus while bone marrow and liver remain relatively inert (Pepelko 1970; Ou et al. 1980). This difference between man and rat can be explained anatomically by the larger capacity of human bone marrow which is sufficient for the increased production of erythrocytes under hypoxic conditions, while the rat has to rely on extramedullary erythropoiesis in the spleen (Seifert and Marks 1985), and sometimes even in the liver (Crosby 1983). In our study haematopoietic changes in the spleen and liver under conditions of hypoxia at a simulated altidude of 6,000 m and after return to a normal pressure environment were investigated. Both young and adult rats showed a similar response to hypoxia. Neither during hypoxia nor after return to sea-level pressure could extramedullary haematopoiesis be detected in the liver. In contrast, the spleen exhibited significant changes.

One week after exposure to hypoxia a markedly increased erythropoiesis was found in the splenic cords; early erythroid cells were more dominant than in controls. This suggests that the erythroblasts proliferate in the splenic cords and that under conditions of hypoxia the increase in splenic weight and size is due to an increase in extramedullary erythropoiesis, while neither granulopoiesis nor thrombopoiesis are stimulated by hypoxia.

Thus in rats the spleen appears to be an important organ in the physiological response to hypoxia. In contrast, in human adults only certain pathological conditions accompanied by extramedullary haematopoiesis cause erythropoiesis localized mainly in the splenic sinuses (Rappaport 1970; Stutte 1984). The difference between the erythropoietic sites in the splenic red pulp of man and rat may be explained by the fact that the spleen in small animals such as rats remains a normal haematopoietic organ through adulthood (Crosby 1983), whereas in humans it ceases to have haematopoietic functions after birth (Freedman and Saunders 1981). Recent investigations suggest that haemopoiesis in the fetal human spleen may be limited or even nonexistent (Ishikawa 1985). In contrast to the proliferation of local erythroblasts in the rats' splenic cords that occurs with an increased erythropoietic activity in the bone marrow, splenic haematopoiesis in man is mediated by the immigration of erythroid cell precursors from the blood (Freedman and Saunders 1981) and their proliferation in the splenic sinuses. It may be assumed that erythroid cell precursors are restricted to the vascular region, since in myelofibrosis almost only myeloid progenitor cells and megakaryocytes are seen in the perivascular regions.

Haemosiderin stores in the spleen were progressively reduced during the animals'exposure to hypoxia. This phenomenon may be attributed to an increased iron consumption by the stimulated erythropoiesis. One week after the animals had been returned to sea-level pressure the number of erythroblasts in the splenic cords was sharply diminished, while the number of macrophages containing haemosiderin and/or phagocytosed erythroid cells increased. This finding constituted a characteristic morphological change in the splenic red pulp. It may be assumed that the increased splenic erythropoiesis after cessation of its appropriate stimulus returns to normal mainly through erythrophagocytosis by macrophages.

It is unknown, however, whether erythroid cells are phagocytosed at random or whether cells already damaged by metabolic injuries during splenic pooling are preferentially ingested by macrophages (Jandl and Aster 1967). It seems probable that erythrophagocytosis occurs as a random process, since it was seen not only during the first week after return to a normal pressure environment but also to a lesser degree in the third and fourth week and even in controls. The rapidly increasing number of macrophages that appear after the termination of the hypoxic stimulus may be derived from macrophages already present in the splenic cords (Simon and Burke 1970), and from circulating monocytes (Wintrobe 1981). The splenic enlargement during this period of time is mainly caused by an increase in the number of macrophages containing haemosiderin and/or phagocytosed erythroid cells (Azen and Schilling 1964).

A slight increase in haemosiderin deposits in Kupffer cells after cessation of hypoxia indicates that splenic macrophages release iron into the circulation that is partly taken up by the liver.

Since it has been observed that the spleen continues to release erythrocytes long after cessation of the hypoxic stimulus (Yoffrey et al. 1966), it has been suggested that a persistent stimulus initiated by hypoxia or a committed pool of erythroid precursor cells is present in the spleen (Ou et al. 1980). In our study peripheral erythrocyte counts as well as haemoglobin and haematocrit values began to decrease immediately after termination of hypoxia and returned to normal within three weeks.

These changes of the erythrocyte counts correspond to the mean erythrocyte life span in rats of 60 days (Fryers and Berlin 1952), which is not affected by hypoxia (Ou and Smith 1978). Moreover, splenic erythropoiesis three weeks after cessation of hypoxia was markedly reduced in comparison with controls. It is suggested that after termination of hypoxia splenic erythropoiesis is inhibited as part of a rebound phenomenon (Moffatt et al. 1964) and accompanied by erythrophagocytosis by macrophages. If some erythroid cells from the splenic pool of erythroid precursor cells are still relased from the spleen (Ou et al. 1980), their number is insignificant.

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