Virchows Arch. A Path. Anat. and Histol. 364, 265—273 (1974)

Electron Microscopy of Thrombocytes in the Orthotopic Porcine Liver Homograft during the Late Rejection (Phagocytosis of Thrombocytes by Kupffer Cells)

L. Cossel

Institute of Pathology, Karl-Marx-University, Leipzig, GDR

Received June 6, 1974

Summary. Electron microscopical observations of thrombocytes in the orthotopic porcine liver homograft in later stages after transplantation are described and demonstrated. The most remarkable finding was a phagocytosis of apparently unaltered thrombocytes by the Kupffer cells. Besides single or small groups of thrombocytes in the sinusoids with partly close topographical relations to the endothelial lining and intrasinusoidal accumulation of blood platelets were observed. The possible significance of these findings is discussed with particular reference to the long-term prognosis of liver transplantation.

Electron microscopical studies of orthotopic porcine liver homografts revealed findings, which are interesting in view of the interrelationship between the reticuloendothelial system in the liver (Kupffer cells) and the thrombocytes, and as regards information on the influence of liver transplantation on the thrombocytes and thus on the host's blodd clotting mechanism.

Materials and Methods

The results reported in this paper were obtained from an orthotopically transplanted allogeneic porcine liver. The tissue was removed by exploratory excision made during laparotomy performed 138 days after transplantation. The transplantation technique was a modification of the technique described by Starzl (1964) and Starzl *et al.* (1965). The transplantation was performed by the transplantation group of the Clinic of Surgery, Karl-Marx-University, Leipzig under the direction of Schwarzer. For electron microscopy liver tissue was separated, within 30 to 90 seconds, into 1 mm^3 pieces on a cooled glass plate and immersed in icecooled, 1%, isotonic OsO_4 solution buffered at a pH value of 7.2, the duration of fixation being 2 hours. The material, after having been in contact with tyrode solution for a period of 15 minutes, is dehydrated in an ascending acetone series, 0.5% of phosphotungstic acid and 0.5% of uranyl acetate being added at the 70% acetone level. After this, the material is imbedded in Micropal and then polymerized in an incubator at a temperature of 60°C. An Om U2 type ultramicrotome (C. Reichert, Optische Werke AG, Vienna), was used for the preparation of ultrathin sections. The ultrathin sections were observed with an SEM 3–2 type 100 kv electromagnetic electron microscope (Werk für Fernsehelektronik, Berlin-Oberschöneweide) as well as with a D2 type 50 kv electrostatic electron microscope (VEB Carl Zeiss, Jena).

Results

A varying number of thrombocytes was observed in the sinusoids. The blood platelets lie separately or in small groups. There were also accumulations of blood platelets filling up almost the total lumen of the sinusoids. The slices of the throm-

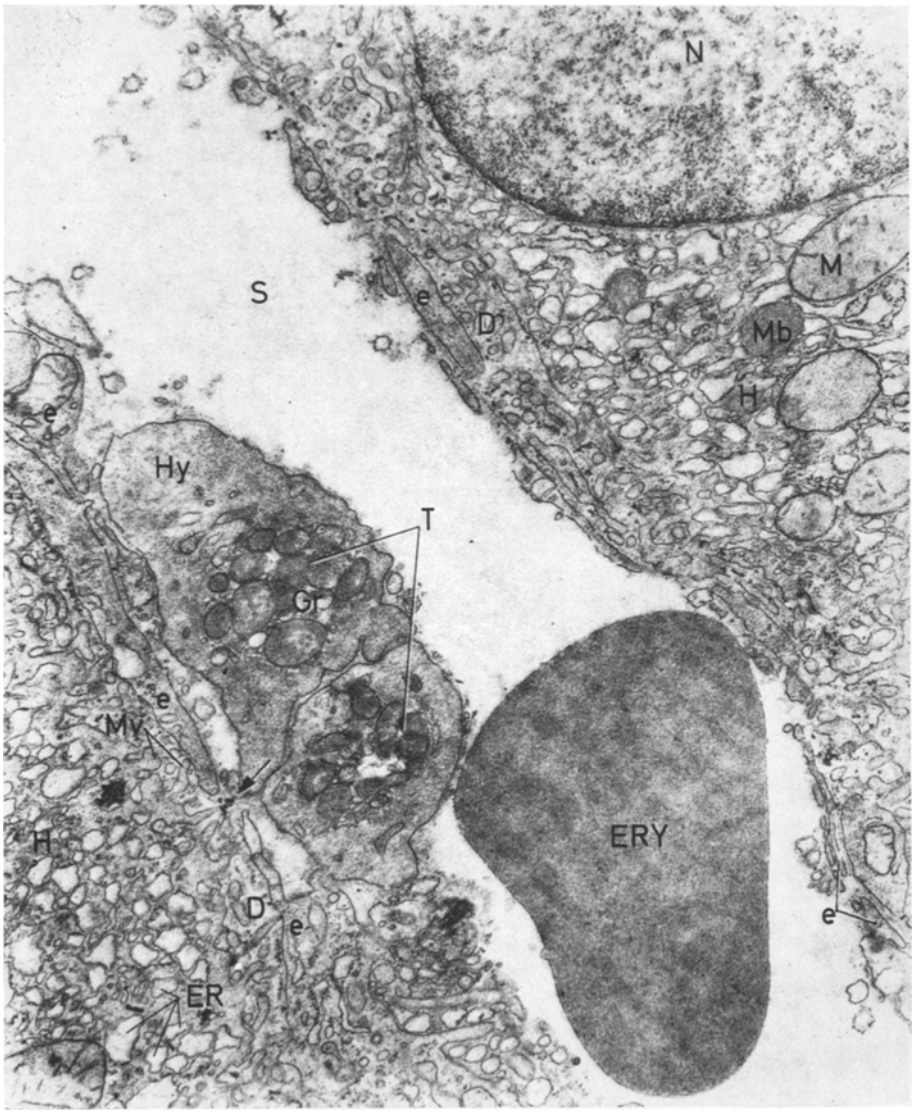

Fig. 1. Two platelets (*T*) with granulomere (*Gr*) and hyalomere (*Hy*) in close vicinity of an endothelial pore (↑) in the partially two layered endothelial sinusoid lining (*e*). Erythrocyte (*ERY*) in the sinusoid lumen (*S*). *D* Space of Disse between the endothelial lining (*e*) and the hepatocytes (*H*). Microvilli (*Mv*), increased and dilated endoplasmic reticulum (*ER*), mitochondria (*M*), microbodies (*Mb*) and nucleus (*N*) of the hepatocytes. Orthotopic porcine liver homograft 138 days after transplantation. × 14200

bocytes are roundish, oval or longish-shaped (Figs. 1–3). They display the granulomere in their center and the organelle-free hyalomere at the periphery (Figs. 1–3). Some of the thrombocyte slices show short, stout pseudopodia-like processes. The thrombocytes are often found near defects of cell membranes and colliquation necroses of liver epithelial cells together with small lymphocytes,

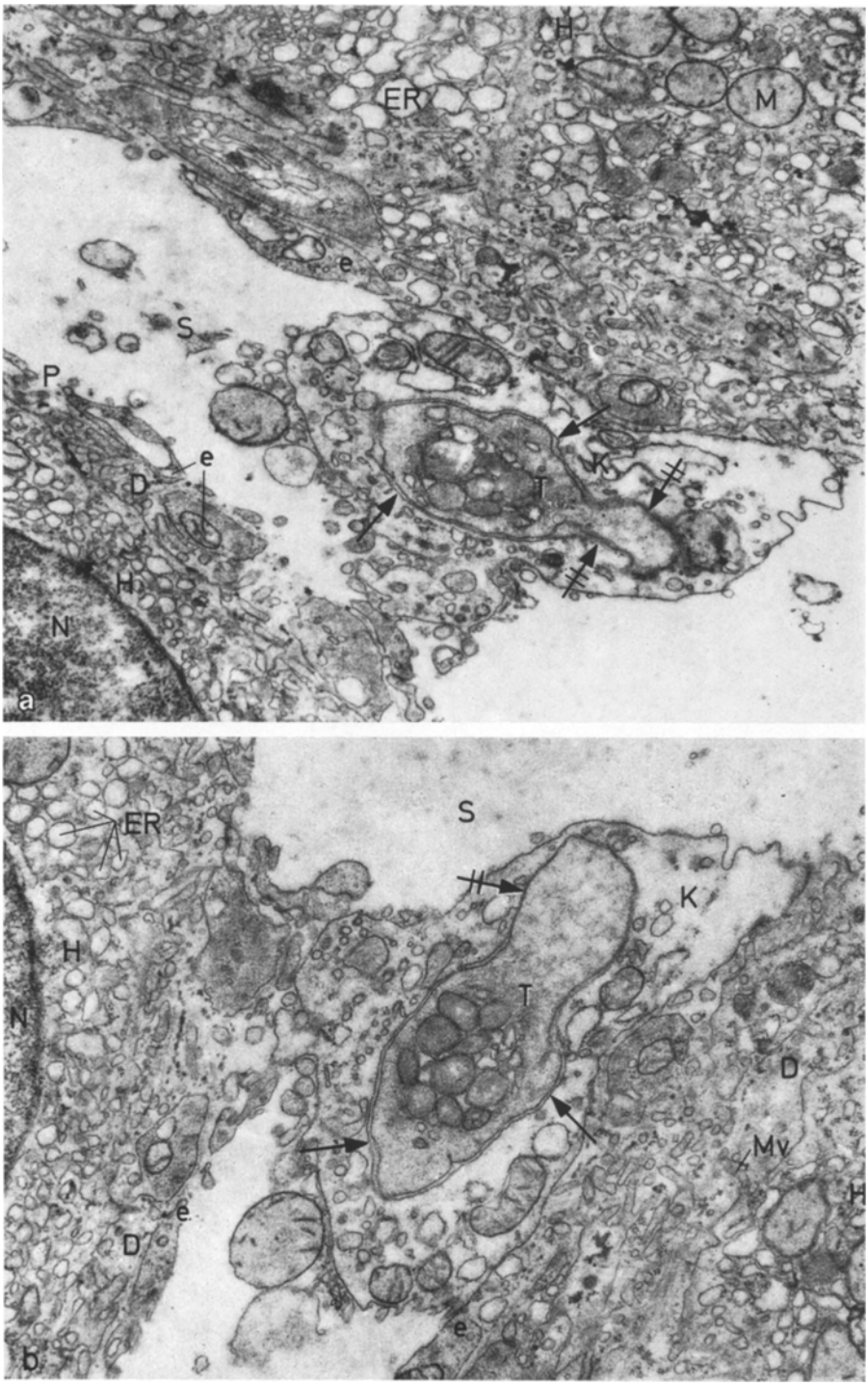

Fig. 2a and b. Relatively intact platelets (*T*) within Kupffer cells (*K*). The platelets are surrounded by membranes of the Kupffer cells. Between these membranes and the cell membranes of the platelets is a narrow space (↑). This space is focally not clearly visible (focal membrane fusion) (⇞). *S* sinusoid lumen containing liver cell debris. *D* Space of Disse between the endothelial sinusoid lining (*e*) and hepatocytes (*H*). *P* endothelial pore. Microvilli (*Mv*), increased and dilated endoplasmic reticulum (*ER*), mitochondria (*M*) and nucleus (*N*) of the hepatocytes. Orthotopic porcine liver homograft 138 days after transplantation. (a) × 14200, (b) × 16500

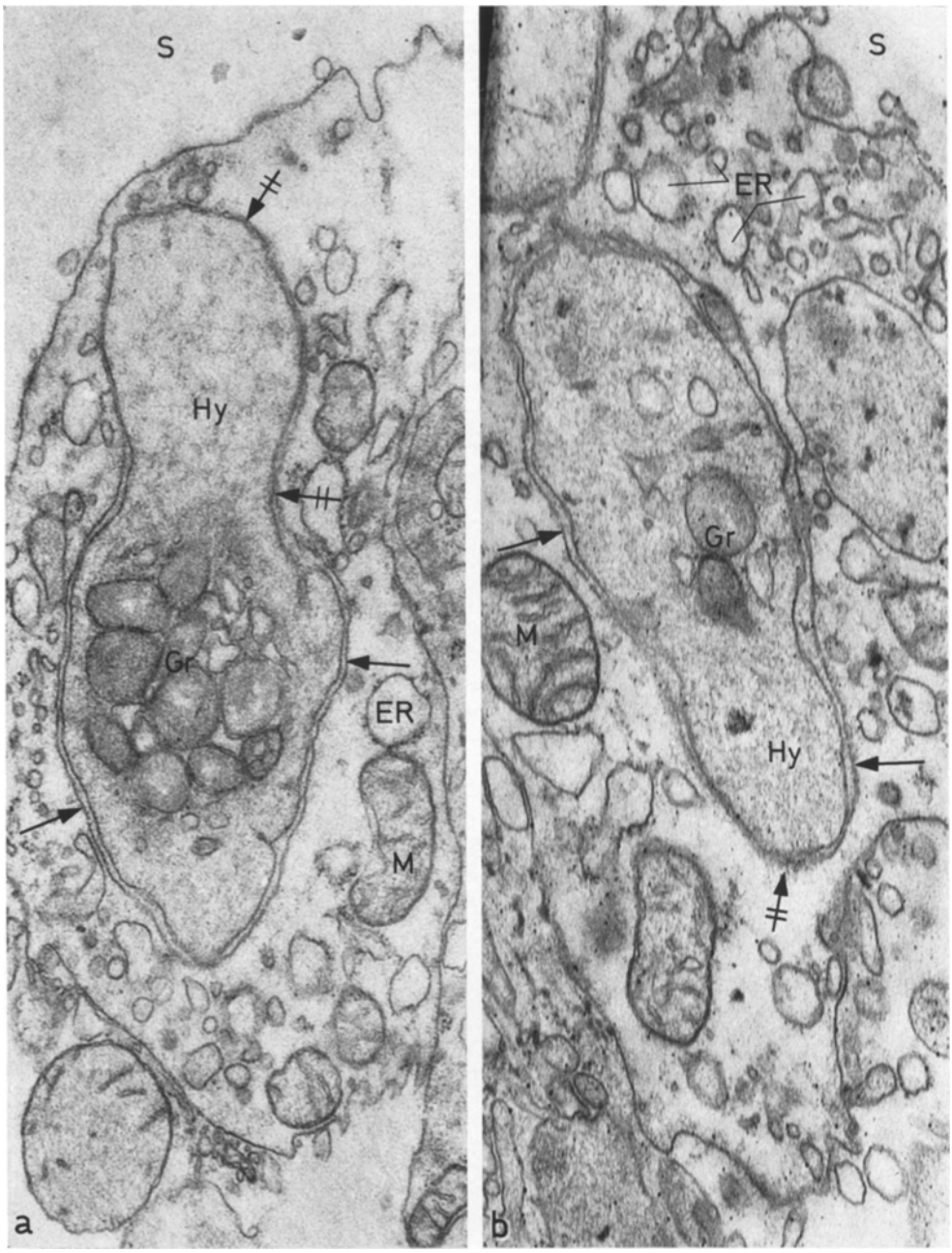

Fig. 3a and b. Platelets in Kupffer cells viewed at a higher magnification. The platelets and the surrounding membranes of Kupffer cells are separated by a narrow space (↑). Membranes of platelets and Kupffer cells seem to be fused at several points (⇗). The platelets have not undergone any significant changes. *Gr* granulomere, *Hy* hyalomere. Partially dilated smooth and rough surfaced endoplasmic reticulum (*ER*) and mitochondria (*M*) in the Kupffer cells. *S* sinusoid lumen. Orthotopic porcine liver homograft 138 days after transplantation. (a) × 28400, (b) × 38400

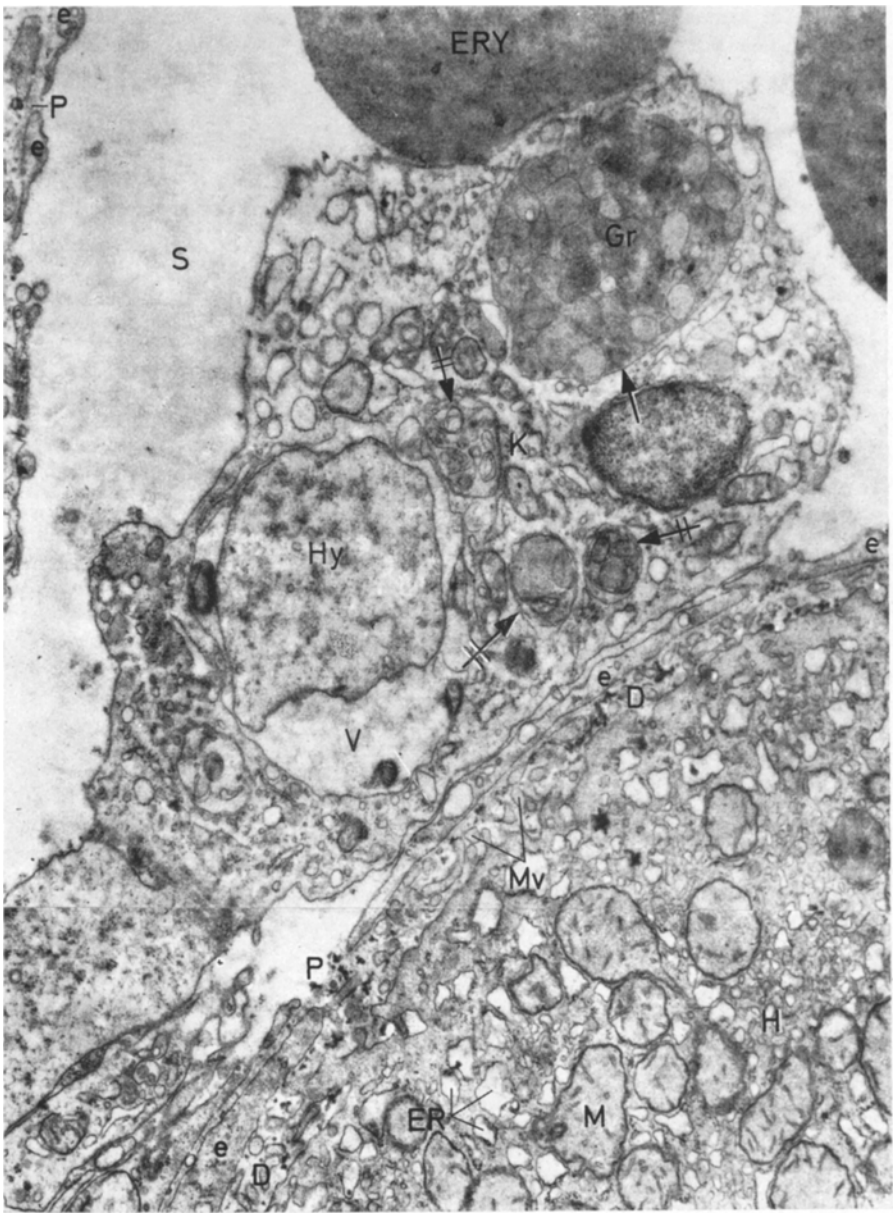

Fig. 4. Kupffer cell (*K*) with electron dense portion of platelet granulomere (*Gr*), partially bounded by a single membrane (↑), and a part of low-electron-density cytoplasm (platelet hyalomere) (*Hy*) in a membrane bounded vacuole (*V*). The crossed arrows (‡) point at cytosomes possibly containing markedly altered organelles of a phagocytized platelet. *S* sinusoid lumen with two erythrocytes (*ERY*), *e* endothelial sinusoid lining with pores (*P*), *D* space of Disse. Microvilli (*Mv*), *H* hepatocyte. *ER* endoplasmic reticulum, *M* mitochondria. Orthotopic porcine liver homograft 138 days after transplantation . × 15200

granulocytes and macrophages (activated Kupffer cells) (Cossel *et al.*, 1974). Other blood platelets lie at focal interruptions of the endothelial sinusoid lining or at the sinusoid wall, often in the vicinity of the normally present endothelial pores (Fig. 1). In the neighbourhood of thrombocytes focal basement-like structures are visible in the sinusoid wall between the endothelial cells of the partially two layered endothelial lining and in the space of Disse between the endothelial lining and the hepatocytes.—Very rarely fibrin fibres were seen in the lumen of sinusoids by the side of thrombocytes.

Further, thrombocytes were observed within the Kupffer cells (activated endothelial cells) (Figs. 2, 3). They are surrounded by a membrane of the Kupffer cell (Figs. 2, 3). The electron microscopical structure of these blood platelets is not altered (Figs. 2, 3). There is an electron optically empty space between the cell membrane of the thrombocyte and the surrounding membrane of the Kupffer cell (Figs. 2, 3). Focally these membranes, however, are in close contact, and they seem to be fused (Figs. 2, 3). In other Kupffer cells one sees parts of the granulomere of thrombocytes with abnormal electron- dense and partly blurred structure, which are closely bounded by a simple membrane (Fig. 4). Besides, parts of low-electron-density cytoplasm without organelles are observed in membrane bounded vacuoles of Kupffer cells (Fig. 4). Furthermore the light and electron microscopical examinations exhibited the features of a late rejection in the transplant.—At the time, the tissue was removed, there were not any clinical indications of haemorrhagic diathesis or thrombocytopenia.

Discussion

Only few references were found in the literature regarding results of electron microscopical observations of thrombocytes in the transplanted liver. Hutchison *et al.* (1968) and Groth (1969) describe a sequestration of single undamaged blood platelets into the widened perisinusoidal spaces of the homografts without illustrations during the early phase after the transplantation lasting from hours to some days, which is marked by a transient increase of fibrinolysis and thrombocytopenia. These observations were made on orthotopic liver homografts in human and dogs. Hutchison *et al.* (1968) suppose that the primary cause of this findings is mechanical entrapment of the platelets within injured grafts. Such an extravasation of platelets into the space of Disse was not observed in the studies presented in this paper. Porter (1969) saw thrombocytes together with lymphocytes, macrophages, and fibrin in the wall of central veins and veins in portal tracts four days after orthotopic allogeneic liver transplantation in dogs by electron microscopy. No communications were found in the literature on electron microscopical observations on thrombocytes in liver homografts which were made after transplantation as late as in the present study.

The observed focal occurrence of thrombocytes in the sinusoids is explained most readily as a sequel of necrotic processes in the late rejection (cp. Cossel *et al.*, 1974) in progress at the time, the tissue was removed. By this the tissue thrombokinase releasing into the sinusoids increasingly attracts thrombocytes and leads to a focal accumulation—the initial reversible stage of thrombosis. As in the first hours and days after transplantation (Hutchison *et al.*, 1968; Groth, 1969), later phases of thrombus formation and complete intrasinusoidal thrombosis were not observed. It is also possible that the accumulation ot thrombocytes in

the sinusoids is caused by contact with antigen-antibody complexes (Porter *et al.*, 1967; O'Brien, 1970; Evans *et al.*, 1970, Sharma *et al.*, 1972). However the changes described and demonstrated in the present study do not appear to have such an immunologic basis.

The occurrence of basement membrane-like structures in the sinusoid wall near the thrombocytes and the apposition of thrombocytes to the endothelial sinusoid lining particularly in the region of the endothelial pores (Fig. 1) has been observed even without liver transplantation. This was interpreted as a morphological equivalent of the influence of the thrombocytes on the barrier function of the vascular wall (Cossel, 1972 a, b). Obviously, these findings and the occurrence of thrombocytes at sites with interrupted sinusoid lining are the expression of such a mechanism also in the transplanted liver.

The Figures 2 and 3 may be regarded as early phases and Fig. 4 as advanced stages of phagocytosis of blood platelets by Kupffer cells (activated endothelial cells). It cannot be excluded with certainty that the thrombocytes lie in deep invaginations of the cytoplasm of the Kupffer cells and their intracellular site is simulated by the position of the section plane. However, this possibility is highly improbable. Shirasawa and Chandler (1971) believe the focal membrane fusion between the platelets and the phagocytic vacuole (cp. Figs. 2, 3) observed by electron microscopy during the phagocytosis of platelets by granulocytes in vitro, to be evidence of complete phagocytosis. The portions of cytoplasm with low electron density and without cell organelles, which are surrounded by a membrane and lie in the vacuoles of the Kupffer cells (Fig. 4) may be phagocytosed parts of the hyalomere of the thrombocytes. Similar findings were obtained by Shirasawa and Chandler in the above mentioned investigations. They were regarded as phagocytosed platelets.

Normally, the blood platelets are destroyed in the reticuloendothelial system of liver, spleen, and, to a minor degree, of the lung (Rein, 1960; Schulz, 1968; Gehrmann and Bleifeld, 1969; Huhn and Stich, 1969; Aster, 1969; Simon and Burke, 1970; Edwards and Simon, 1970), if they are not used up in latent and actual blood coagulation and physiological endothelial sealing. Further, platelets are assumed to be destroyed by monocytes within vascular system (Huhn and Stich, 1969). The standard publications on the liver (Popper and Schaffner, 1961; Rouiller, 1964) do not mention a phagocytosis of thrombocytes by Kupffer cells. Popper and Schaffner believe that the degradation of leucocytes and erythrocytes in the Kupffer cells is not the normal way of destruction of these cells. In the available literature, such as monographs on the ultrastructure of the liver (David, 1964, 1967; Hübner, 1968; Tanikawa, 1968), electron microscopical pictures of the phagocytosis and destruction of thrombocytes in Kupffer cells could not be found. French and Barcat (1968) electronmicroscopically describe the phagocytosis of platelets by cells of the reticuloendothelial system in the liver of mice, rats, and guinea pigs. But in this study is only one electron micrograph illustrating this phenomenon in the spleen. In the literature, there are electron micrographs of the phagocytosis of platelets by splenic macrophages of normal albino rabbits (Simon and Burke, 1970), of rats following the injection of colloidal carbon (French and Barcat, 1968), and in human idiopathic thrombocytopenia (Firkin *et al.*, 1969), by endothelial cells (Marchesi, 1964), by granulocytes in vitro (Shirasawa and Chandler, 1971), by monocytes in vitro (Movat *et al.*, 1965), and by human

blood monocytes (Huhn and Stich, 1969). The electron micrographs, published in these papers, are partly similar to the findings presented in Fig. 4, partly the thrombocytic origin of the phagocytosed material lying in membrane bound vacuoles is not clearly recognizable. In orthotopic homografts in the early phases after liver transplantation in man and dogs Hutchison *et al.* (1968) electronmicroscopically describe macrophages situated in the widened spaces of Disse and in the spaces around the small central and portal venous tributaries. In these large mononuclear cells thrombocyte fragments and occasionally ingested whole platelets are described by the authors. These observations are not exemplified by electron micrographs so that a comparison with the findings demonstrated here is not possible. Interpretation suffers from this fact and poor possibilities of comparison of the findings in Figs. 2 and 3 with electron microscopical illustrations of the physiological and pathological destruction of thrombocytes in the Kupffer cells and reticulum cells. These findings, however, suggest a phagocytosis of platelets which are not essentially altered in their fine structure (Figs. 2, 3). Changes due to ageing processes (for detailed bibliographies see Schulz, 1968; Huhn and Stich, 1969) are not evident in the thrombocytes within the Kupffer cells (Figs. 2, 3). Therefore, it is not probable, that these pictures represent electron microscopical equivalents of the physiological degradation of effete platelets. It would rather appear that the Figs. 2 and 3 are the morphological equivalent of an increased reaction of macrophages of the transplant (Kupffer cells) against host cells (thrombocytes) circulating in the blood. In this context it may be mentioned that increased phagocytosis of erythrocytes was found in the Kupffer cells of transplanted livers. Possibly, such processes cause the thrombocytopenia observed clinically and in animal experiments after liver transplantation (for detailed bibliographies see Groth, 1969; Najarian and Simons, 1972). Since thrombocytopenia does not occur regularly during the late stages after liver transplantation (Hutchison *et al.*, 1968; Groth, 1969), obviously there is a possibility of compensation by increased formation of platelets in the host organism. Further investigations must show to what extent the electron microscopical findings presented here are of general importance for liver transplantation. However, these findings give rise to the assumption that increased phagocytosis of thrombocytes by the Kupffer cells of the transplanted liver might in the long run lead to considerable strains on the thrombocytopoiesis of the host organism. This would entail blood clotting disturbances and thus impair the long-term prognosis after what was at first successfull transplantation.

I should like to thank Miss H. Schütz and Mrs. A. Kästner for their most valuable technical assistance.

References

Aster, R.H.: Studies of the fate of platelets in rats and man. Blood **34**, 117–128 (1969)

Cossel, L.: Elektronenmikroskopische Befunde an den Lebersinusoiden nach Injektion gefäßabdichtender Substanzen in die Pfortader. Zbl. allg. Path. path. Anat. **116**, 401–409 (1972a)

Cossel, L.: Zur Lokalisation des Einflusses der Blutgerinnung auf die Durchlässigkeit der Gefäßwand. Dtsch. Gesundh.-Wes. **27**, 2233–2243 (1972b)

Cossel, L., Mahnke, P.-F., Schwarzer, R.: "Killer"-lymphocytes in action ? (Light and electron microscopicla findings in orthotopic liver homografts). Virchows Arch. A Path. Anat. and Histol. **364**, 179–190 (1974)

David, H.: Submikroskopische Ortho- und Pathomorphologie der Leber. Berlin: Akademie-Verlag 1964

David, H.: Elektronenmikroskopische Organpathologie. Berlin: Volk u. Gesundheit 1967
Edwards, V.D., Simon, G.T.: Ultrastructural aspects of red cell destruction in the normal rat spleen. J. Ultrastruct. Res. **33**, 187–201 (1970)
Firkin, B.G., Wright, R., Miller, S., Stokes, E.: Splenic macrophages in thrombocytopenia. Blood **33**, 240–245 (1969)
French, J.E., Barcat, J.A.: The fine structure of platelets and platelet aggregates in vivo. Progr. Biochim. Pharmacol. **4**, 550–555 (1968)
Gehrmann, G., Bleifeld, W.: Lebensdauer und Abbauort menschlicher Thrombocyten bei Thrombopenien. In: R. Marx, Der Thrombocyt, S. 144–152. München: J. F. Lehmann 1969
Groth, C.G.: In: T.E. Starzl and C.W. Putman (ed.), Experience in hepatic transplantation, p. 159–175. Philadelphia-London-Toronto: W. B. Saunders Comp. 1969
Hübner, G.: Die pathischen Reaktionen des Lebergewebes. Eine elektronenmikroskopische Studie. Veröffentlichungen aus der morphologischen Pathologie, H. 78. Stuttgart: G. Fischer 1968
Huhn, D., Stich, W.: Fine structure of blood and bone marrow. München: J. F. Lehmann 1969
Hutchison, D.E., Genton, E., Porter, K.A., Daloze, P.M., Huguet, C., Brettschneider, L., Groth, C.G., Starzl, T.E.: Platelet changes following clinical and experimental hepatic homotransplantation. Arch. Surg. **97**, 27–33 (1968)
Marchesi, V.T.: Some electron microscopic observations on interactions between leukocytes, platelets, and endothelial cells in acute inflammation. Ann. N.Y. Acad. Sci. **116**, 774–788 (1964)
Movat, H.Z., Weiser, W.J., Glynn, M.F., Mustard, J.F.: Platelet phagocytosis and aggregation. J. Cell Biol. **27**, 531–543 (1965)
Najarian, J.S., Simons, R.L.: Transplantation. Berlin-Wien-München: Urban & Schwarzenberg 1972
O'Brien, J.R.: An introductory review of platelet physiology In: Platelets and the vessel wall fibrin deposition, ed.: G. G. Schettler, p. 1–5. Stuttgart: G. Thieme 1970
Popper, H., Schaffner, F.: Die Leber. Stuttgart: G. Thieme 1961
Porter, K.A.: Pathology of the orthotopic homograft and heterograft. In: T.E. Starzl and C.W. Putman (ed.), Experience in hepatic transplantation, p. 422–471. Philadelphia-London-Toronto: W. B. Saunders Comp. 1969
Rein, H.: Physiologie des Menschen, 13/14. Aufl. hrsg. von M. Schneider. Berlin-Göttingen-Heidelberg: Springer 1960
Rouiller, Ch. (ed.): The liver, morphology, biochemistry, physiology, vol. I 1963, vol. II 1964. New York-London: Academic Press 1964
Schulz, H.: Thrombocyten und Thrombose im elektronenmikroskopischen Bild. Berlin-Heidelberg-New York: Springer 1968
Sharma, H.M., Moore, S., Merrik, H.W., Smith, M.R.: Platelets in early hyperacute allograft rejection in kidneys and their modification by sulfinpyrazone (anturan) therapy. Amer. J. Path. **66**, 445–460 (1972)
Shirasawa, K., Chandler, A.B.: Phagocytosis of platelets by leucocytes in artificial thrombi and in platelet aggregates induced by adenosine diphosphate. Amer. J. Path. **63**, 215–230 (1971)
Simon, G.T., Burke, J.S.: Electron microscopy of the spleen. III. Erythro-Leukophagocytosis. Amer. J. Path. **58**, 451–469 (1970)
Starzl, T.E.: Experimental and clinical homotransplantation of the liver. Ann. N.Y. Acad. Sci. **120**, 739 (1964)
Starzl, T.E., Marchioro, J.L., Porter, K.A.: Experimental clinical observations after homotransplantation of the whole liver. Rev. Int. Hepat. **15**, 1447 (1965)
Tanikawa, K.: Ultrastructural aspects of the liver and its disorders. Tokyo: Igaku shoin LTD 1968

Prof. Dr. Lothar Cossel
Institute of Pathology
Karl-Marx-University
DDR — 701 Leipzig
Liebigstraße 26
German Democratic Republic