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Absence of macrophage and presence of plasmacellular iron storage in the terminal duodenum of patients with hereditary haemochromatosis

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Summary. Biopsy specimens of the terminal duodenum obtained from 11 patients with hereditary haemochromatosis were examined by light and electron microscopy. Stainable iron was found in the lamina propria of the terminal duodenum in only 4 patients, all of whom were in an advanced stage of the disease. The iron was localized in the basal parts of the villi, sparing their tips, and between the crypts of Lieberkühn. The iron-storing cells could be identified as plasma cells, in which ferritin and haemosiderin were localized within lysosomes and ferritin molecules scattered in the cell sap. There was no storage of iron in macrophages. These observations demonstrate the impaired iron-storing capacity of macrophages in hereditary haemochromatosis, which may be related to the increased iron absorption in this iron storage disease.

Key words: Macrophages – Plasma cells – Duodenum – Hereditary haemochromatosis

Introduction

Iron overload in hereditary haemochromatosis (HH) is considered to be the result of an inappropriately high absorption of iron by the gut from a normal diet (Bothwell et al. 1979). Iron absorption studies have been inconclusive (Heinrich 1970), because they are obviously dependent on the stage of iron overload of the individual patient examined. While iron absorption is increased in young patients during the phase of iron accumulation, it may be normal in untreated patients with long-standing disease (Bothwell et al. 1979). For any given stage, however, absorption of iron is always inappropriately high with respect to body iron stores (Walters et al. 1975). The post-absorption excretion of iron (Björn-Rassmussen et al. 1980) has been constantly found to be very low at different stages of HH (Björn-Rasmussen 1983).

The biochemical mechanism underlying HH is unknown and the anatomical site of the defect in the gut has not been identified. Recently, a four-compartment model of mucosal iron transport with rate constants of uptake of iron from the gut lumen, retention in mucosal storage, and transfer of mucosal iron to the plasma was proposed. This last step has been suggested to be involved in the increased iron absorption in HH (McLaren et al. 1988). Peculiarities in the pattern of iron storage in the small intestine of haemochromatotic patients such as an absence of ferritin bodies within enterocytes and a lack of iron-storing macrophages in the apical villous stroma were described more than 20 years ago (Astaldi et al. 1966; Cattan et al. 1967). In a recent immunohistochemical study with monoclonal antibodies for human isoferritins the enterocytic defect was confirmed but no HH-specific pattern of iron storage in lamina propria cells was found (Fracanzani et al. 1989).

By applying the Prussian blue reaction on semithin sections and using electron microscopy we noticed that iron storage in the lamina propria of patients with HH was confined to plasma cells; macrophages did not take part. This incompetence of duodenal macrophages for iron storage corresponds to the decreased iron storage capacity previously observed in macrophages and their precursors of other organs, tissues, and in cell systems (Brink et al. 1976; Düllmann et al. 1980, 1982; Fillet and Marsaglia 1975; Fillet et al. 1989; Flanagan et al. 1989; Green et al. 1978; McLaren et al. 1979; Sheldon 1935). Its significance for the increased iron absorption in HH will be discussed.

Materials and methods

Diagnosis of HH in the 11 patients was based on a proven iron overload, the absence of any disturbance of erythropoiesis, and an increased frequency of the HLA-A3 antigen (Table 1). As controls, 7 patients with normal serum iron status were used. These

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Table 1. Iron status data and localization of iron in the terminal duodenum of 11 patients with hereditary haemochromatosis

Patient no.	Age (years)	Sex	HLA		Grade of iron overload	Blood removed (l)	Transferrin saturation (%)	Serum ferritin (µg/l)	Macrophageal iron	Plasmacellular iron	Enterocyte ferritin bodies
			A3	B7							
1	41	M	+	+	IV	0	81	ND	0	+	0
1	41				IV	1.2	89	ND	0	+	0
1	45				0 (IV)	50.4	7	23	0	+	0
2	41	M	+	+	IV	0	87	5400	0	+	+
3	52	M	+	+	IV	58.8	69	ND	0	+	0
4	61	M	−	−	IV	0	86	3500	0	0	0
5	52	M	+	+	IV	0	100	5200	0	0	0
6	50	M	+	+	III	5.3	77	ND	0	0	0
6	52				0	13.7	45	ND	0	0	0
6	53				0	15.7	19	ND	0	0	0
7	43	M	+	+	III	0	85	ND	0	0	+
8	26	M	−	−	III	0	84	1940	0	0	0
9	42	M	+	−	III	0	81	4600	0	0	0
10	34	F	+	+	II	16.6	81	ND	0	0	0
11	38	M	+	−	IV	0	79	5530	0	+	0
1–11		(A3+)	81.8%								
Control population		(A3+)	26%		Normal control ($n=7$)		41±11	53±25	0	0	+

underwent routine gastrointestinal examination for suspected stomach and small-bowel disease.

HH was graded as follows:

Stage I. Borderline iron overload, proven by liver biopsy or quantitative phlebotomy, but not detectable by iron status, including ferritin determination.

Stage II. Slight iron overload, detected by elevated transferrin saturation (>60%) mostly in conjunction with hyperferritinaemia; no signs of liver impairment.

Stage III. Moderate iron overload with functional and/or fibrotic liver impairment, mostly reversible by iron depletion.

Stage IV. Heavy iron overload with cirrhosis of the liver and possible, more widely spread organ damage, mostly irreversible by iron depletion.

Stage 0. Therapeutically reversed iron overload without residual organ damage.

Stage 0 (IV). Therapeutically induced absence of iron overload with permanent organ damage such as cirrhosis of the liver.

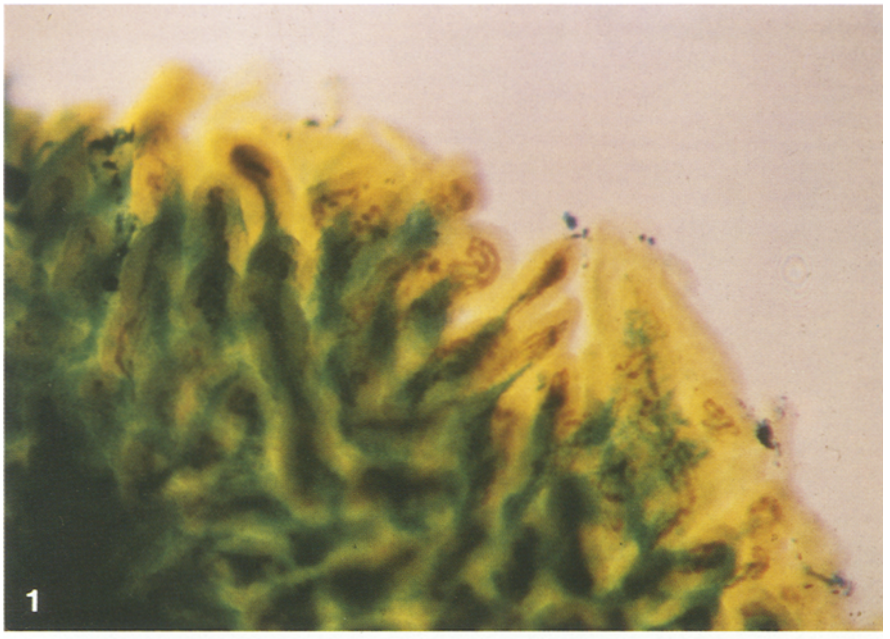
Peroral biopsies of the terminal duodenum were performed with the Crosby capsule from the region of the ligament of Treitz. The biosy specimens were taken after a period of fasting 10–12 h. The correct position of the Crosby capsule was checked by X-ray.

The specimens were fixed in 3% buffered glutaraldehyde and divided into two parts. One part was dehydrated with ethanol, stained en bloc by Prussian blue reaction, again dehydrated and transferred to benzene benzoate (2:1) in order to render it transparent. The other part was postfixed in buffered 1% osmium tetroxide. Dehydration was performed in graded ethanol and the specimens were embedded in Epon 812. Semithin and ultrathin sections were cut on a Reichert ultramicrotome (OM U3). In order to remove the epoxy resin, semithin sections were treated with sodium methanate (Matakas 1968). Prussian blue staining was followed by periodic acid-Schiff counterstaining. Ultrathin sections were examined unstained or after staining with uranyl acetate and lead citrate. Photographs were taken in a Philips EM 301.

Serum iron levels and iron binding capacity were measured by standard techniques. Serum ferritin concentrations were determined by radioimmunassay (Riagnost, Hoechst). HLA antigens A and B were determined by a microlymphocytotoxicity test in the laboratory of Dr. V. Müller, Zentralinstitut für Transfusionsmedizin, Hamburg.

Results

En bloc staining demonstrated iron in the lamina propria of the terminal duodenum in 4 of the 11 patients, each being in stage IV of the disease. It was localized in the basal parts of the villi and in deeper layers of the lamina propria (Fig. 1). On semithin sections it appeared mainly as iron-positive granules of nearly equal size and sporadically as a slight bluish tint of the cytoplasm in medium-sized cells with round to oval eccentrically located nuclei (Fig. 2). Electron microscopically, these cells could be differentiated from macrophages (Fig. 5) and identified by their size and abundant rough endoplasmic reticulum as plasma cells (Fig. 3). Within them, most of the iron was stored in lysosomes in the form of ferritin and haemosiderin. (Figs. 4, 5). The lysosomal ferritin molecules were partially arranged in a paracrystalline array. Furthermore, ferritin molecules were scattered in the cytosol of the plasma cell (Figs. 4, 5). Iron storage in macrophages was not found (Fig. 5). Repeated Crosby biopsies in one patient during venesection therapy revealed a decrease but no disappearance



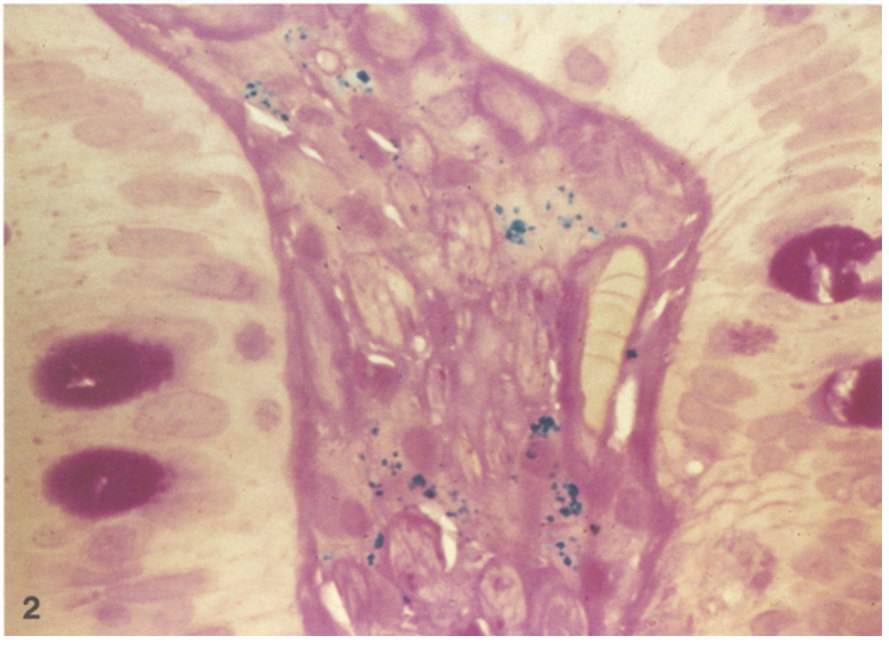


Fig. 1. Iron is not present at the tips of the duodenal villi but in deeper layers of the lamina propria. Terminal duodenum. Stage IV of hereditary haemochromatosis (HH). Biopsy specimen cleared after Prussian blue reaction, ×55

Fig. 2. Well-outlined iron positive granules of nearly equal size and some slight bluish tint of the cytoplasm in medium-sized cells of the basal stroma of a duodenal villus. Terminal duodenum. Stage IV of HH. Semithin section. Prussian blue reaction, PAS counterstain, ×900

of the plasmacellular iron deposits even when iron deficiency anaemia was therapeutically induced (Table 1).

In 2 of the 11 patients ferritin bodies were found in the apical cytoplasm of enterocytes. The iron status data and the localization of iron in the terminal duodenum of the 11 patients are summarized in Table 1.

Discussion

Iron was demonstrated by the Prussian blue reaction in the lamina propria of the terminal duodenum in only 4 of 11 haemochromatotic patients. Its pattern of distribution, sparing the tips of the villi, was similar to that described by Astaldi et al. (1966) in 1 case and to that found by Cattan et al. (1967) in 6 of their 8 patients with HH. Both authors found the iron in cells which they described as macrophages which were either rather small or of medium size (Astaldi et al. 1966), or contained the stainable iron in compact, well-outlined granules of equal size (Cattan 1979, 1983). Using electron microscopy, we identified these iron-storing cells in the duodenal mucosa of haemochromatotic patients as plasma cells, not macrophages.

In our patients with HH, iron storage by duodenal plasma cells was restricted to the most advanced stage

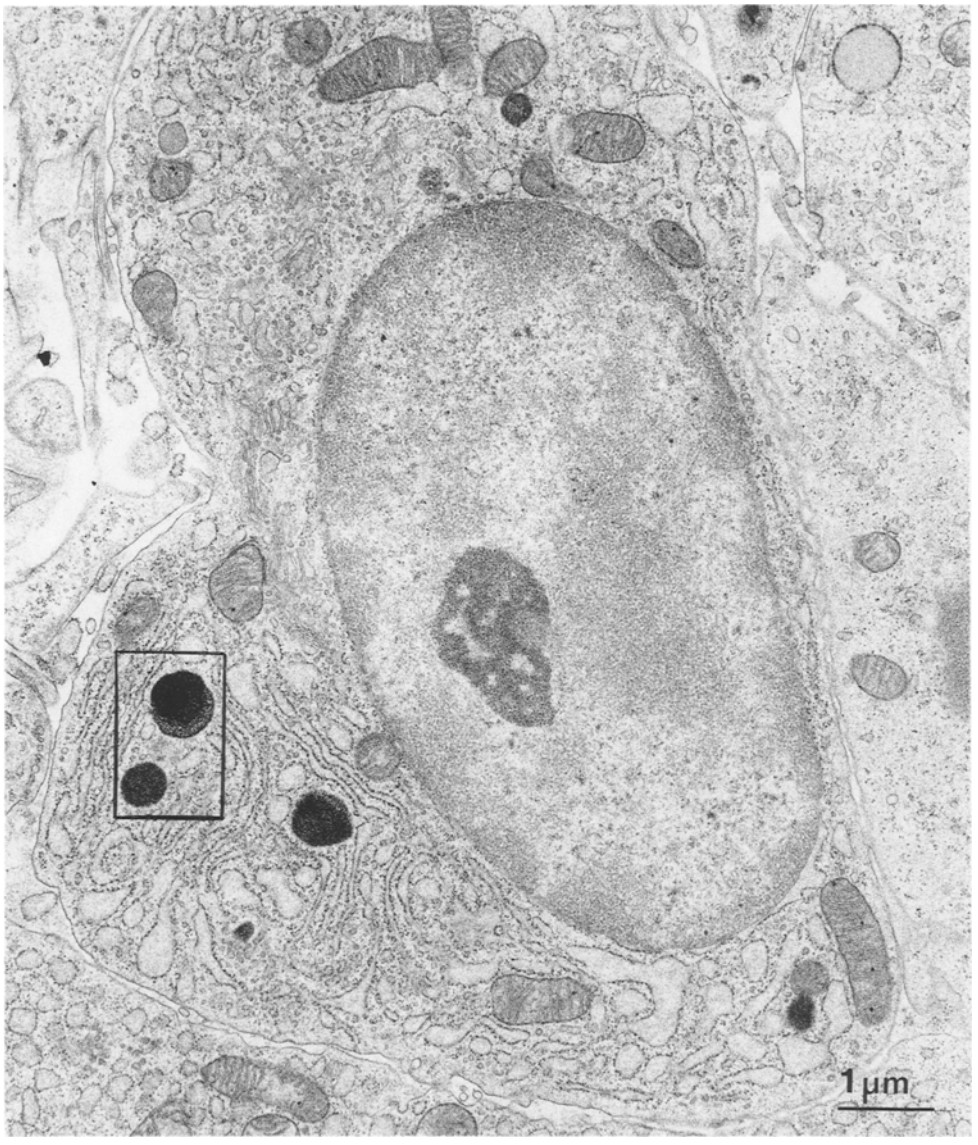


Fig. 3. Iron-containing plasma cell in the lamina propria of the duodenum. The area enclosed in the rectangle is magnified in Fig. 4 to demonstrate the different forms of stored iron. Terminal duodenum. Stage IV of HH. Uranyl acetate lead citrate, ×17100

of the disease (stage IV). Five patients in precirrhotic stages (stage II+III) and a further 2 patients in stage IV revealed no iron storage in the cells of the duodenal lamina propria. Absence of stainable iron in duodenojejunal specimens was first described by Cattan et al. (1967) in 2 of their 8 haemochromatotic patients. The higher incidence of this finding in our group may be due to the fact that the majority belong to the precirrhotic stages.

Iron-storing plasma cells in HH have been described, in a few cases, only in the bone marrow (Koszewski 1952; Goodman and Hall 1966). We have also observed them there in the precirrhotic stages, as well as in the cirrhotic stage of the disease (Düllmann and Wulfhekel 1988). The iron of the bone marrow plasma cells is quite refractory to venesection therapy. This seems also to be the case in duodenal plasma cells, as seen in the follow-up of patient 1 (Table 1).

Lack of stainable iron in macrophages of the terminal duodenum in HH not only concerns the villous apex, as already described (Astaldi et al. 1966; Cattan et al. 1967) but, as shown here, the whole lamina propria. Impaired iron storage in macrophages in HH has already been described in other organs, in the so-called RES, and in the precursors of macrophages, the monocytes (Brink et al. 1976; Düllmann et al. 1980, 1982; Fillet and Marsaglia 1975; Fillet et al. 1989; Flanagan et al. 1989; Green et al. 1978; Sheldon 1935).

The inability of the duodenal macrophage to store iron lends further support to the postulated importance of mucosal macrophages for the regulation of intestinal iron absorption (Cattan 1979, 1983; Cattan et al. 1967). According to the "turntable theory" (Cattan 1983), under normal conditions continuous competition takes place between villous macrophages and the plasma iron carrier transferrin for the intestinally absorbed iron. This

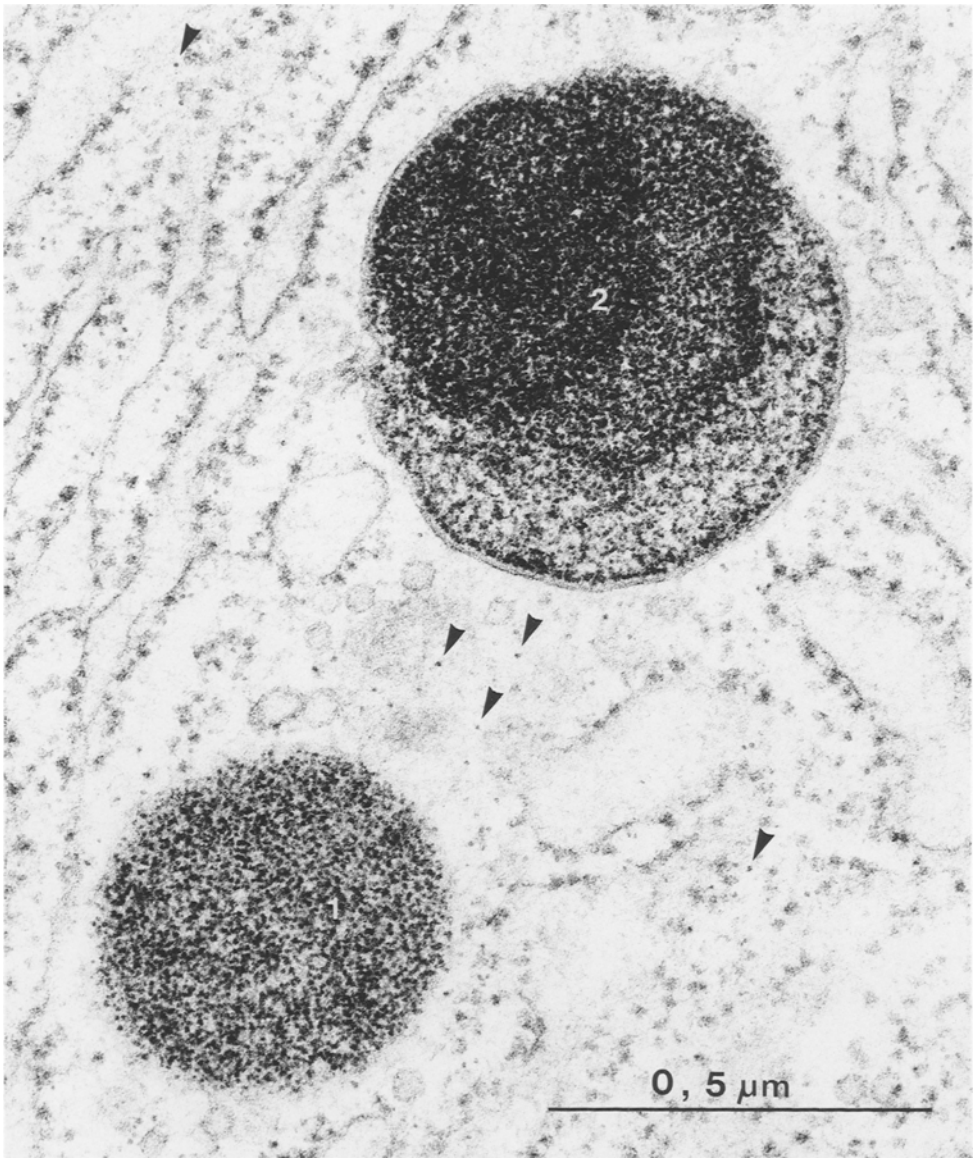


Fig. 4. Part of the plasma cell of Fig. 3. Two lysosomes (1, 2) are filled with ferritin particles and amorphous masses of haemosiderin. Free ferritin molecules (*arrowhead*) are scattered in the cytosol. Terminal duodenum. Stage IV of HH. Uranyl acetate lead citrate, ×135000

mucosal iron store is subject to potential renewal through transepithelial migration of iron-loaded macrophages into the lumen of the gut.

The inability of intestinal macrophages to store iron is in good agreement with the iron absorption data showing an increased mucosal transport index (Powell et al. 1970) and an increased mucosal transfer of iron (McLaren et al. 1988) found in patients with HH. It is in addition consistent with the observation of a decreased intestinal iron excretion (Björn-Rasmussen 1983). Post-absorption iron excretion (Björn-Rasmussen et al. 1980) is a process lasting up to 4 weeks, which must be related to the activities of iron-storing cells of the mucosa such as macrophages, which have a much larger life-span than enterocytes.

In secondary haemochromatosis such as transfusional iron overload there is a converse situation: iron absorption is downregulated and post-absorption excretion of iron is very high (Björn-Rasmussen 1983); duodenal macrophages are heavily iron-laden especially at the tips of the villi and they can be observed crossing the columnar epithelium into the gut lumen (Astaldi et al. 1966; Cattan 1983; Crosby 1963; our own observation).

Absence of ferritin bodies in the enterocytes of haemochromatotic patients has already been related to the high absorption of iron (Crosby 1963). Excessive iron in this situation cannot be removed in the form of ferritin within sloughed enterocytes. In 2 of our patients, however, ferritin bodies were found, as was the case in 3 of 24 haemochromatotic patients studied recently (Fracanzani et al. 1989). Thus Crosby's statement that the intestinal epithelium fails to form ferritin bodies in HH (Crosby 1977) cannot be generalized.

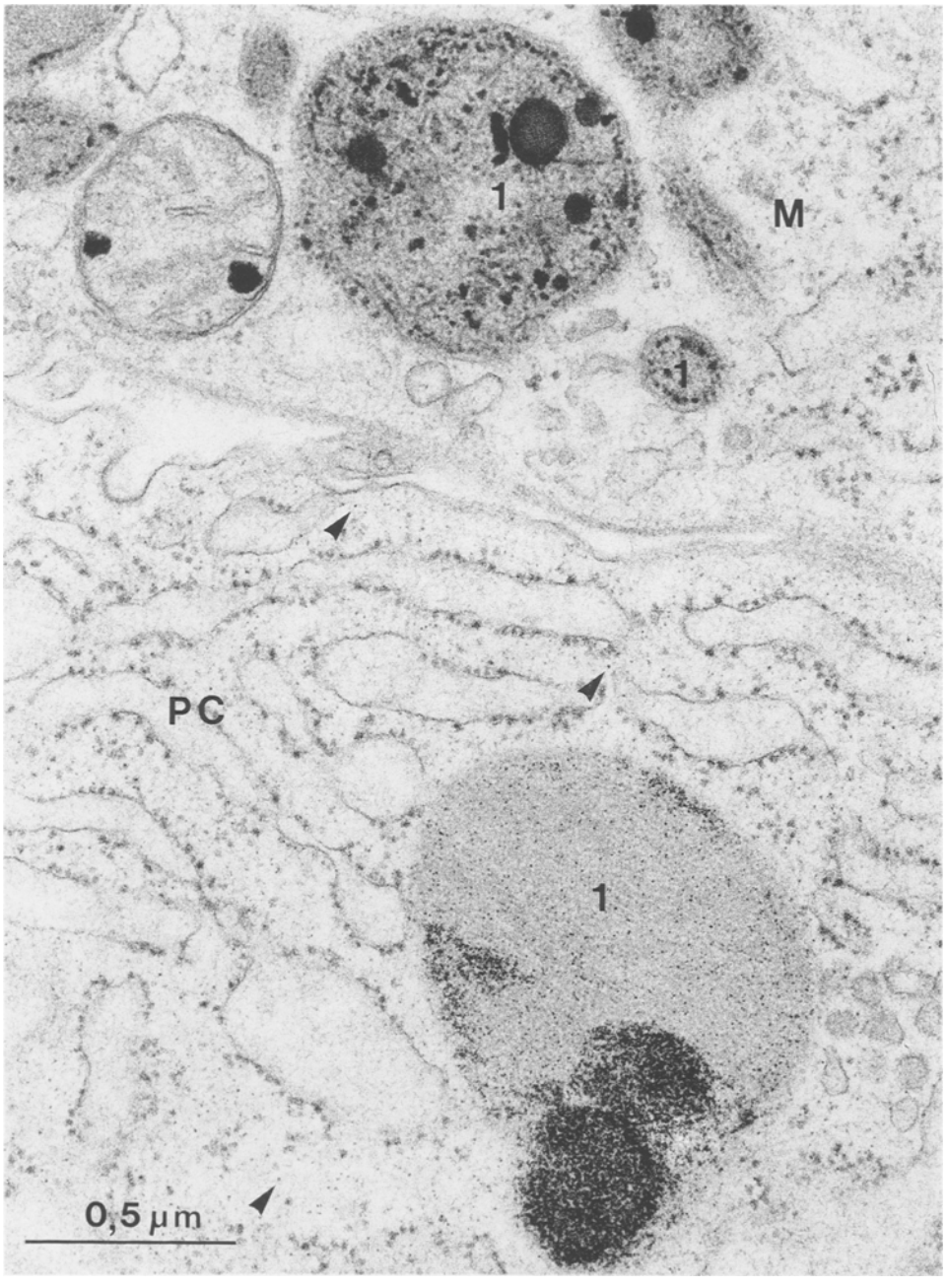


Fig. 5. Plasma cell (*PC*) and adjacent intestinal macrophage (*M*). Within the plasma cell, lysosomes (1) containing ferritin and haemosiderin, as well as free ferritin molecules (*arrowhead*) of the cytosol, are seen. The iron storage compounds are neither present in lysosomes (1) nor in the cell sap of the macrophage. The electron-dense material within the lysosomes and the mitochondria of this macrophage would show up hardly in unstained sections, thus revealing its osmiophilic nature. Terminal duodenum. Stage IV of HH. Uranyl acetate lead citrate, ×75000

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