# *Foamy Cells Associated With Platelet Phagocytosis*

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**In order to gain an insight into the mechanism for the formation of foamy cells (macrophages with foamy cytoplasm) frequently seen in spleens affected by idiopathic thrombocytopenic purpura (ITP), these cells were experimentally induced in mice by subcutaneous injection of platelets with or without accompanied administration of corticosteroid. The light- and electron-microscopic features of experimentally reproduced foamy cells were essentially similar to those seen in the spleens of ITP patients. Corticosteroid had no significant effect on the formation of foamy cells. Most macrophages with foamy cytoplasm contained various amounts of phospholipids, which were derived from platelet membranes. By electron microscopy, myelinlike materials were frequently demonstrated in the cytoplasm of foamy cells. Although lysosomal enzyme activity was revealed in the macrophages that contained morphologically recognizable platelets, there was no demonstrable activity in the cells that contained myelinlike materials. From these results, the following conclusion has been suggested as the mechanism for the formation of foamy cells. Under the state of accelerated phagocytosis of platelets by the macrophages, such as in ITP, the amount of ingested platelet membranes is beyond the capacity of lysosomal digestion. Thus, the incompletely degraded membrane constituents, especially membrane-derived phospholipids, remain in the macrophages, and they are most responsible for the foamy appearance of these macrophages. (Am J Pathol 1984, 114:104–111)**

FOAMY CELLS (macrophages with foamy cytoplasm) frequently observed in the spleens of patients with idiopathic thrombocytopenic purpura (ITP) reveal the characteristic manner of staining for phospholipids and myelinlike materials in electron microscopy.[1-9] It is suggested that myelinlike materials in these cells are chiefly derived from phagocytized platelets. To date, it has not been shown that such foamy cells can be experimentally induced by platelets. In addition, it still remains unknown whether or not steroid therapy, which is usually applied to ITP patients, is related to the appearance of foamy cells in the spleen.

The main purpose of this study was to offer a satisfactory explanation for the mechanism of the formation of foamy cells found in the spleens of patients with ITP. We describe the histologic, immunohistologic, and ultrastructural characteristics of foamy cells experimentally reproduced in mice by subcutaneous injection of platelets with or without steroid administration and of the platelets sensitized with platelet antibody.

## Materials and Methods

### Isolation of Murine Platelets

The blood obtained with 1% EDTA-2Na from ICR mice was centrifuged at 3000 rpm for 15 minutes. We centrifuged the buffy coat at 1000 rpm for 10 minutes to obtain platelet-rich plasma, which was washed by 1% EDTA-2Na saline three times. Platelet concentrate obtained by centrifugation at 3000 rpm for 15 minutes was suspended in 0.01 M phosphate-buffered saline (PBS) at pH 7.2.

### Anti-Platelet Antibody

Antiserums were raised in Japanese white rabbits by subcutaneous injection of 2 ml of platelet suspension ($5 \times 10^9$ platelets in 0.01 M PBS) from ICR mice

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in complete Freund's adjuvant, followed by a booster injection 2 weeks later. The serum collected 2 weeks after the booster injection was absorbed by the murine serum and erythrocytes. The specificity of this antiserum was confirmed by the double-immunodiffusion technique.

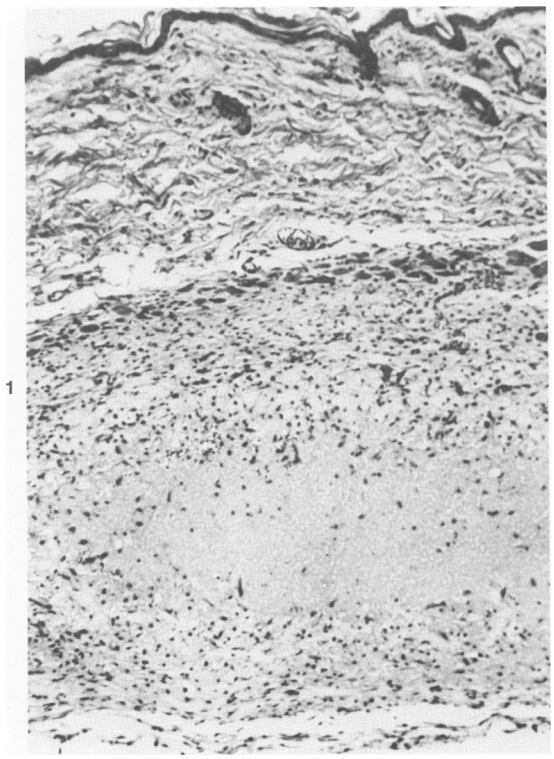
### Reproduction of Foamy Cells

A small volume of platelet suspension ($8 \times 10^8$ platelets/0.05 ml) were subcutaneously injected to AKR and ICR mice. The tissue materials for observations were obtained from the lesions at 3 and 6 hours and on Days 1, 2, 4, 5, 7, 9, 11, 13, 14, and 21 after the injection. Twelve mice were administered steroid (hydrocortisone, 0.02 mg) every day after the platelet injection, and the material was obtained 5 and 7 days later. Murine platelets were incubated with anti-ICR murine platelet antibody for 30 minutes at 37 C. The mixture was centrifuged at 10,000 rpm for 15 minutes. The precipitate was suspended in 0.01 M PBS and subcutaneously injected into the mice. The tissues from the area of the injection were removed at 5 and 7 days after the injection.

### Methods for Histology, Immunohistochemistry, and Electron Microscopy

For histologic examination, the tissue fragments were fixed in 10% buffered formalin. Sections from paraffin blocks were stained with hematoxylin and eosin (H&E). Unlabeled antibody peroxidase–antiperoxidase (PAP) staining with a minor modification of Sternberger's method[10] was employed for the demonstration of murine platelet antigen. The tissues were also fixed in formalin–calcium. Frozen sections were stained with Sudan III, Sudan black, Baker's acid hematin,[11] Smith-Dietrich[12] for demonstration of phospholipid, acid phosphatase,[13] and β-glucuronidase.[14]

For transmission electron microscopy, the subcutaneous tissues were fixed in 2.1% glutaraldehyde at 4 C and postfixed in 1% osmium tetroxide. They were dehydrated in gradient ethanol and embedded in Epon 812. Semithin sections stained with alkaline toluidine blue were examined with a light microscope for selection of the appropriate areas. The ultrathin sections cut with an LKB ultramicrotome were stained with uranyl acetate and lead citrate. They were examined in a Hitachi H-300 electron microscope.



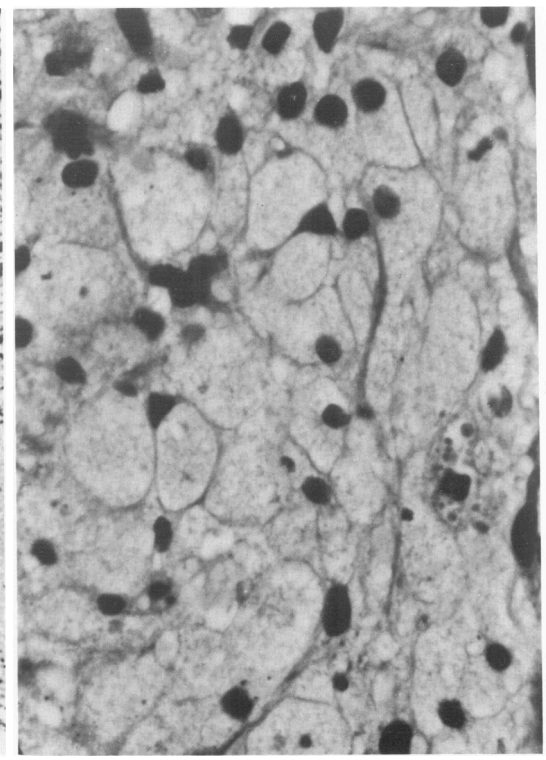


**Figure 1**—A large number of foamy cells are demonstrated in the peripheral area of platelet mass. (H&E)

**Figure 2**—These foamy cells have an eccentric nucleus and pale cytoplasm. (H&E)

Subcutaneous tissues fixed in cold 2.1% glutaraldehyde in cacodylate buffer were incubated in the solution containing lead nitrate and β-glycerophosphate for the demonstration of acid phosphatase activity. The selected tissues at Days 2, 5, and 7 were fixed in cold periodate, lysine–paraformaldehyde (PLP). Frozen sections were incubated in the solution containing anti-murine antibody (Fab′) conjugated with peroxidase and reacted with 3,3′-diaminobenzidine (DAB). Then they were postfixed in 1% osmium tetroxide and processed for electron microscopy.

## Results

Until 2 days after the injection of platelets, miliary nodules with whitish cut surface were clearly noted in the subcutaneous tissue. During Days 4 and 7, these nodules were gradually dispersed and almost completely disappeared at Day 14.

### Microscopic Findings

The mass of platelets injected into the subcutaneous tissues was stained pinkish red with H&E. At 3 and 6 hours, a few neutrophils infiltrated around the mass of platelets, and after 24 hours a large number of neutrophils and several macrophages were noted around and within the mass. After 2 and 4 days, large numbers of macrophages migrated around and into the central area of the mass. Large macrophages with foamy cytoplasm (foamy cells) were scattered around the mass at this stage. At Day 7, a mantle of these foamy cells surrounding the platelet mass and accompanied by a proliferation of capillaries was noted in the subcutaneous tissue (Figure 1), giving a granulomatous appearance to the area. The foamy cells had an eccentric nucleus, clear cytoplasm with irregular stippling, and poorly defined foamy vacuolation (Figure 2). These cells were stained orange-red with Sudan III, black with Sudan black, grayish black with Baker's acid hematin, and blackish blue with Smith–Dietrich stain for demonstration of phospholipid (Figure 3). Moderate activity of acid phosphatase and β-glucuronidase was demonstrated in the macrophages located in the peripheral area of the nodule. On the contrary, this enzyme activity was markedly decreased in the foamy cells. Although some foamy cells contained trace activity, the others exhibited complete absence of this enzyme activity.

At Days 9–14, the number of foamy cells was gradually decreased and replaced by macrophages with usual appearance and fibroblasts. Subcutaneous nodules induced by platelet injection were completely resolved at Day 21.

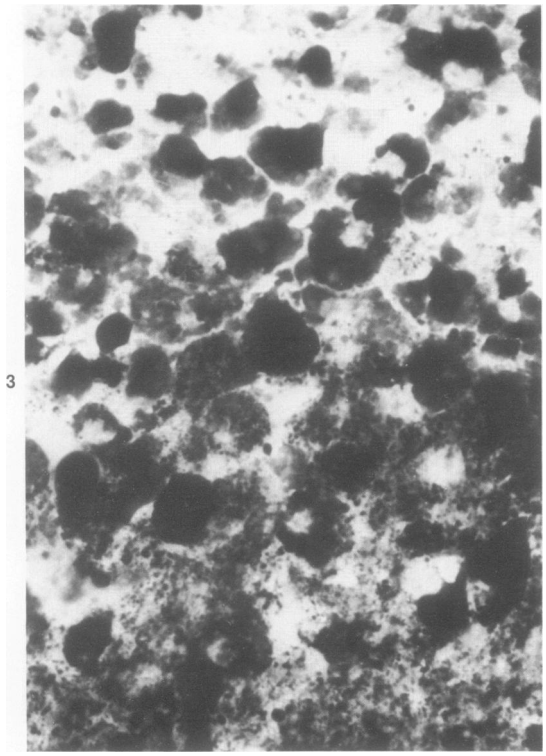


**Figure 3** — The foamy cells are stained blackish blue. (Smith–Dietrich reaction) material immunoreactive for anti-murine platelet antibody. (PAP)

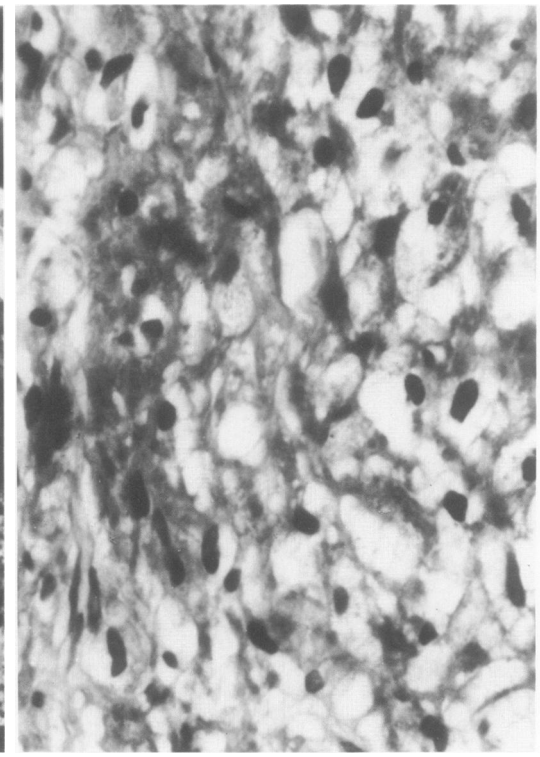


**Figure 4** — Several macrophages and some foamy cells contain

### Immunohistochemical Findings

At Day 2, a few macrophages revealed platelet-associated antigens in their cytoplasm. A small amount of material positive for anti-murine platelet antibody were also noted in occasional neutrophils. At Day 7, some foamy cells surrounding the platelet mass exhibited platelet antigens in the cytoplasm (Figure 4).

### Electron-Microscopic Findings

At Day 2, many neutrophils and several macrophages phagocytized the intact-appearing or partially degraded platelets. A few macrophages had various kinds of degraded structures of platelets. The membrane surrounding the engulfed platelets became denser, and platelets revealed some darkening of the cellular matrix and condensation of their specific granules (Figure 5). As the intracellular digestion advanced, the phagocytized platelets were condensed into coarsely granular or mottled inclusions. At this stage, structures recognizable as platelets could hardly be seen. Some myelinlike material was noted in the phagocytic vacuoles. A large amount of product immunoreactive for anti-murine platelet antibody was recognized in most of the phagocytic vacuoles (Figure 6). At Days 5 and 7, there were many macrophages that contained abundant myelinlike material. These macrophages usually had moderately developed Golgi apparatus, a few mitochondria, and occasional lysosomal dense bodies (Figures 8 and 9). Based on the light-microscopic study of semithin sections stained with toluidine blue, the foamy cells demonstrable by light microscopy were estimated to correspond to the macrophages containing abundant myelinlike material. Acid phosphatase activity was noted around and within digested platelets (Figure 7). However, there was no demonstrable activity in most of myelinlike material. At Days 9 and 11, there were many dense bodies and some myelinlike material in many macrophages (Figure 10). At this stage several fibroblasts were demonstrated in the lesion.

Histologic and ultrastructural findings of the subcutaneous nodules induced by the injection of sensitized platelets and observed at Days 5 and 7 were essentially similar to those of the lesions produced by untreated platelets, except for the more numerous foamy cells in this group (Figure 11).

Administration of corticosteroid had no significant influence on the histologic and ultrastructural findings described above. Under the experimental conditions employed in this study, this agent had neither promotive nor suppressive effects on the appearance of foamy cells.

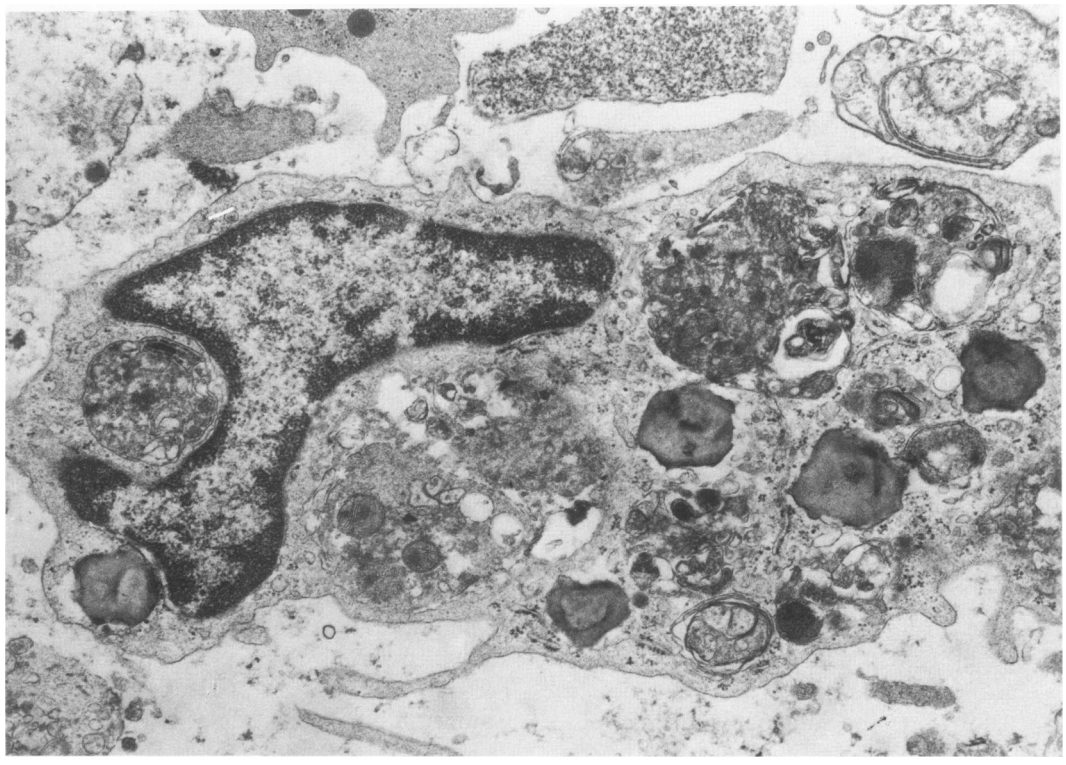


**Figure 5**—A macrophage contains several platelets in various stages of digestion. (×17,700)

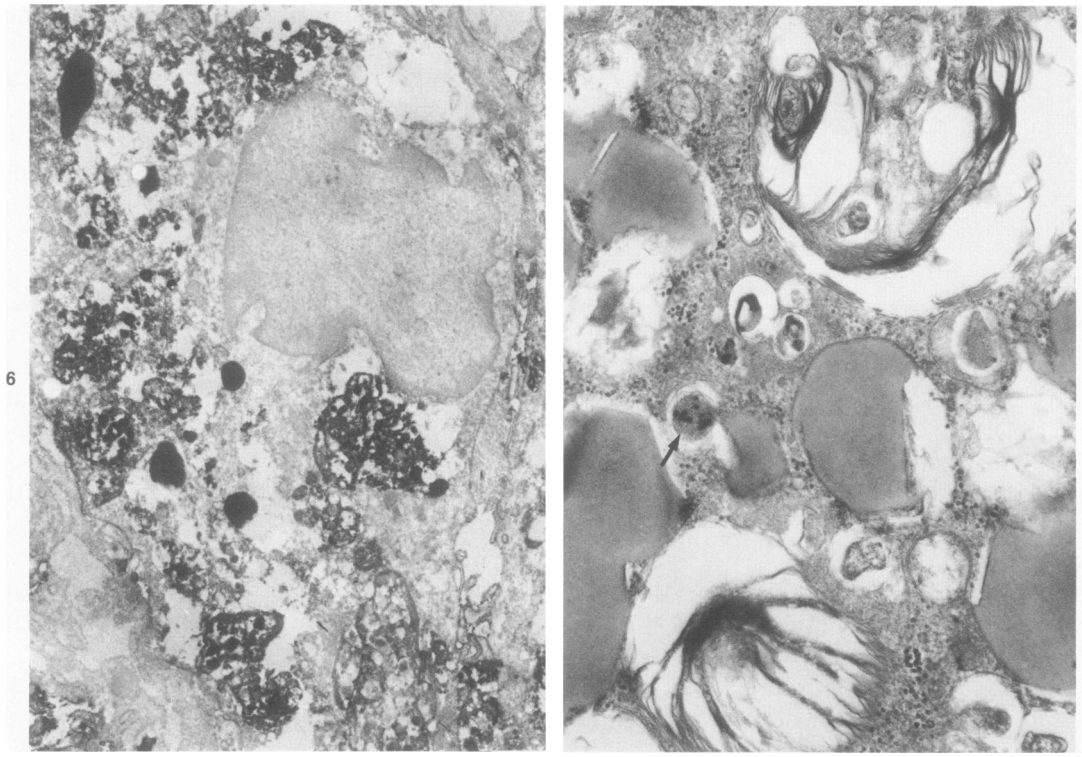


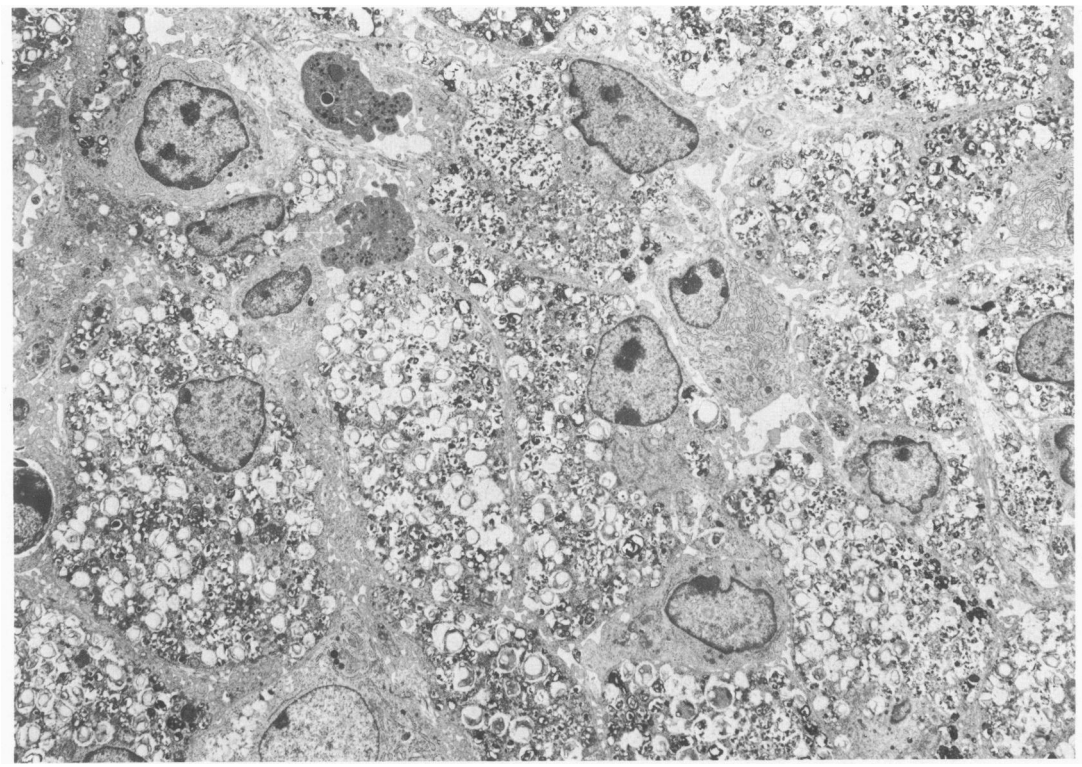
**Figure 6** — A large amount of product immunoreactive for anti-murine platelet antibody is recognized in macrophages. (× 7000) **Figure 7** — Some material positive for acid phosphatase can be seen in a lysosome (*arrow*), but no activity is demonstrated within myelinlike material. (× 23,000)

## Discussion

Presently, it is generally accepted that the spleen plays a central role in the pathogenesis of ITP and that splenectomy is the therapeutic measure of choice in the management of patients with this disease. The light- and electron-microscopic findings of the removed spleen and observations concerning platelet phagocytosis and foamy macrophages have been described by many investigators.[1-9] In order to provide additional evidence for the mechanism of formation of foamy macrophages in ITP spleens, they were experimentally reproduced in mice by subcutaneous injection of platelets. In the preliminary experiment, we administered platelets into the tail vein of the mice. Most of the infused platelets were trapped in the pulmonary capillaries, and the animals died within a few days. Therefore, a time-course observation was impossible in these animals. The best way to introduce platelets seemed to be via the splenic artery after laparatomy. But we found it technically very difficult to inject a large number of platelets into the mice by this route. These were the main reasons we injected platelet suspensions into the subcutaneous tissue.

The light- and electron-microscopic features of the foamy cells experimentally induced in this study are essentially similar to those seen in the spleen from patients with ITP. As morphologic characteristics common between the two, they contain phospholipids and myelinlike material in their cytoplasm, as revealed by histochemistry and electron microscopy, respectively. In previous reports it was suggested that the phospholipid content[1] and the cytoplasmic inclusions[5,6,9] observed in the foamy macrophages in the spleen are derived from platelets as a result of platelet phagocytosis. The present observations provide more direct evidence supporting this concept. Furthermore, this experimental investigation clarifies the role of steroid therapy and anti-platelet antibody in the appearance of foamy cells and demonstrates more precisely than hitherto the sequence in which these cells are produced.

Several investigators have suggested that the foamy cells in the spleen from patients with ITP are related to steroid therapy.[3,4] On the contrary, others insist that the appearance of these cells are not necessarily related to this agent.[2,6,7] Because corticosteroid is known to stabilize the cell membrane, it may be expected that the administration of corticosteroid would inhibit platelet phagocytosis and intracellular degradation and thus reduce the number of foamy cells. Contrary to this expectation, administration of



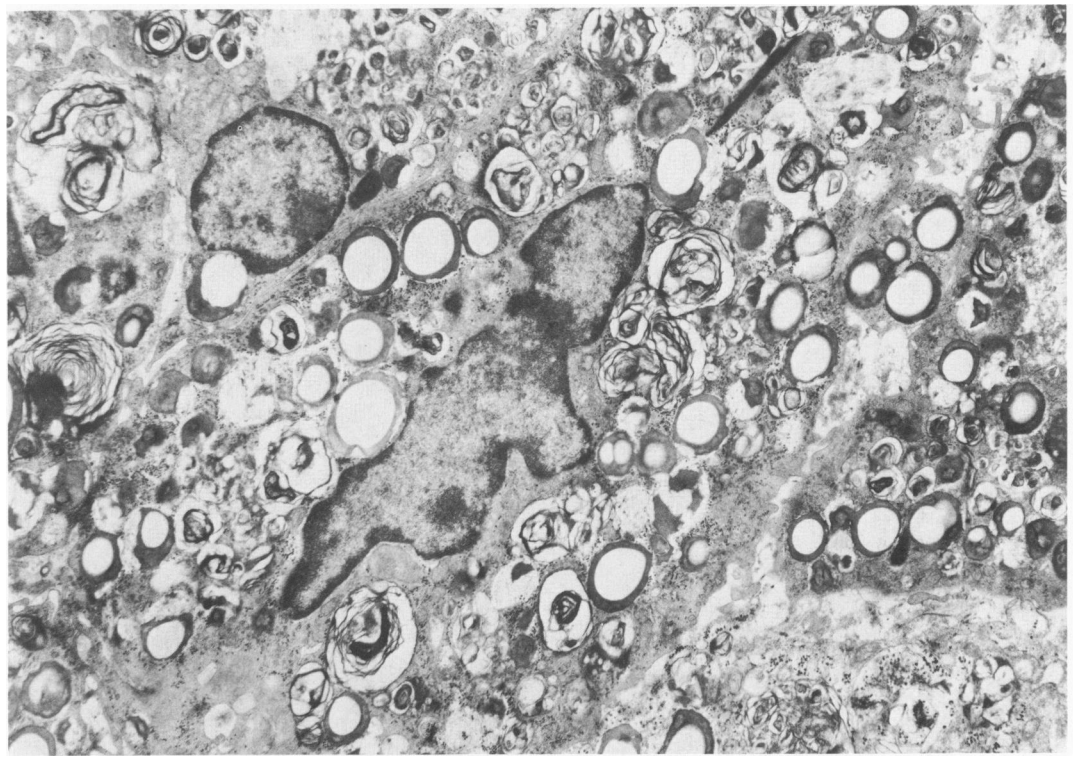


**Figure 8** — There are many macrophages that contain large amounts of myelinlike material. (× 2000)

**Figure 9** — A large amount of myelinlike material and dense bodies is noted in a macrophage. (× 7000)

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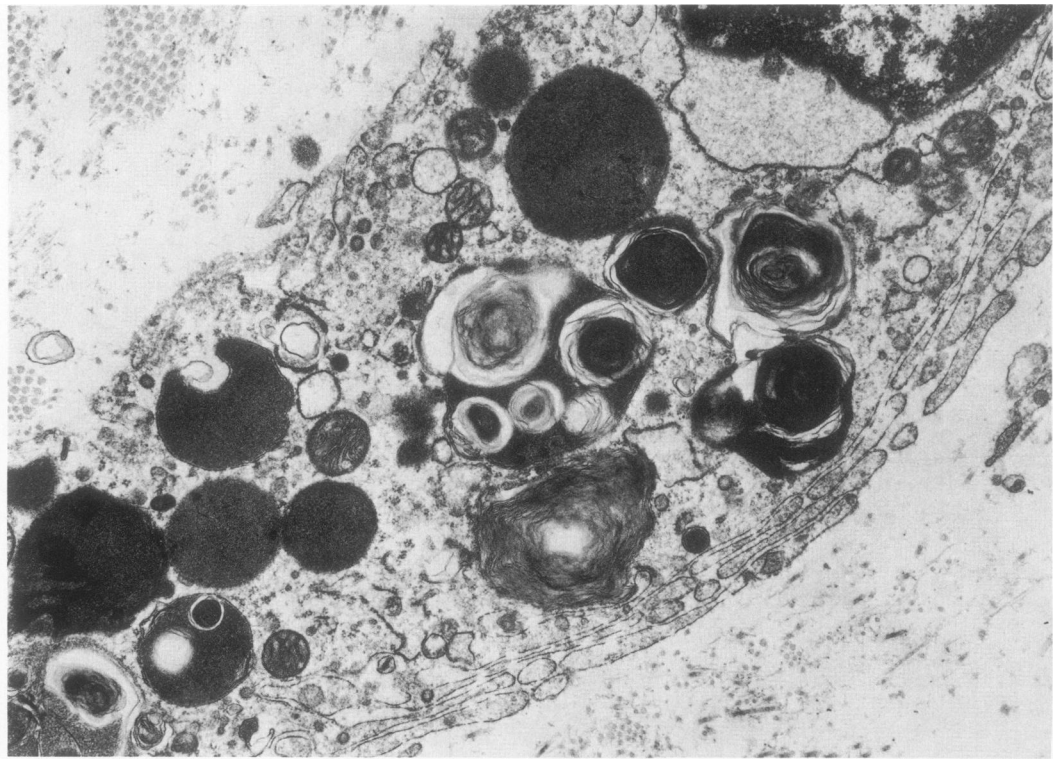

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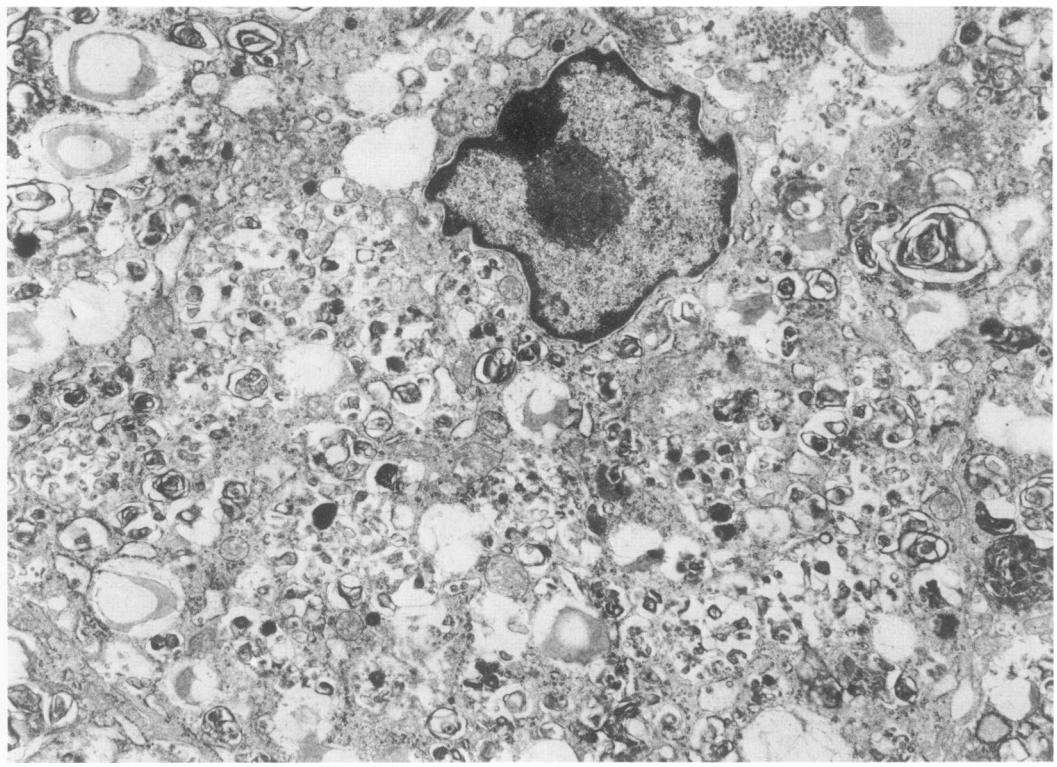

**Figure 10** – A macrophage has dense bodies and some myelinlike material. (×15,000) **Figure 11** – Myelinlike material and dense bodies are noted in a foamy cell induced by subcutaneous injection of sensitized platelets. (×8500)

this agent neither accelerated nor inhibited the formation of foamy cells under the experimental conditions employed in this study.

It is widely accepted that platelets in patients with ITP are sensitized with anti-platelet antibody, and they are easily phagocytized by the splenic macrophages. In this experiment, foamy cells were more conspicuous when sensitized platelets were injected to the subcutaneous tissue. This suggests that the platelets coated with immunoglobulins are more susceptible to phagocytosis than those without antibody. However, the ultrastructural findings in foamy cells were essentially the same between the two groups. Although the binding of immunoglobulin to the platelet seems to be a prerequisite for accelerated phagocytosis, it is not necessarily related to the formation of foamy cells.

The findings obtained in this study indicate that the incompletely degraded platelet membranes are most responsible for the phospholipid content and myelinlike material of the foamy cells. Histochemical studies revealed a weak activity of acid phosphatase and β-glucuronidase in occasional foamy cells. Within the macrophages that contained myelinlike structures, a trace activity of acid phosphatase was demonstrated by electron microscopy. Although immunoreactive platelet antigen was demonstrated in the macrophages that contained morphologically recognizable platelets, it was hardly revealed in most of foamy cells. These findings may lead to the proposal that the foamy cells have already consumed their lysosomal enzymes in the process of platelet digestion, and that the remaining lysosomal enzymes are not sufficient to digest myelinlike material further. The phospholipids within the phagocytic vacuoles seem to be vulnerable to lysosomal digestion, because lysosomal enzymes of macrophages are shown to contain phospholipase.[15] As a possible explanation for a reduced reaction for lysosomal enzymes, we suggest that the macrophages have enough lysosomal enzymes to digest proteinous substances, but phospholipase activity to digest membrane phospholipids is insufficient. On the basis of these speculations, it appears possible that in case of accelerated platelet phagocytosis, such as in ITP, the amount of ingested cellular membranes is beyond the capacity of lysosomal digestion. Thus, the incompletely degraded membrane constituents, especially phospholipids, remain in the macrophages for a certain period of time and lead them to transform into lipid-laden macrophages with a foamy appearance.

An alternative explanation for the decreased activity of lysosomal enzymes in foamy cells is that the enzyme may be inducible, and extensive platelet phagocytosis results in the expansion of macrophage cytoplasm and in a dispersion of enzymes over a large area. It is reasonable to assume that the expansion of the cytoplasm may give a weaker reaction. At present, we have no conclusive evidence to exclude this possibility. However, electron microscopy revealed complete absence of acid phosphatase in some foamy cells. In addition, lysosomal enzyme activity was demonstrated in the expanded cytoplasm of macrophages that contained morphologically recognizable platelets. Because of these findings we favor the possibility of enzyme exhaustion in the foamy cells. More experimental evidence is necessary for a definite answer to this question.

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