SOME MORPHOLOGIC CHARACTERISTICS OF WHIPPLE'S DISEASE

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Following Whipple's original description[1] of the disease which is now known by his name, investigations have emphasized the alteration in lipid metabolism which forms one important aspect of the total disease picture. This emphasis received initial impetus from the fact that the name originally proposed for the disease by Whipple himself was intestinal lipodystrophy. This term, while descriptive of some of the obvious features of the disorder, unfortunately neglected an important morphologic feature noted by Whipple in his original description. This was the presence, in addition to the increased quantity of fat and fat-containing cells, of many large cells resembling macrophages which contained finely vacuolated, frothy nonosmiophilic cytoplasm. There is now virtually unanimous agreement that (a) these cells do not contain lipid stainable by any of the usual histochemical procedures; (b) most of them contain large quantities of periodic acid-Schiff (PAS) positive material, probably glycoprotein; and (c) as a morphologic feature they appear to be unique and therefore may be of greater diagnostic significance than the histologically impressive but nonspecific accumulation of lipid in the affected tissues. It is of interest that the PAS stain, not available at the time of Whipple's study, has since been applied to the formalin-preserved specimens from his original case, and it has been demonstrated that the large foamy histiocytes originally described in the submucosa did contain PAS-positive material.[2]

The term "sickle form particle containing" (SPC) cell was coined by Haubrich, Watson and Sieracki[3] in 1958 to describe these large PAS-positive histiocytes. This term derived from the fact that a touch preparation prepared from a biopsy specimen containing such cells, stained by the PAS technique, showed clusters of distinct particles. These particles were highly PAS-positive, measured 2 to 3 μ in major dimension, and in contour bore a striking resemblance to sickled erythrocytes. Such PAS-positive histiocytes have been identified in a number of body tissues and organs other than intestine.[3]

This research was supported in part by United States Public Health Service Research Grant No. C-2738.

Accepted for publication, November 3, 1962.

* Recipient of USPHS Senior Research Fellowship No. SF-37.

Several combined light and electron microscopic studies of the distinctive morphologic features of the histiocytes have been carried out.[3-6] Haubrich and co-workers[3] identified in their electron micrographs the large frothy cells which were seen with the light microscope, and within the cytoplasm of these cells they observed densely packed structures interpreted as the sickle form particles seen in touch preparations. More detailed observations on the structure of these particles were made by Cohen, Schimmel, Holt and Isselbacher[4] who, by the technique of serial thick and thin sections, demonstrated identity of the ovoid cytoplasmic particles in the electron micrographs with the PAS-positive granules (sickle particles) observable with the light microscope.

Whipple described a second type of particle as "bacilliform" in appearance but came to no definite conclusion concerning its significance. Similar particles have been identified by a number of other investigators.[3-6] Chears and Ashworth[5] and Yardley and Hendrix,[6] in recent investigations on the ultrastructural features of Whipple's disease, emphasized the possible etiologic significance of the bacilliform bodies and their relationship to the PAS-positive granules.

It is our purpose to present further information concerning the fine structure of the cytoplasmic particles, to consider the etiologic significance of the bacilliform bodies, and to present observations on the intercellular relationships of the epithelium in Whipple's disease.

Methods

Biopsy material for examination was obtained via peroral jejunal biopsy.[7] The patient was a 48-year-old man. Onset of illness occurred 3 years prior to biopsy, and steroid therapy in the form of Meticorten® alternating with ACTH was started 7 months prior to biopsy; definite clinical improvement was noted. Details of the clinical course have been published.[7]

For electron microscopy a portion of the biopsy specimen was fixed immediately upon removal from the biopsy tube in a solution of 1 per cent osmium tetroxide buffered to a pH of 7.4 in veronal acetate buffer. After fixation for 1½ hours the specimen was dehydrated and carried into prepolymerized methacrylate by successive 10-minute changes in the following fluids: buffer, 70 per cent ethanol, 80 per cent ethanol, 95 per cent ethanol, 3 changes of absolute ethanol, equal volumes of absolute ethanol and monomer, and 3 changes of methacrylate monomer. Polymerization was carried out at 65° C. Sections were cut with either a glass or a diamond knife on a Servall Porter-Blum microtome. Sections were mounted on formvar-coated copper specimen grids and examined either directly or after lead hydroxide post stain with an RCA EMU-2E electron microscope. Photographs were made at instrument magnifications of 2,230 to 10,070, and further photographic enlargement made to the values indicated in the figure legends.

For light microscopy a larger portion of the biopsy specimen was fixed immediately in Bouin's solution and paraffin-embedded sections were prepared utilizing conventional histologic techniques. Representative sections were stained with hematoxylin and eosin (H and E), Masson, Brown-Brenn, and the periodic acid-Schiff (PAS) stains.

OBSERVATIONS

Structural Features

The over-all histologic pattern was characteristic of Whipple's disease. Many of the villi were thick, with bulbous edematous tips, but a number of villi appeared normal. In most zones, especially in the normal-appearing villi, the striate border was well preserved and normal in appearance. In some zones, however, it was slightly irregular and somewhat attenuated. This was most pronounced in those villi which appeared to be the most severely affected. The intestinal epithelium was relatively normal in appearance throughout most of the biopsy specimen, there being the usual complement of goblet cells scattered amongst the surface epithelium. The crypt epithelium was also relatively normal in appearance. A few argentaffin cells were identified and Paneth cells were plentiful. Within the affected villi, large numbers of the large characteristically foamy histiocytes lay just beneath the basement membrane (Figs. 1 to 6). In addition to the infiltrate of foamy histiocytes in the lamina propria there were large numbers of other types of cells in the exudate. For the most part these consisted of small macrophages and lymphocytes, but there were occasional plasma cells and eosinophils as well as occasional neutrophils.

In some of the most severely affected villi, numerous intercellular spaces were noted between the columnar cells of the surface epithelium. These varied in major dimension from 1 to 5 μ. In the paraffin sections the spaces appeared empty. In most of the electron micrographs of the epithelial layer, intercellular empty spaces corresponding to those seen with the light microscope were visible (Fig. 7). The width of these spaces varied from about 0.1 μ to 3 or 4 μ, and their profiles were generally triangular or irregularly polyhedral. In the electron micrographs the spaces appeared to be more widespread than had been anticipated from the paraffin sections, apparently because many of them were too small to be distinctly visible with the light microscope. In most of the electron micrographs the spaces contained small quantities of finely granular material, and occasionally a few fine fibrils. We were never able to observe continuity of the intercellular spaces with the lumen surface of the mucosa, the nearest approach being to approximately 8 to 10 μ from the lumen border of the cell. Neither were we able to demonstrate continuity with the subepithelial space or the basement membrane of the mucosal layer. It seemed likely that the spaces were confined to the epithelial layer itself, although to rule out conclusively the possibility of rare connections with the lumen surface or the subepithelial extracellular space, more thorough studies would be required.

The present case differed from Whipple's original case and from a

number of other published cases in the complete absence of bacilliform bodies.

Histiocytes

With the light microscope 3 different forms of histiocytes could be identified. The first (Fig. 1) had a grossly foamy appearance with the H and E stain. The individual vacuoles were fairly distinct and gave the impression of being separate cytoplasmic objects, an impression borne out by the PAS stain (Fig. 2). Within this it could be seen that highly specific PAS-positive material was present as discrete granules and clumps, corresponding roughly in size to the individual vacuoles seen with the H and E stain. Form 2 (Fig. 3) contained only a very small number of much smaller vacuoles or none at all. Such cells (Fig. 4), were highly PAS-positive, but the stained material was distributed diffusely and uniformly throughout the cytoplasm, with only a few very tiny and indistinct granules. The third form (Fig. 5) resembled the first, as seen in the H and E sections, but differed in that with the PAS stain (Fig. 6) only some of the vacuolated spaces contained PAS-positive material, and in extreme cases some cells appeared to be almost entirely PAS-negative. Approximately two thirds of the histiocytes were classified as form 1, about one third as form 2, while less than 5 per cent were form 3. There appeared to be no site of predilection for either of the 3 forms; however, the granular (form 1) histiocytes were usually more numerous in the tips of the bulbous affected villi, whereas form 2 histiocytes occurred chiefly in the submucosa, deep to the crypts. Form 3 cells did not appear to be specifically localized.

The electron microscopic appearance of a typical histiocyte is depicted in Figure 8. The nucleus was rather small and usually had a circular profile although minor irregularities were frequently seen. The cytoplasm contained abundant endoplasmic reticulum of both smooth-surfaced and rough-surfaced varieties, the former usually predominating. The cytoplasmic bodies corresponding to the PAS-positive granules had a varied and complex structure (Figs. 9 and 10). They were limited by a membrane which in many places could be seen to be a double structure measuring approximately 100 Å in width. The bodies contained irregular masses of dense osmiophilic substance including homogeneous, faintly granular material and fibrils of varying length and width (Fig. 10). In places where the latter were favorably oriented they could be seen to have an axial periodicity which was of the order of 60 to 80 Å (Fig. 9 inset). Other types of linear structures were also seen (Figs. 9 and 10). No periodicity could be detected in these, and they were more suggestive of a laminar membrane system rather than filaments.

Most of the histiocytes contained moderately large quantities of ferritin within the cytoplasm (Figs. 9 and 10) distributed usually in a diffuse fashion without sites of predilection except that the cytoplasmic bodies contained virtually none.

Discussion

The significance of the 3 morphologic types of histiocytes in Whipple's disease is not clear. There may be some relationship to Rowlands and Landing's histochemical studies [8] which have indicated the presence of more than one molecular form of glycoprotein within the cells. We should like to emphasize, however, that although we arbitrarily divided the histiocytes into 3 different categories, transitional forms could be found and it is possible that the varying appearances might be related only to different degrees of deposition of PAS-positive substance.

The absence of bacilliform bodies may bear on the unresolved question whether the PAS-positive material within the histiocytes is of intrinsic or extrinsic origin. Evidence in favor of extrinsic origin from a phagocytized bacterial product was suggested by Chears and Ashworth.[5] It was first fully discussed by Yardley and Hendrix,[6] who suggested that the PAS-positive material in the histiocytes might be derived from a hypothetical capsular polysaccharide of phagocytized bacteria. Their data and argument strongly supported the suggestion that at least part of the PAS-positive substance might have such an origin. Notwithstanding this, the fact that at no time were any bacilliform bodies found in the present case, and the existence of a large population of strongly PAS-positive histiocytes compel one to consider the possibility that at least some of the PAS-positive material was not of bacterial origin.

The cytoplasmic particles in the histiocytes have been described differently by various observers. Haubrich and co-workers [3] described them as clusters of minute vacuolated bodies. Although it was evident that the particles had a complex fine structure, little further detail of the fine structure was given. Cohen and co-workers [4] stated that the internal structure of the particles, which they called ovoid bodies or inclusions, consisted of closely packed membranes, granules and vesicles. Although the electron microscopic appearance of the particles in our case was similar, there were certain differences. These were (a) the absence of any evidence of vacuoles within the particles themselves, and (b) the presence of material which we interpreted to be fibrillar and to exhibit an axial periodicity of approximately 60 to 80 Å.

We concurred with Cohen and co-workers [4] in the observation of inclusions. In many ways these membrane systems were reminiscent of the double membranes of mitochondria; however no morphologic evi-

dence other than the similarity suggested a closer relationship. The relationship of the bodies to the vesicles of the endoplasmic reticulum was difficult to assess. In rare cases the outer membrane of the body appeared to be continuous with an adjacent vesicle, usually of the smooth-surfaced variety, but there was no suggestion of extensive connection with the intracanalicular system of the cell. It was noted that most of the histiocytes containing the ovoid bodies also contained an exceptionally well-developed endoplasmic reticulum (Figs. 8 and 9) giving the cell a vacuolated appearance. In all cases the vesicles of the endoplasmic reticulum were external to the ovoid bodies. The nature of the fibrils and granular material that made up large portions of the bodies was not clear. Our knowledge of the physical form, degree of aggregation and other characteristics of glycoproteins is not sufficiently precise to allow a definite statement concerning the relationships of the fibrils and their periodic structure to the molecular make-up of the specific glycoproteins present in the histiocytes. Such molecules are known to be highly asymmetrical, however, and it seems reasonable to speculate that the fibrils represented aggregations of individual glycoprotein molecules.

The intra-epithelial intercellular spaces observed in the present specimens may be analogous to the intercellular spaces demonstrated by Chears and Ashworth.[5] In their material, however, the spaces contained dense precipitates of both granular and fibrillar osmiophilic material in quantities sufficient to fill the spaces almost entirely. It is, of course, possible that these spaces represented artifacts of preparation; their presence in tissues fixed and embedded by different methods argues against this possibility.

The bacilliform particles noted by Whipple have since been described as occurring in both intracellular and extracellular locations.[3-6] Haubrich and co-workers[3] suggested that they might be products of cellular elaboration of PAS-positive substance; Cohen and co-workers[4] mentioned the possibility that they might signify virus substance. Both Chears and Ashworth[5] and Yardley and Hendrix,[6] noting the bacillary appearance of the particles and their probable identity with Whipple's "bacillary" bodies, presented excellent and conservative discussions of their possible etiologic significance. Although the exact nature of the particles has not yet been determined, it seems now almost certain that they are identical with the small Levaditi-positive bacilliform bodies described by Whipple. Published electron micrographs[3-5] indicate that the bodies bear little if any resemblance to any known virus, the appearance being highly suggestive of small bacteria as originally considered by Whipple.

Speculation concerning the particles has naturally centered about whether they have etiologic significance. Although the classical criteria for establishing them as etiologic agents have not been satisfied, a number of observations favor this hypothesis. They have been identified in a number of cases of Whipple's disease but have not, to our knowledge, been found in the intestine in other conditions (the data on this point are sparse). The particles have been noted in contact with histiocytes and within their cytoplasm. The possibility that a bacterial capsular polysaccharide contributes to the formation of intracellular ovoid bodies in histiocytes is credible though as yet unproved. It is a primary point of the present communication to emphasize that PAS-positive material may appear in the absence of such bodies.

If a bona fide case of active, untreated Whipple's disease could be found with complete absence of the bacilliform bodies, it could then be stated with some degree of certainty that they do not play a major etiologic role in the disorder. We would caution against using the absence of bodies in our case as incontrovertible evidence against their etiologic role. It should be remembered that the present patient was in remission at the time of biopsy; it might be logically argued that a prerequisite for remission might be the disappearance of the etiologic agent. Large quantities of PAS-positive material in the absence of bacillary bodies, while suggestive of nonbacterial origin would not preclude the possibility that bacteria present prior to biopsy had contributed insoluble PAS-positive capsular substance. It is not suggested, therefore, that the hypothesis of bacterial etiology be discarded; verification of our negative findings in other cases is an obvious prerequisite to such a step. It does seem reasonable to us, however, that the bacterial theory constitutes only one reasonable but unsubstantiated hypothesis among others, equally plausible.

In considering other etiologic possibilities, the hypothesis offered by Haubrich and co-workers [3] of the production of abnormal PAS-positive material with storage in histiocytes should be mentioned. Adding our own speculations to theirs we would like to suggest tentatively the following concept of pathogenesis: abnormal (functional or genetic) reticuloendothelial cells → synthesis of abnormal PAS-positive substance (? macromolecules; ? glycoprotein) → stimulation of inflammatory reaction with elaboration of collagen and progressive fibrosis → blockage of lymphatics → malabsorption → death.

The therapeutic benefit of cortical steroids [9] and tetracycline has resulted in clinical enthusiasm despite occasional failures. There is a total lack of understanding of the basic mechanism of action of steroids in this disease. If cortisone acts at step 3 in our suggested pathogenetic

scheme, and inhibits the inflammatory reaction or the subsequent fibrosis, it might be expected that occasional therapeutic failures would be encountered when the inflammatory reaction was either not susceptible to inhibition by the drug or had progressed to an irreversible stage before its administration.

An important conflict exists in the observations made to date. On the one hand, objects closely resembling bacteria have been clearly demonstrated in the cytoplasm of histiocytes, probably within cytoplasmic bodies. This feature is clearly suggestive of phagocytosis.[3-6] On the other hand, our own observations and those of others [10-12] in conditions other than Whipple's disease indicate that (1) in phagocytosis and "ingestion" vacuoles, cytoplasmic vesicles have single limiting membranes rather than double ones as seen in the cytoplasmic bodies of Whipple's disease; (2) packets of flattened vesicles, as in these cytoplasmic bodies, have classically been associated with intracellular synthetic systems such as mitochondria, endoplasmic reticulum and the Golgi apparatus; they are usually not associated with phagocytized debris; (3) phagocytized material that cannot be digested usually assumes a nondescript, irregular, disorganized appearance rather than the symmetrical, uniform appearance of these cytoplasmic bodies. Further studies correlating ultrafine structure with function in the process of phagocytosis and macromolecular synthesis will undoubtedly provide answers to the questions here raised.

Summary

Tissue from the intestine in a case of Whipple's disease in remission and on steroid therapy was examined by light and electron microscopy. The morphologic observations of previous investigators were substantially confirmed. The bacillary bodies described in Whipple's original case and in many subsequently reported instances were not, however, identified.

On the basis of differences in PAS reaction and histologic appearance, it was possible to identify 3 types of histiocytes. Conclusions derived from study of the fine structure of the cytoplasmic bodies, while not ruling out phagocytic origin, were interpreted as compatible with primary synthesis by the histiocytes.

Ferritin was identified in the cytoplasm of the histiocytes adjacent to but only rarely within the cytoplasmic bodies.

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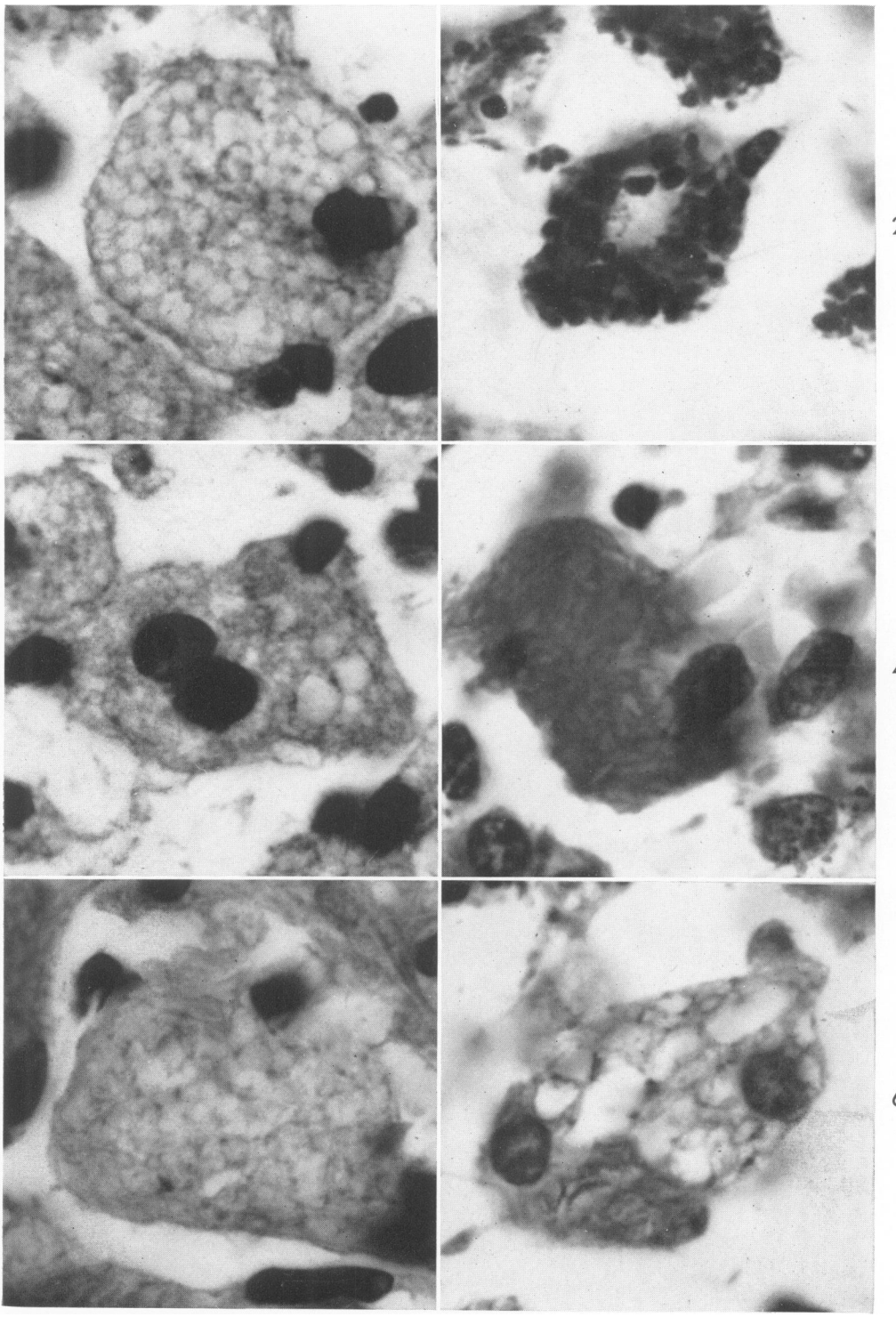
The authors wish to thank Miss Lillemor Wallmark for invaluable assistance in electron microscopy and photography and Mrs. Madeleine Strauss for assistance in preparation of the final copy.

[*Illustrations follow*]

Legends for Figures

FIGS. 1 to 6. The 3 different forms of histiocytes in the submucosa of the intestine in Whipple's disease are illustrated. The characteristic appearance of the type 1 histiocyte is depicted in Figures 1 and 2; type 2 histiocyte in Figures 3 and 4; and type 3 histiocyte in Figures 5 and 6. Figures 1, 3 and 5, hematoxylin and eosin stain; Figures 2, 4 and 6, periodic acid-Schiff stain. × 1,800.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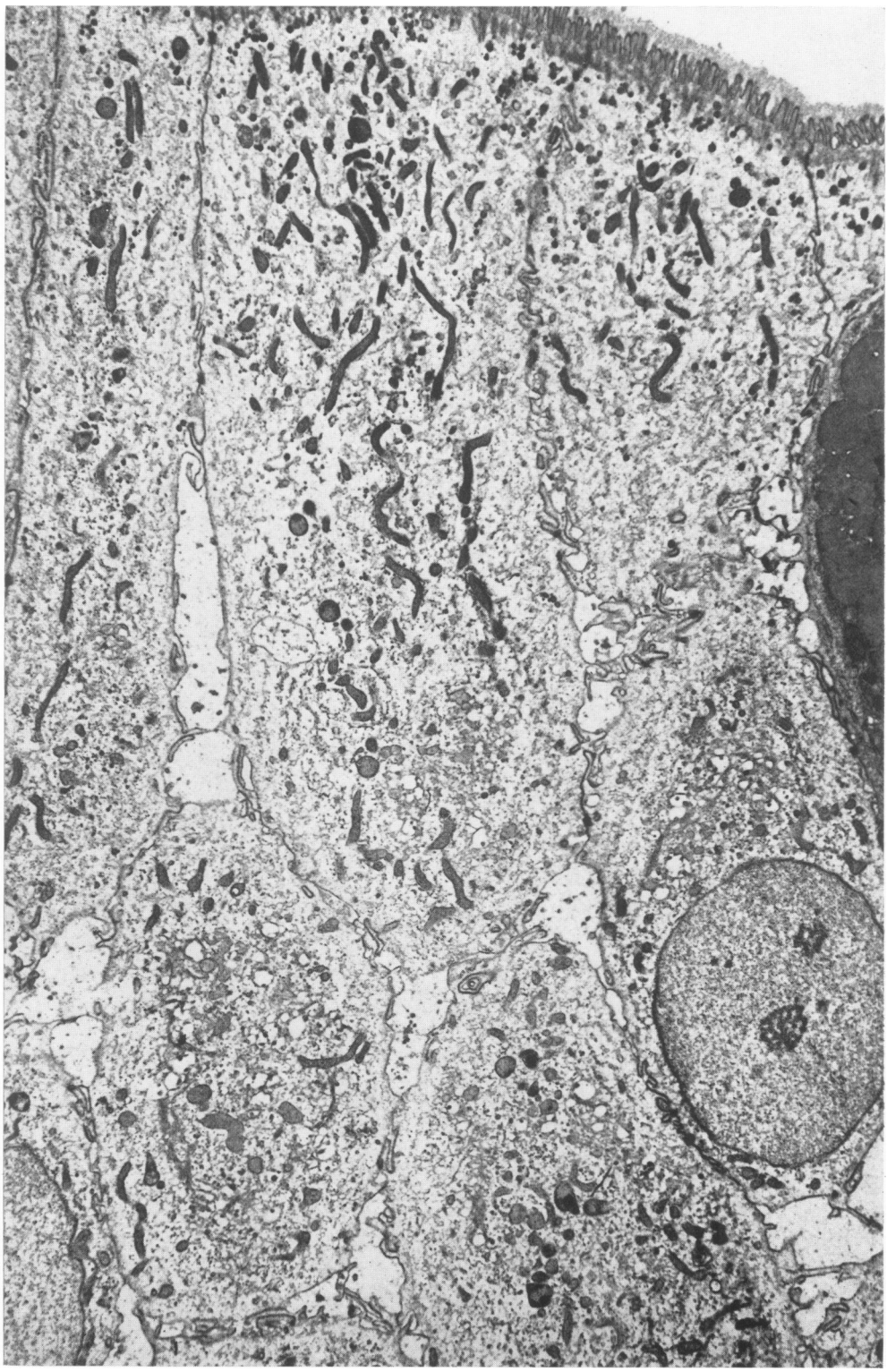

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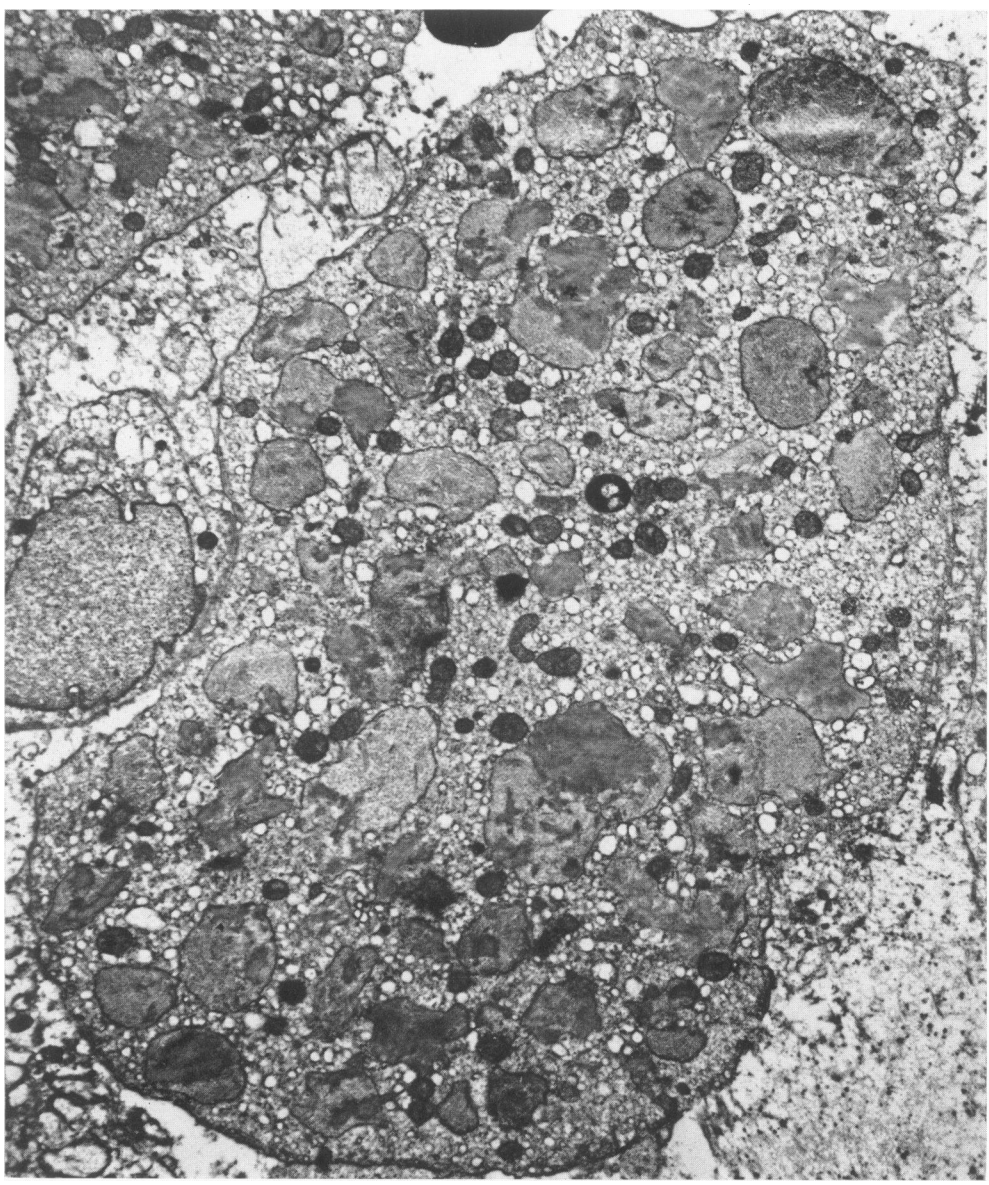

FIG. 7. Intramucosal interepithelial spaces are evident in the intestinal mucosa. The microvilli are relatively normal in appearance. No changes are noted in the mitochondria, endoplasmic reticulum, nuclei or Golgi substance. A small amount of finely granular material can be seen in the intercellular spaces. × 7,000.

FIG. 8. A histiocyte thought to be type 1. Definite correlation between the electron microscopic structure and the morphologic types demonstrated in paraffin sections has not been made. Mitochondria are plentiful, as are vesicles of the endoplasmic reticulum. The cytoplasmic bodies have a complex and varied structure shown in greater detail in Figures 9 and 10. Although the intervening cytoplasm is stippled with numerous vesicles of endoplasmic reticulum, no vesicles or vacuoles can be identified within the bodies themselves. × 7,000.

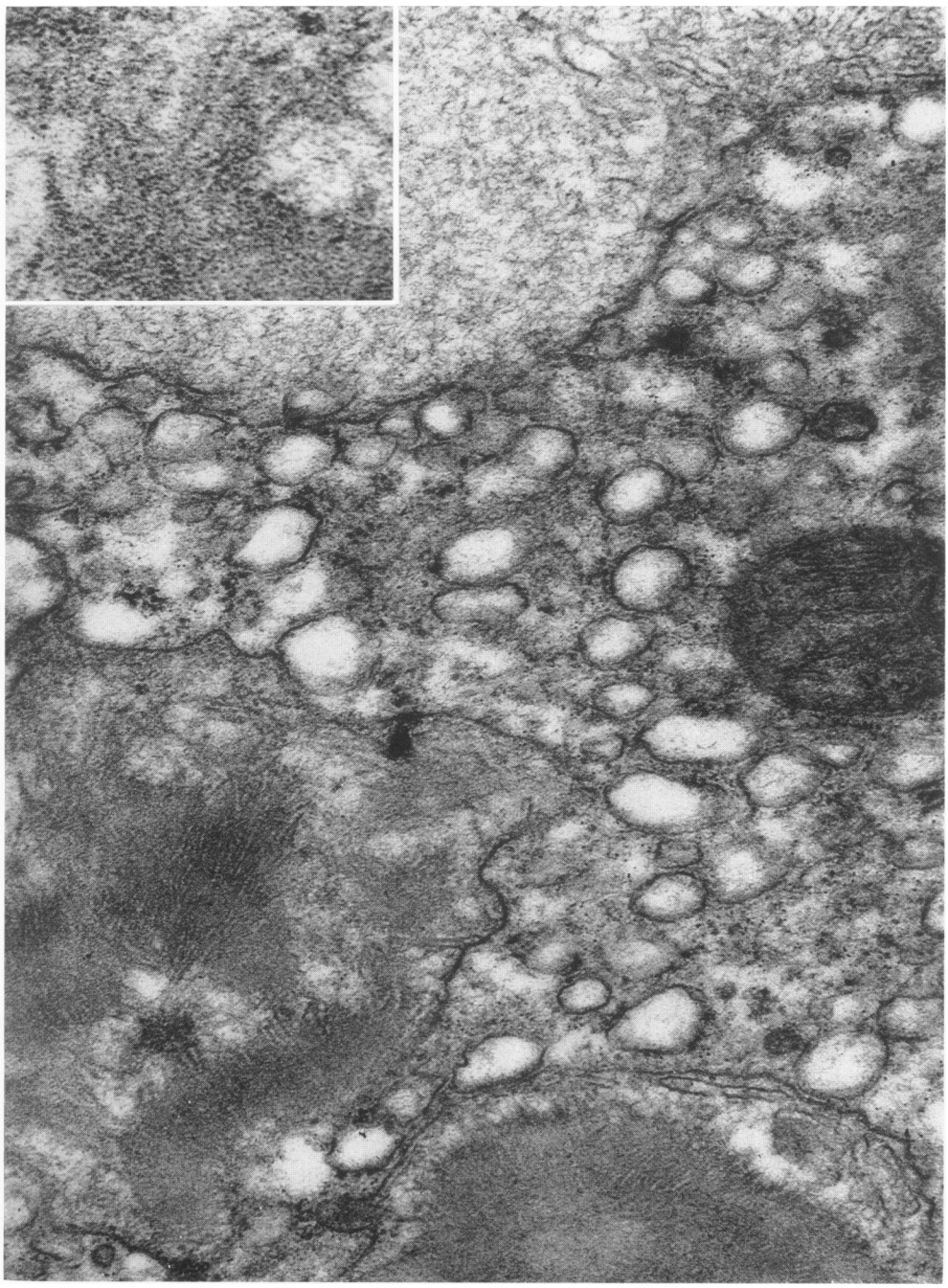

FIG. 9. The cytoplasm of a histiocyte contains a mitochondrion and numerous vesicles of endoplasmic reticulum as well as portions of 3 cytoplasmic bodies. The double membrane that limits each of these bodies can be seen in greater detail in Figure 10. Three different morphologic structures are identified within the substance of the bodies: packets of membranes, fibrils, and tiny granules. The inset depicts fibrils favorably oriented to display the 60 to 80 Å axial periodicity. × 56,000. Inset, × 100,000.

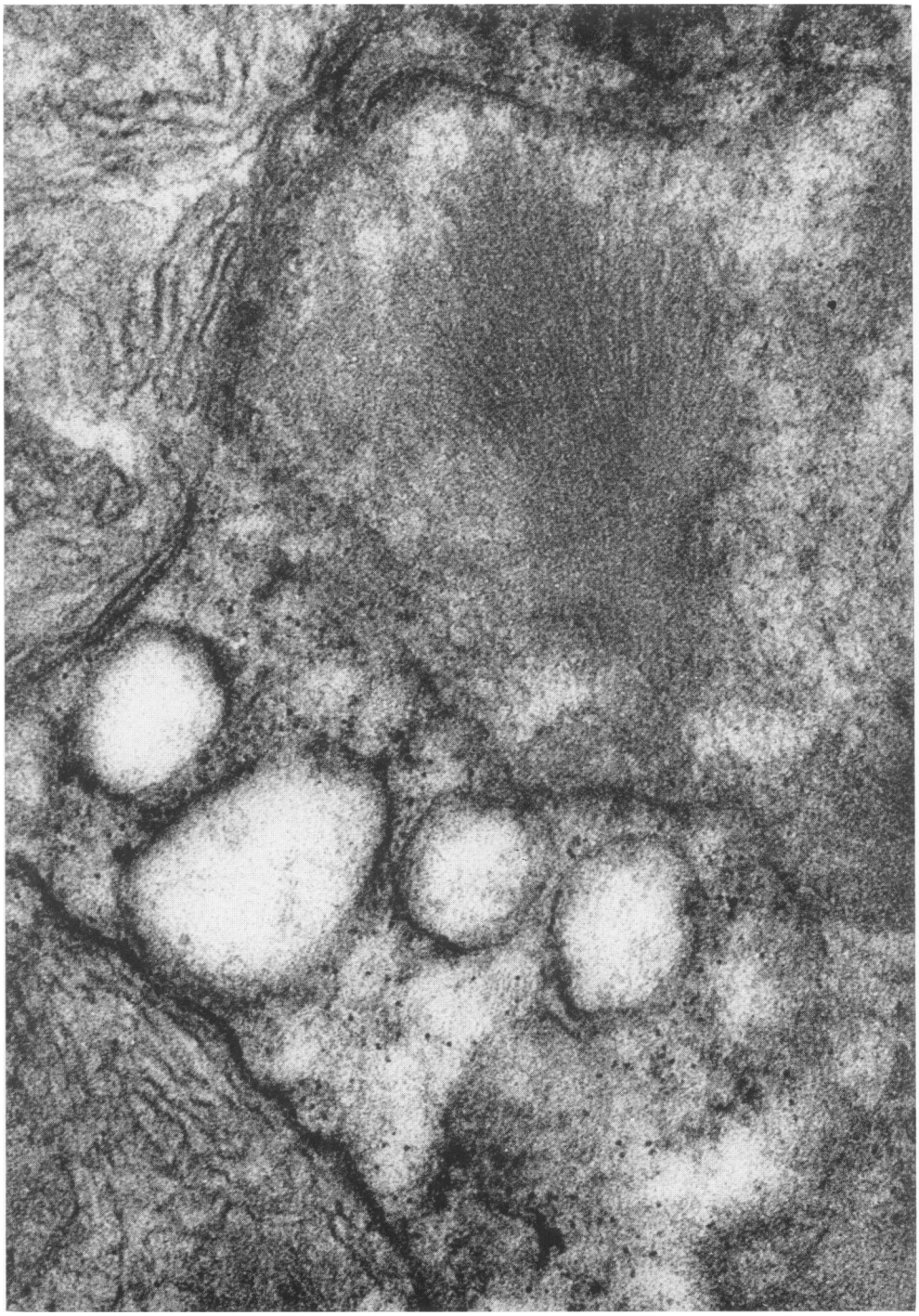

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FIG. 10. A uniform double limiting membrane of the cytoplasmic bodies is illustrated, and ferritin appears in the intervening tongues of cytoplasm. Also seen are details of the closely packed membranes, fibrils and granules described previously. × 100,000.