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ULTRASTRUCTURE OF SCORBUTIC HUMAN SKIN

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Abstract. An electron microscopic study on a clinically typical, biochemically documented human scurvy case showed: (1) Loss of cytoplasmic processes and rounding of the dermal fibroblasts. (2) Decreased and discontinuous, rough-surfaced endoplasmic reticulum and development of active lysosomes in the dermal fibroblasts. (3) Vacuolization and ballooning of endothelial cells of vessels. (4) Junctional separation of adjoining endothelial cells, forming gaps in endothelial lining and detachment of basal lamina from endothelium in small venules and lymphatics. (5) A great degree of multiplication of the subepidermal and perivascular basal laminae. (6) A marked decrease of collagenous component of the dermis with an increase of amorphous to finely fibrillar material.

Only primates, Indian fruit bats, red-vented bulbuls, and guinea pigs suffer from scurvy, since they lack an enzyme system which converts gulonolactone to ascorbic acid (7, 16). In this sense scurvy could be regarded as an inherited inborn error of metabolism of carbohydrate (3). In guinea pigs ascorbic acid is rapidly oxidized into carbon dioxide and evaporated through the respiratory system, whereas in humans it is slowly metabolized into oxalate and excreted in urine (15). For this reason, it is easy to produce experimental scurvy in guinea pigs by a commercially available scorbutogenic diet. It is, however, more difficult to produce scurvy in man with acute deprivation of ascorbic acid. For example, the work of Crandon et al. (8) indicates that it takes 161 days to produce perifollicular hemorrhage in man. Ross & Benditt (21) and Gore et al. (9) have performed electron microscopic studies on scorbutic guinea pigs. Ross & Benditt (21) described defective collagen formation by pathologically changed fibroblasts, and Gore et al. (9) demonstrated similar changes in the capillaries. Their data, however, were derived from artificially produced wounds of the skin and the heart of scorbutic guinea pigs respectively. So far, no human scurvy case has been studied with the electron microscope.

Recently, we had a case of clinically typical and biochemically well-documented scurvy. This report deals with the fine structure of petechial lesions from this patient.

CASE REPORT

L. B., a 65-year-old white male, deaf-mute, was admitted to the City of Memphis Hospitals with a chief complaint of weakness. Since the patient had no relatives, the history was obtained from the manager of the rooming house where he lived. According to this informant, the patient had become progressively weaker over several months, eventually reaching the point of being unable to care for himself. He had an extremely poor food intake for many months, consisting chiefly of cottage cheese, beer, and an occasional hamburger. For the two weeks immediately prior to admission he had no appreciable food intake. He denied any fruit consumption for several years.

Physical examination revealed an obviously malnourished, dehydrated male with a blood pressure of 100/60, pulse of 100, temperature 97.4°F, weight of 115 lbs., and a height of 5 ft., $6\,^1/_2$ inches. The patient had mild cheilosis with atrophic gums. He had only four teeth, two of them loose. Examination of the skin revealed follicular papules on the extremities and trunk, with "corkscrew" hairs on the lower extremities. There were numerous petechial hemorrhages with pigmentation secondary to hemosiderin deposits. On the lower extremities there were numerous, very extensive, ecchymotic areas. The rest of the examination revealed only mild testicular atrophy, extreme muscular weakness, and a total conduction deafness.

The following laboratory tests were negative or within normal limits: urinalysis, lumbar puncture, alkaline phosphatase, serum proteins, PBI, uric acid, intermediate PPD, X-rays of chest, skull, abdomen, legs, and pelvis. Other laboratory results on admission include a hematocrit of 25 and a total WBC count of 7500/mm^3, with

Table I. *Absorbic acid*

	Patient	Control
Plasma (mg%)	0.12–0.17	0.9–1.1
Buffy coat (mg%)	Undetected	39
Urine (mg/24 h)	2.0	15.0

a differential of 2 bands, 54 segs, 4 eosinophils, 1 basophil, 34 lymphocytes, 4 monocytes, and 1 metamyelocyte. Initial reticulocyte count was 8.1%. Red cell morphology showed basophilic stippling with slight hypochromia and both macrocytosis and microcytosis. Initial BUN was 53 mg%, which fell to 9 mg% after hydration. Serum bilirubin was 2.7 mg%, 1.7 mg% direct. Blood study revealed abnormal platelet function manifested by prolonged bleeding time and abnormal platelet aggregation. Bone marrow showed slight hypercellularity.

Ascorbic acid studies using the method of Roe & Kuether (20) and that of Maickel (17) revealed a plasma ascorbic acid of 0.12–0.17 mg%, but buffy coat ascorbic acid was undetectable (Table I). An ascorbic acid loading test was done using 1 mg of ascorbic acid per pound of body weight intravenously. The patient excreted less than 1% of the injected material in 5 hours. Normal subjects excrete 50–60% of the injected dose in 4 hours. This indicated a low tissue saturation of ascorbic acid.

Six days after admission the patient was transferred to the Clinical Research Center of the University of Tennessee. He was maintained on a balanced diet which contained less than 4 mg of ascorbic acid per day. The first skin biopsy was obtained during this period. Following the completion of these studies, patient was placed on total of 400–800 mg of ascorbic acid per day for 7 days. By the time of discharge, he had improved remarkably with restoration of physical strength, improvement in hematological status, and almost complete clearance of his skin lesions. The second biopsy was done after discharge.

MATERIAL AND METHOD

In the first biopsy, four specimens were taken from his right leg by a 4-mm punch. Lesions which showed perifollicular hemorrhage and follicular hyperkeratosis were selected. The second biopsy specimens were taken from four areas adjacent to each corresponding site of the first biopsy. Specimens were cut into small pieces, approximately 1.5 mm across, and immediately fixed in 5% cold glutaraldehyde buffered to pH 7.4 with phosphate buffer. After four hours fixation and overnight rinse in a plain phosphate buffer solution, all tissue blocks were re-fixed in 1% osmic acid in veronal buffer at pH 7.4 for one hour, dehydrated through graded concentrations of ethanol and propylene oxide and embedded in Araldite. Thin sections, 400 A to 600 A, were cut with a diamond knife mounted on a Porter-Blum MT2 Ultra-Microtome, picked up on uncoated grids, stained with uranyl acetate and lead citrate (19), lightly coated with evaporated carbon, and observed with a Hitachi HU 11C electron microscope.

RESULTS

Fibroblasts. Since the major role of ascorbic acid in collagen synthesis is now attributed to the hydroxylation of proline to hydroxoproline (4), the dermal fibroblasts were the first to be examined. In contrast to normal dermal fibroblasts which show an elongated shape (Fig. 1 *a*) and many projected cytoplasmic processes (12), the majority of the dermal fibroblasts from the lesions of this patient appeared shrunken and round (Fig. 1 *b*). Rough endoplasmic reticulum was not only diminished in amount but also showed morphological changes; i.e., in contrast to continuous, elongated arrays of cisternae (Fig. 1 *a*) many of the fibroblasts from this patient showed a discontinuous, round profile (Fig. 1 *b*). Many of these fibroblasts contained lysosomes (Fig. 1 *b*), in which aggregates of ferritin (hemosiderin) were found (Fig. 1 *b*). There were transitional forms between fibroblasts and typical phagocytes. An impression was therefore gained that many fibroblasts acquired phagocytic ability to remove extravasated erythrocytes and their derivatives.

Collagen and elastic fibers. In the dermis the most remarkable change was a decreased number of collagen fibers. In contrast to densely distributed strands of collagen fibers in the normal skin, patchy aggregates of fibers were found in widely separated groups. Individual fibers of larger diameter did not show abnormality (Fig. 2), but they were separated by amorphous to finely fibrillar material (Fig. 2). It appeared that such amorphous to fibrillar material represented building material of collagen fibers (e.g. tropocollagen molecules) which failed to polymerize into larger fibers. It may be that the empty spaces between patchy aggregations of collagen represented dissolved material composed of a still finer variety of tropicollagen which is soluble in water, alcohol, propylene oxide, etc. used in tissue preparation. Elastic fibers did not seem to be much affected.

Basal lamina. The basal lamina of the epidermis and many of the dermal vessels, particularly medium-sized vessels, showed multiplication (Fig. 3). Many of these laminae showed not only swell-

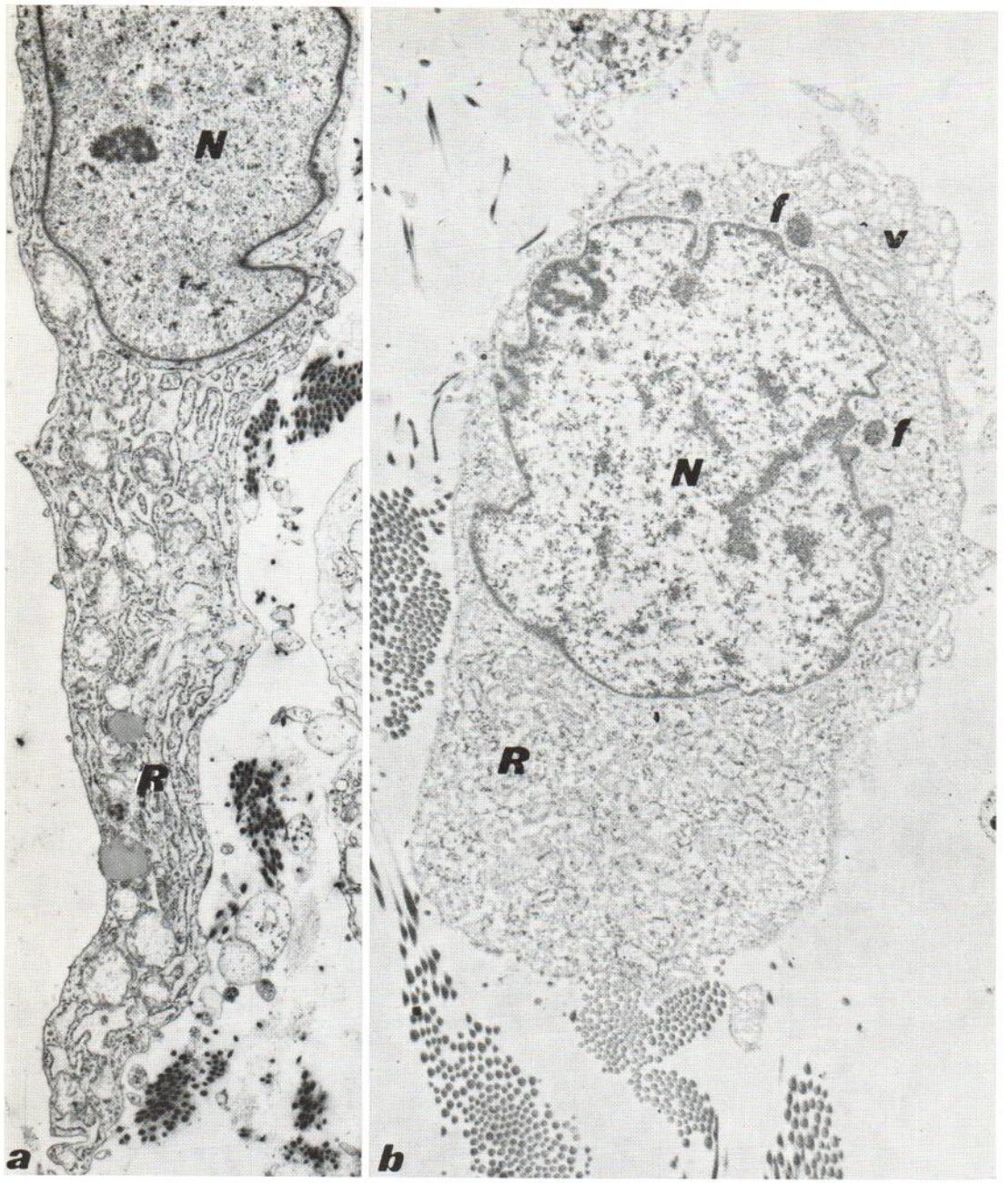

Fig. 1. (*a*) An elongated dermal fibroblast from normal skin. The cytoplasm is filled with a number of sinusoidal arrays of rough-surfaced endoplasmic reticulum (*R*) × *N*-nucleus. × 8500. (*b*) A shrunken fibroblast from the petechial lesion. Rough-surfaced endoplasmic reticulum (*R*) is markedly diminished and had become either aggregates of ribonucleo-protein particles or small, round vesicles (*v*). *f*, Ferritin particles in lysosomes; *N*, nucleus. × 9375.

ing but also actual detachment from both endothelial cells and from smooth muscle cells (Fig. 4). In some instances, dissolution of such laminae into an amorphous substance was observed (Fig. 4). However, in contrast to Stolman's light microscopic observation (22), the perivascular smooth muscle cells per se did not show degenerative changes. In rare instances, vaguely defined perio-

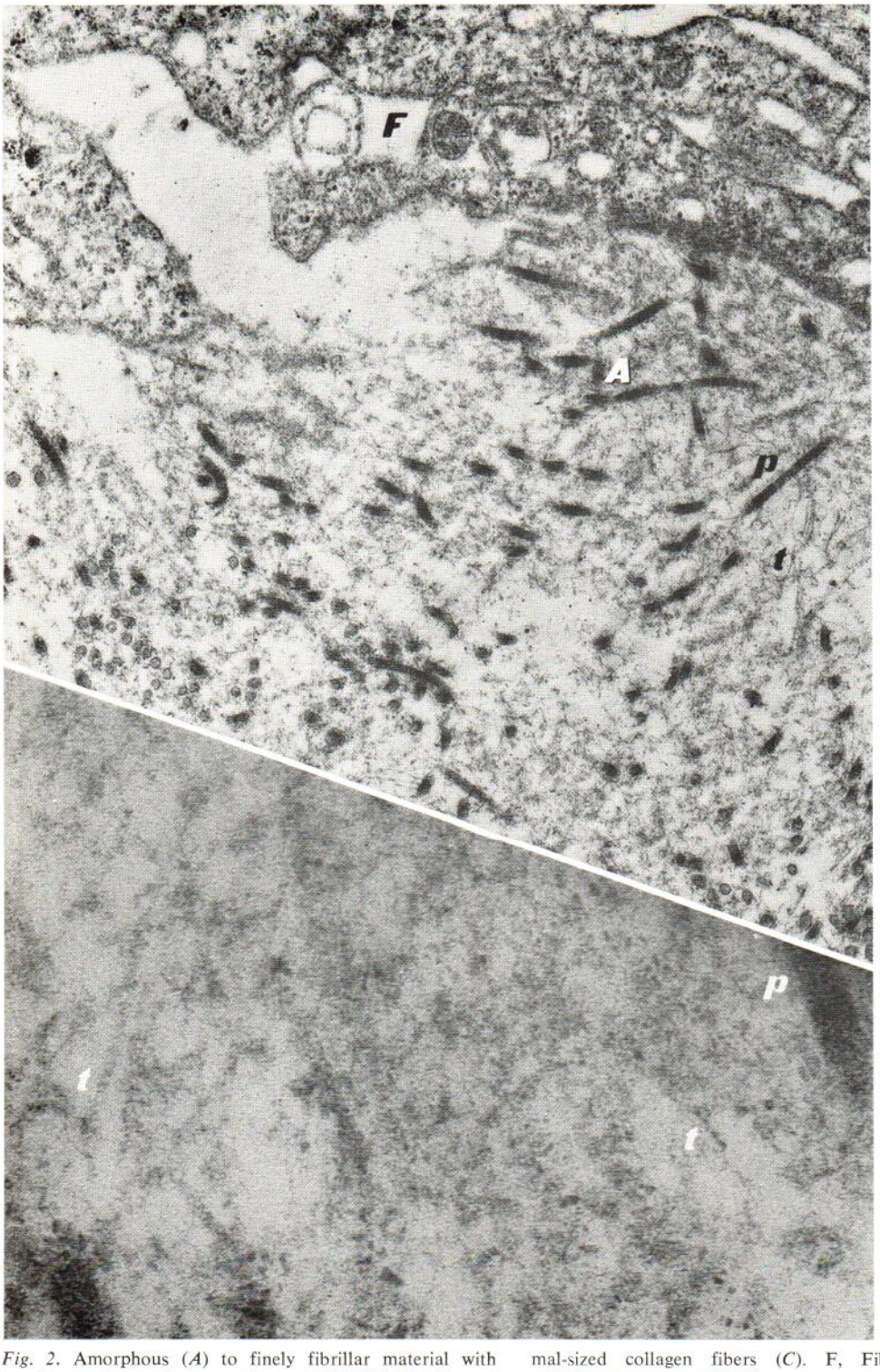

Fig. 2. Amorphous (*A*) to finely fibrillar material with beading pattern of tropocollagen (120–150 A) (*t*) and precollagen (200–550 A) (*p*) fills the spaces between normal-sized collagen fibers (*C*). F, Fibroblast. Upper: × 27,000. Lower: × 260,400.

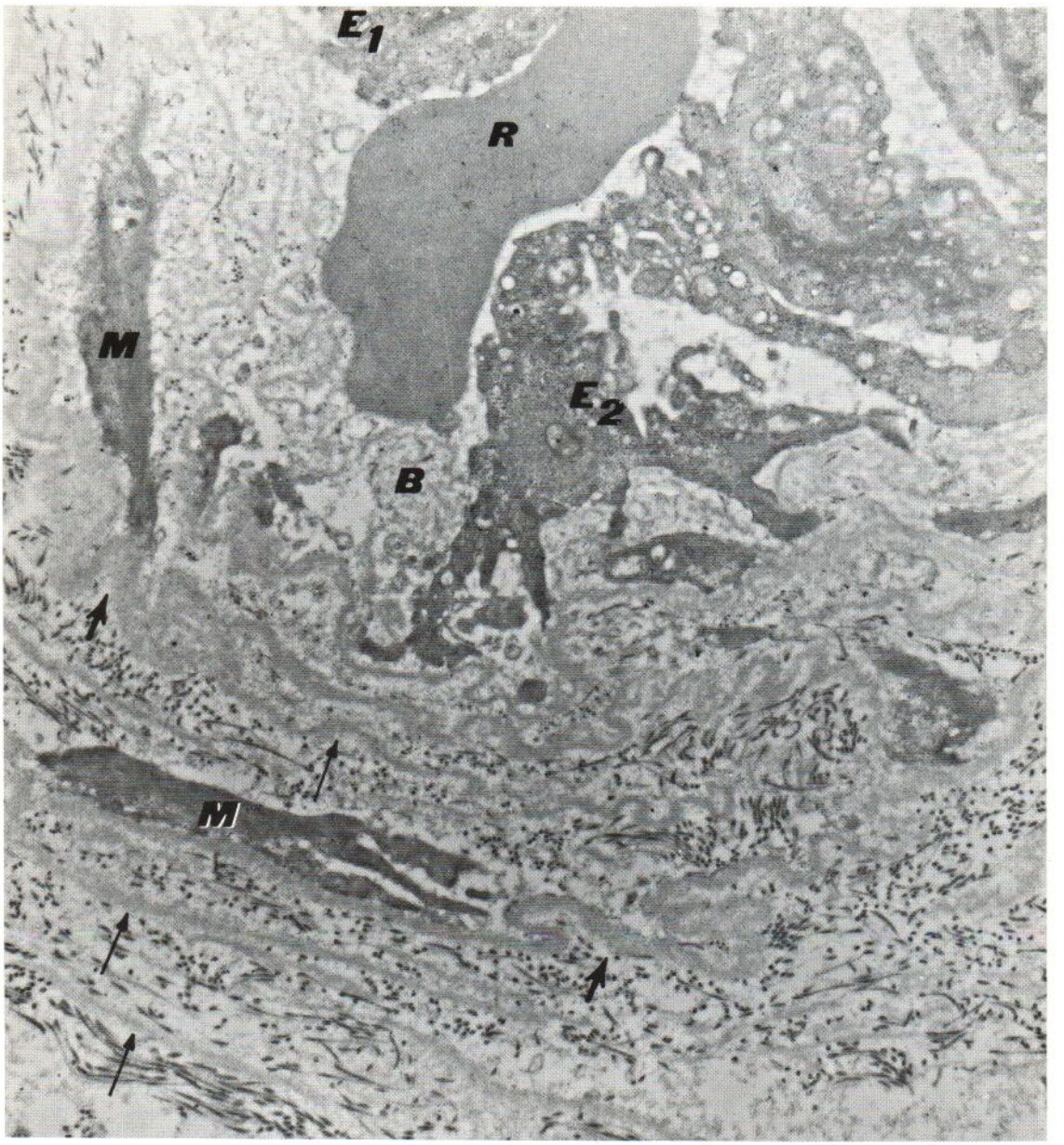

Fig. 3. An erythrocyte (*R*) is extravasating through the separation of two endothelial cells (E_1, E_2). It touches swollen basal lamina-collagen complex (*B*) which exists between endothelium (intima) (E_1, E_2) and muscle cells (media) (*M*). Note multiplication of basal laminae (arrows) and increased amorphous material between them. Also note that some basal laminae are abnormally thick (thick arrows). × 15,800.

dicity could be observed in this substance. The basal laminae which surround arrectores pilorum muscles also became swollen and ill-defined in many areas.

Vessels. Changes of the basal lamina of dermal vessels have been described above. Deficient formation of the dermal collagen also affected perivascular collagen and reticulum fibers. Thus, many vessels appeared to be floating freely in almost empty perivascular spaces (Fig. 5). Such a defect apparently provided less support to the vascular walls. In particular, small venules and

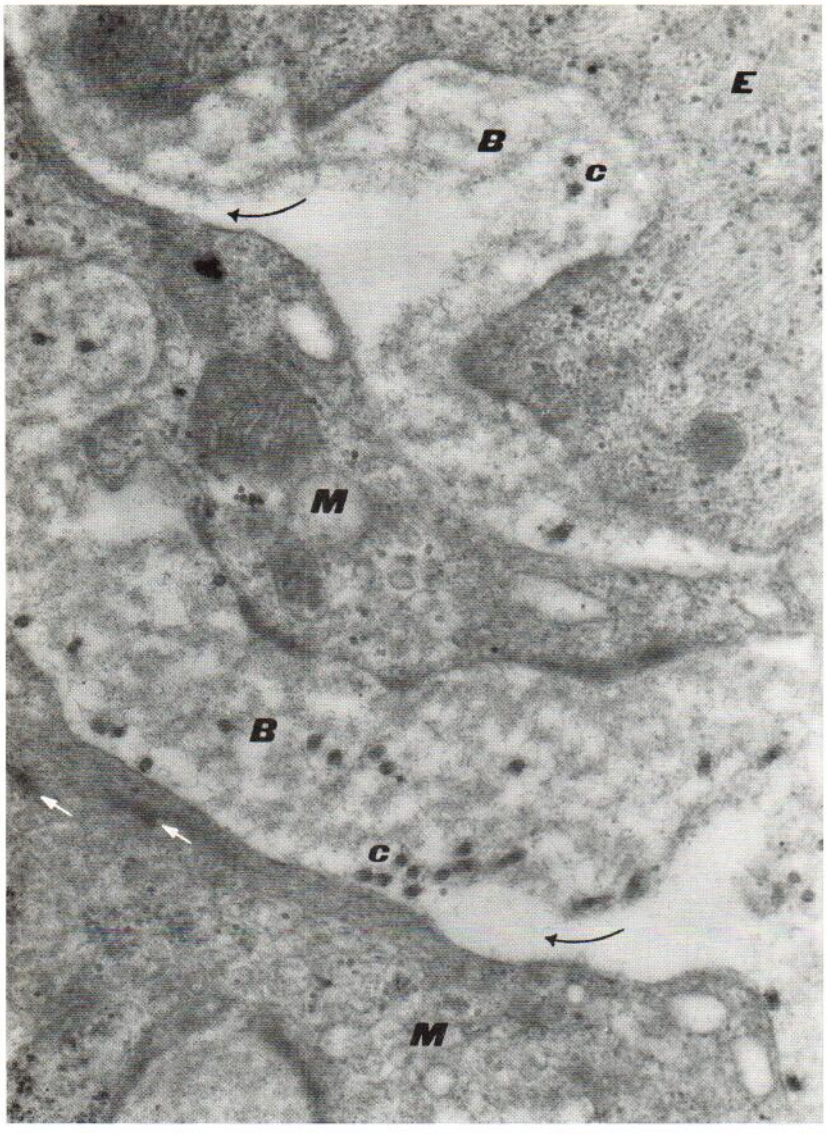

Fig. 4. A high-magnification view of swollen basal lamina-collagen complex (*B*) which fills the spaces between endothelial cells (*E*) and smooth muscle cells (*M*) of a medium-sized vessel. *c*, Collagen fiber; *M* smooth muscle cells; *E*, endothelial cell; White arrows, characteristic black speck of smooth muscle cells; Black curved arrows, detachment of basal lamina from muscle cells. × 41,700.

lymphatics which lack the support of smooth muscle coat or pericytes suffered the most and often showed breakage of the peri-endothelial basal lamina (Fig. 6). Also found was detachment of endothelial cells from the basal lamina and from each other (Fig. 6).

Endothelial cells of vessels of larger caliber were vacuolated and often ballooned out into the lumen (Fig. 3). Where they had completely degenerated, the integrity of the vascular walls was maintained by swollen, multiplicated layers of basal laminae (Figs. 3 and 7 *a*). In some instances only a few basal laminae held the vascular lumen (Fig. 7 *b*). These degenerative changes would seem to be responsible for the extravasation of erythrocytes. Some endothelial cells showed a marked hyperplasia of rough-surfaced endoplasmic reticulum (Fig. 7 *b*). This was interpreted as representing a regenerative process, or an active synthesis of material for the formation of basal laminae.

Hyperkeratosis. Many hyperkeratotic hair follicles lacked hair. The innermost layers of the external hair sheath at the follicular orifice retained a few nucleated horny cells, i.e. parakeratotic cells (Fig. 8). Empty follicles were filled with loosely

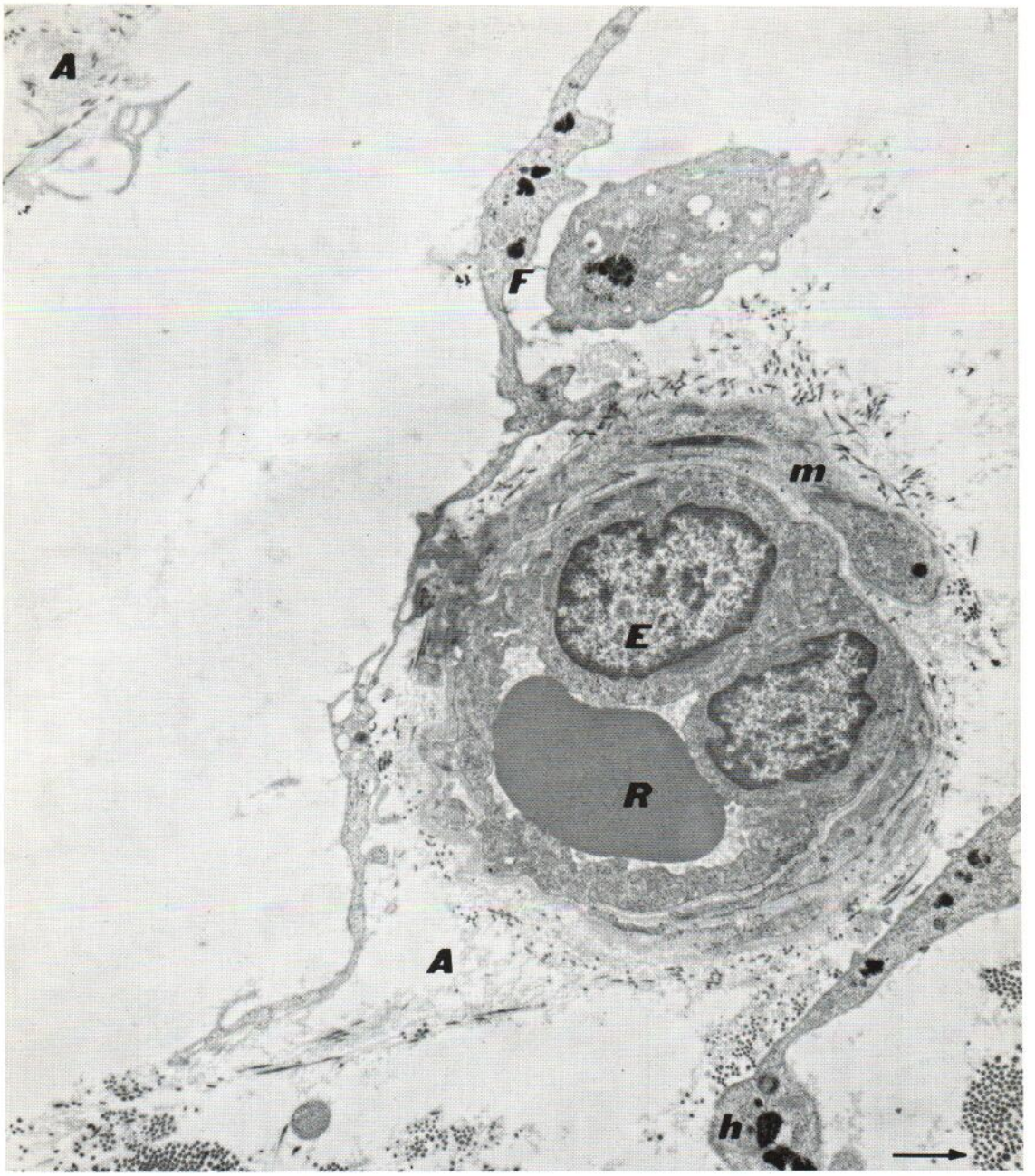

Fig. 5. An arteriole in the mid-dermis appears floating in an empty space which apparently was produced by dissolution of perivascular collagenous tissue. The remaining collagen fibers are extremely variable in size (arrow). Amorphous material (*A*) is increased. *E*, Endothelial cell; *F*, Phagocyte; *h*, hemosiderin in phagocytes; *m*, Peri-endothelial smooth muscle cells; *R*, erythrocyte. × 5,750.

bound shed cells. This may signify that a rapid keratinizing process and overproduction rather than a strong adherence of horny cells is the basis of follicular hyperkeratosis.

Controls. Specimens taken after recovery showed normal fibroblasts, normal collagen, and normal blood vessels. The multiplication of the basal lamina was not observed (Fig. 9).

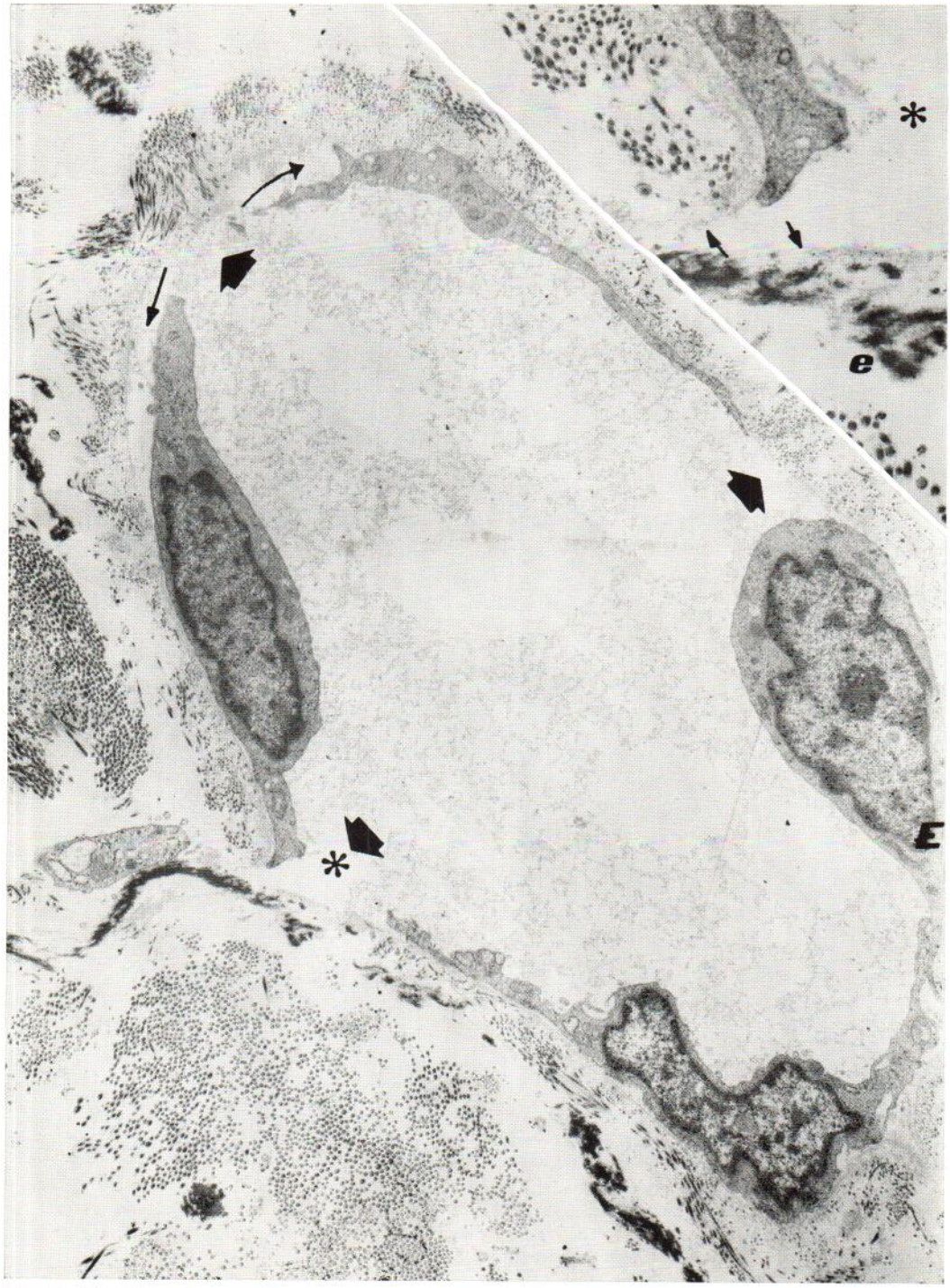

Fig. 6. Disruption of endothelial lining (large arrows) by separation of endothelial cells (*E*) and detachment of some of these from underlying basal lamina (thin arrows) are seen. This vessel is regarded as a venule of the smallest caliber (approximately 20 μ) and not a lymphatic because of the well-developed basal lamina (see insert) and the thickness of endothelial cells (*E*). × 5400. *Inset:* An enlargement of the area marked by *. Note the breakage of the basal lamina (arrows) *E*, Normal elastic fiber. × 16,000.

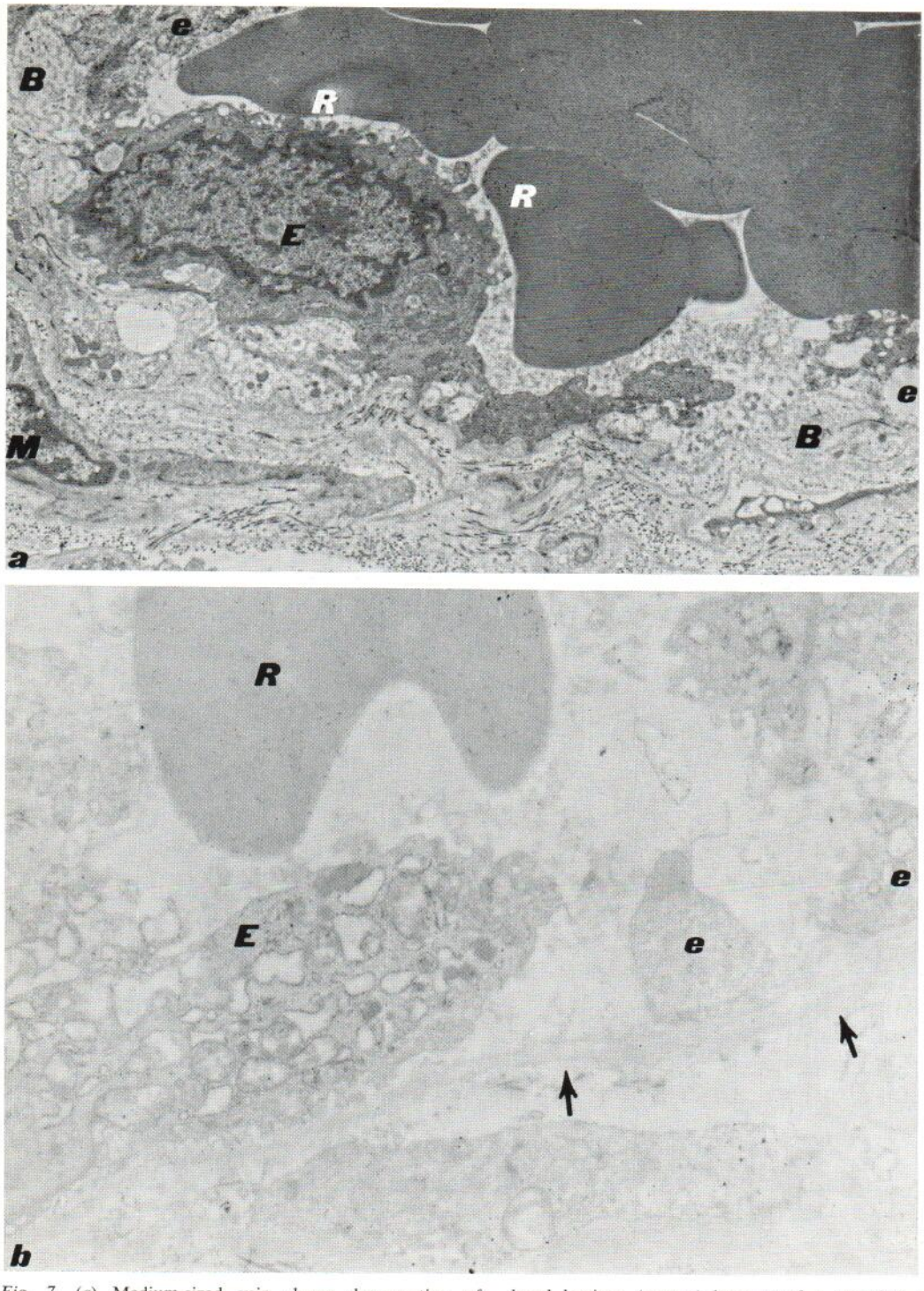

Fig. 7. (*a*) Medium-sized vein shows degeneration of endothelial cells (*e*) and swelling of basal lamina-collagen complex (*B*). *E*, Intact endothelial cell; *M*, smooth muscle cell; *R*, erythrocyte. × 7750. (*b*) The integrity of this small vein is maintained by only a few layers of basal laminae (arrows) because of a complete degeneration of endothelial cells (*e*). One endothelial cell (*E*) adjacent to the degenerated one shows a well-developed endoplasmic reticulum. *R*, Erythrocyte. × 16,000.

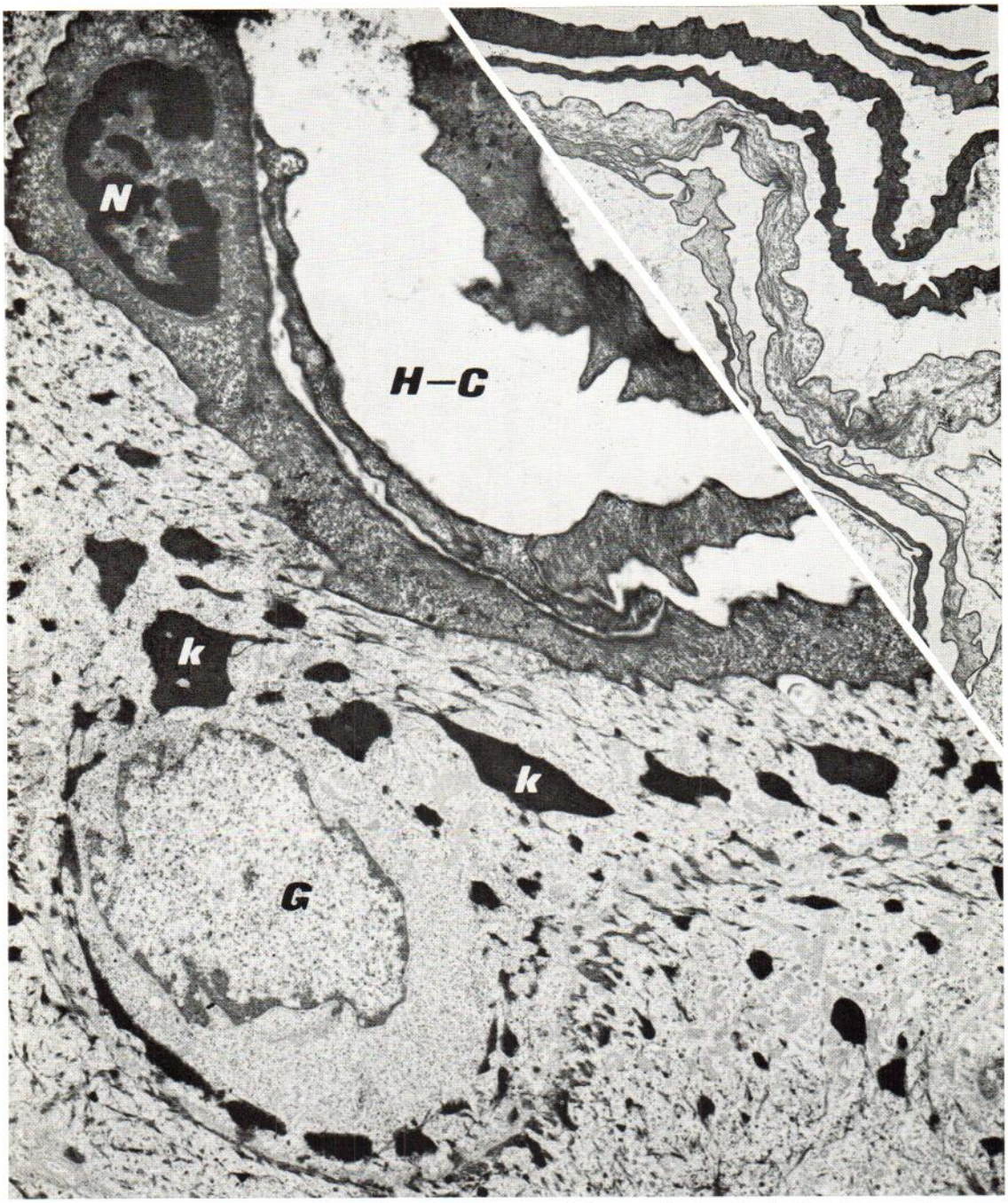

Fig. 8. A nucleus-retaining horny cell (*N*) lines the hair canal (*H–C*) which is plugged with shed, loosely connected horny cells (see inset). *G*, Nucleus of granular cell; *K*, keratohyaline granules. × 5750. Inset: × 2580.

DISCUSSION

Discontinuity of the endothelial lining of small venules, ballooned endothelial cells, and diminished perivascular collagenous support would account for the petechial hemorrhage. Perifollicular location of these petechiae may be due to a mechanical irritation of perifollicular dermal tissue by the movement of hair or elevated areas of folli-

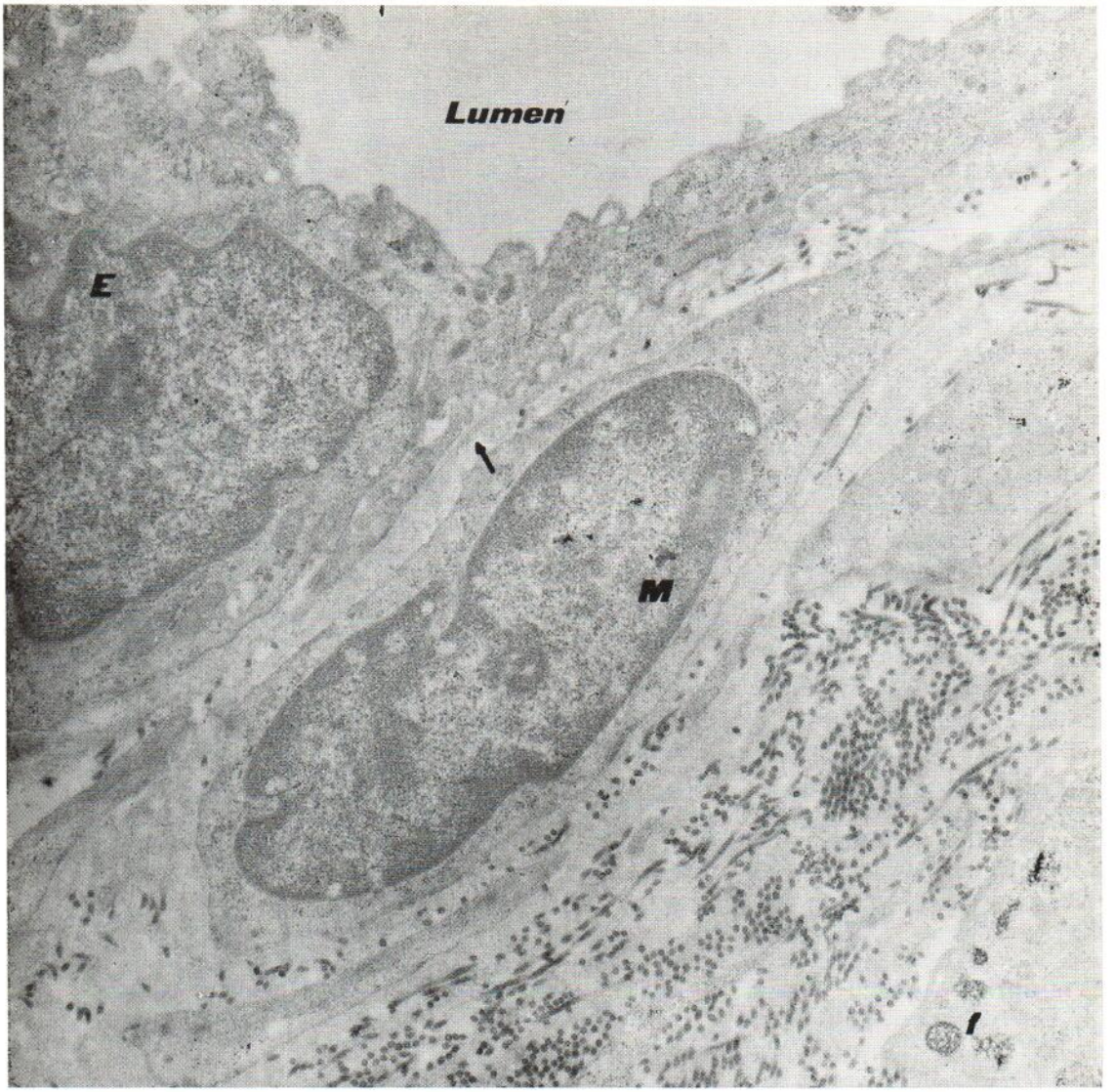

Fig. 9. Biopsy specimen after recovery. A medium-sized venule shows a complete regeneration of the endothelial cells (*E*), absence of the swollen basal lamina-collagen complex and only one layer of the basal lamina (arrow). See Figs. 3, 4, 7 *a* and *b* for comparison. *f*, Ferritin particles in lysosomes of a phagocyte; *M*, perivascular smooth muscle cell. × 14,000.

cular hyperkeratosis which are more subject to traction and friction than other smooth skin surface. Mild parakeratosis at the follicular orifice was found to be the basis of follicular hyperkeratosis.

The supporting system of the vascular wall seems to be very important in the development of hemorrhage. Thus, rupture of the vascular wall has been observed more frequently in small venules and lymphatics. In arteries, arterioles, and capillaries, however, muscle cells, thick elastica interna and pericytes protect the wall, and, in spite of the endothelial disruption, the integrity of the vascular wall was maintained. In experimental scurvy in guinea pigs, Gore et al. (9) described disruption of capillary walls associated with attenuation or even disappearance of the basal lamina underlying the endothelial cells and depletion of pericapillary collagen. However, a much bulkier basement membrane of the renal

glomerulus (1) spared the glomeruli damage even in severely scorbutic guinea pigs. Abnormality of platelets has been shown in experimental (2) as well as in human (6) scurvy. They do not aggregate and fail to adhere to the wall of the glass tubes (2, 6). This may account for the prolonged bleeding time of this patient. An abnormal thromboplastin generation test was also reported in a case of human scurvy (2).

Multiplication of epidermal and perivascular basal laminae has been observed in erythema multiforme (5), colloid milium (13), sun-damaged skin (18), as well as in the normal skin (23). However, a great number of basal laminae such as described in the present report seems to be found only in pathological conditions. It seems that collagen and reticulum fibers which usually abut upon and strengthen the basal lamina are lacking in scorbutic skin. As a defense response, the basal lamina which could be produced by the epithelial cells (14) may have undergone multiplication in order to support the defective vascular wall.

Phagocytic activity of fibroblasts has been described in another hemorrhagic disease, i.e. Kaposi's hemorrhagic sarcoma (10) and that of vascular endothelial cells in chlorpromazine hyperpigmentation (11, 24). Therefore, these phenomena are not peculiar to scurvy.

Ascorbic acid apparently is not necessary for older or "growth" collagen, but is necessary for the formation of "repair" collagen (4). Compatible with this data, strands of mature collagen with normal periodicity were still observed in our material. It is, however, possible that in a very chronic and severe case, with morbidity extending longer than the turn-over period of dermal collagen, the collagenous structure of the skin could be totally affected.

In spite of the different metabolic pathway of ascorbic acid in man, this study revealed that the effect of ascorbic acid deficiency on the human skin is at a fine structural level very similar to that described in guinea pigs.

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