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REMARKABLE CYTOPLASMIC STRUCTURES IN MAST CELLS OF URTICARIA PIGMENTOSA

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Abstract. Some mast cells in the skin of four children with urticaria pigmentosa showed two remarkable cytoplasmic figures, i.e. lucent bands in strata, and electron-dense strips. The lucent bands were thought to originate from the granular endoplasmic reticulum, while the nature of the strips is unknown. The mast cells showing these figures were about to regranulate.

Besides their specific granules, mast cells of urticaria pigmentosa (u.p.) present ordinary cytoplasmic organelles, i.e. granular endoplasmic reticulum, a Golgi zone, mitochondria, ribosomes, centrioles etc. (2). These organelles show more di-distinct figures during regranulation than in the resting phase or during degranulation (3). In this study, hitherto undescribed structures have been observed during the process of regranulation.

MATERIAL AND METHODS

Twenty-two pigmented lesions of fourteen patients with urticaria pigmentosa were biopsied without anesthesia. Two male patients were one year old, while three were 3, 22 and 57 years old. Three female patients were one year, two were 29, four 4, 5, 32 and 55 years old. The specimens were fixed in a 4% glutaraldehyde solution in veronal acetate buffer pH 7.4 with sucrose for one hour at 4°C and washed in the same buffer at 4°C overnight. Afterfixation was carried out in a 1% osmic acid solution in veronal acetate buffer pH 7.4 at 4°C for one hour. After washing, the specimens were dehydrated in alcohol and embedded in Epon 812. Ultrathin sections were stained by uranyl acetate and lead citrate and observed by a Siemens electron microscope.

OBSERVATIONS

Remarkable cytoplasmic figures were found in occasional mast cells located in papules of four children, i.e. three girls of 1, 4 and 5 years, and one boy who was one year old. Two main formations were observed, i.e. lucent bands and dense strips. The lucent bands were arranged in strata showing straight, bent and circular forms (Figs. 1–4). The individual lucent bands had a width of about 150–350 Å and were bounded by a dense single membrane. A 250–750 Å wide space containing fine granular material separated one band from another. One band ended blind or continued into the adjacent band turning over at the end (Fig. 2). The lucent bands were always located at some distance from the Golgi zone (Fig. 1), occasionally showing continuity with the granular endoplasmic reticulum (Fig. 3).

Each dense strip located in the lucent band arrangement (Figs. 4 and 6) or in another cytoplasmic area was enclosed by a single membrane (Fig. 5). The spaces containing a dense strip, were 450–650 Å wide (Figs. 4, 5 and 6). The strips showed straight, bent or circular forms (Fig. 5). They were 170–250 Å wide, of various lengths and of high density. They showed transverse periodical banding with intervals of 130 Å (Figs. 4 and 6).

The mitochondria were large and distinct, and the mast-cell granules showed mature or immature patterns. There were a few honeycomb-like figures indicating granule dissolution. The granular endoplasmic reticulum was distinct, and the Golgi zone was widened (Fig. 1).

DISCUSSION

The stratified appearance of the lucent band array is remarkable and resembles that of the Golgi apparatus. However, the Golgi apparatus is lo-

cated in close relation to centrioles and shows vesicles and saccules arranged less distinctly than the lucent bands and without granular material in the interspaces.

Occasionally, granular endoplasmic reticulum exhibits lamellar structures in plasma cells, in acinous cells of the pancreas (1) and in Earle's L-929 mouse fibroblasts (4). In these cells ribosomes are always situated on their margins.

The marginal continuities of lucent bands with granular endoplasmic reticulum suggest that the structure is a variant of endoplasmic reticulum and that the granular material between the bands may originate from ribosomes.

The dense strips surrounded by a single membrane may show an ultrastructural similarity to lysosomes (Fig. 5 B). However, the nature of those in the stratified areas are unlike these bodies (Fig. 4). The granule figures and the cytoplasmic patterns or organelles suggest that the mast cells containing these specialized structures, are probably in the regranulation phase (mature and immature granules, granular endoplasmic reticulum, distinct mitochondria, and widened Golgi zones in large cytoplasmic areas (2, 3). They could not be found in resting or degranulating mast cells. Accordingly, it seems likely that they are in some way related to granule formation. It should be pointed out that, until now, these remarkable structures have only been found in children.

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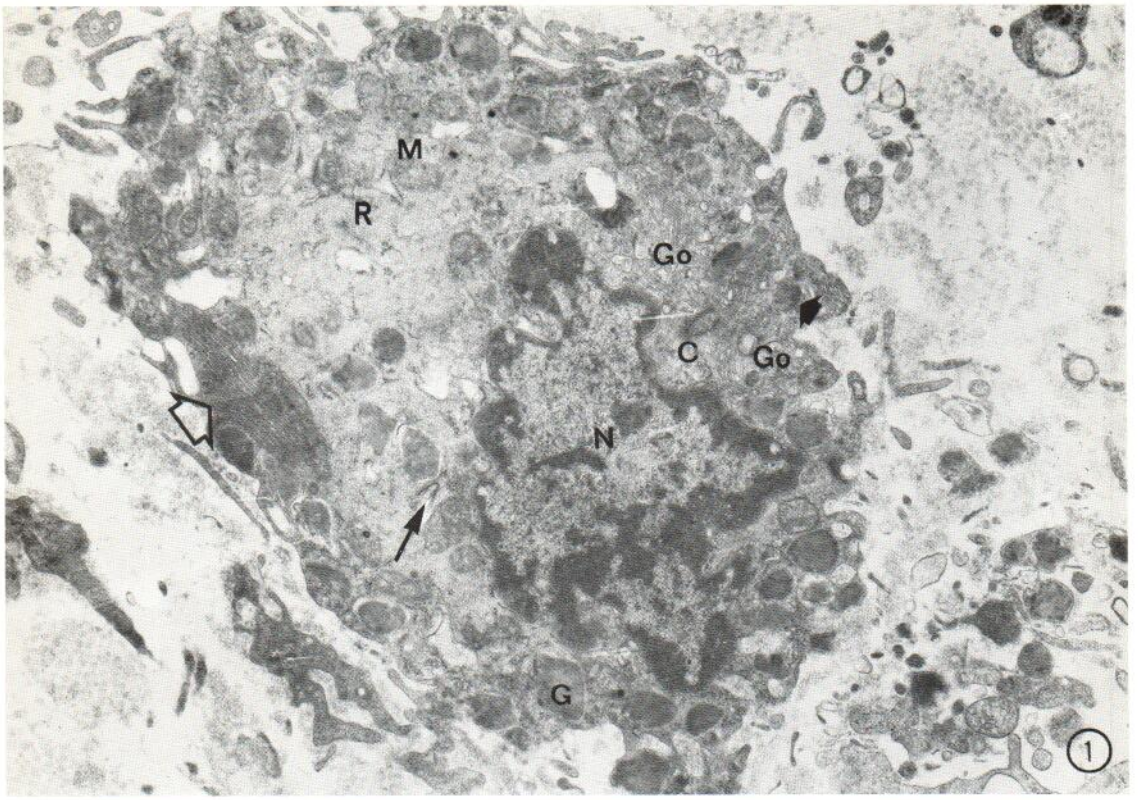
Fig. 1. A mast cell showing nucleus (*N*), centriole (*C*), wide Golgi zone (*Go*), mitochondria (*M*), granular endoplasmic reticulum (*R*), mature granules (*G*), and immature granules (thick arrow). Apart from the Golgi zone, a dense strip (thin arrow), and lucent bands in strata (framed arrow) are seen. × 19,000.

Fig. 2. Lucent bands in strata. The individual lucent bands have a width of 150–350 Å. The boundary lines are dense, and some endings are blind (thin arrows). There may be direct continuity with the adjacent band (thick arrow). Granular material is located in the interspace between two lucent bands. *G*, indicates mast-cell granules; *N*, nucleus. × 58,000.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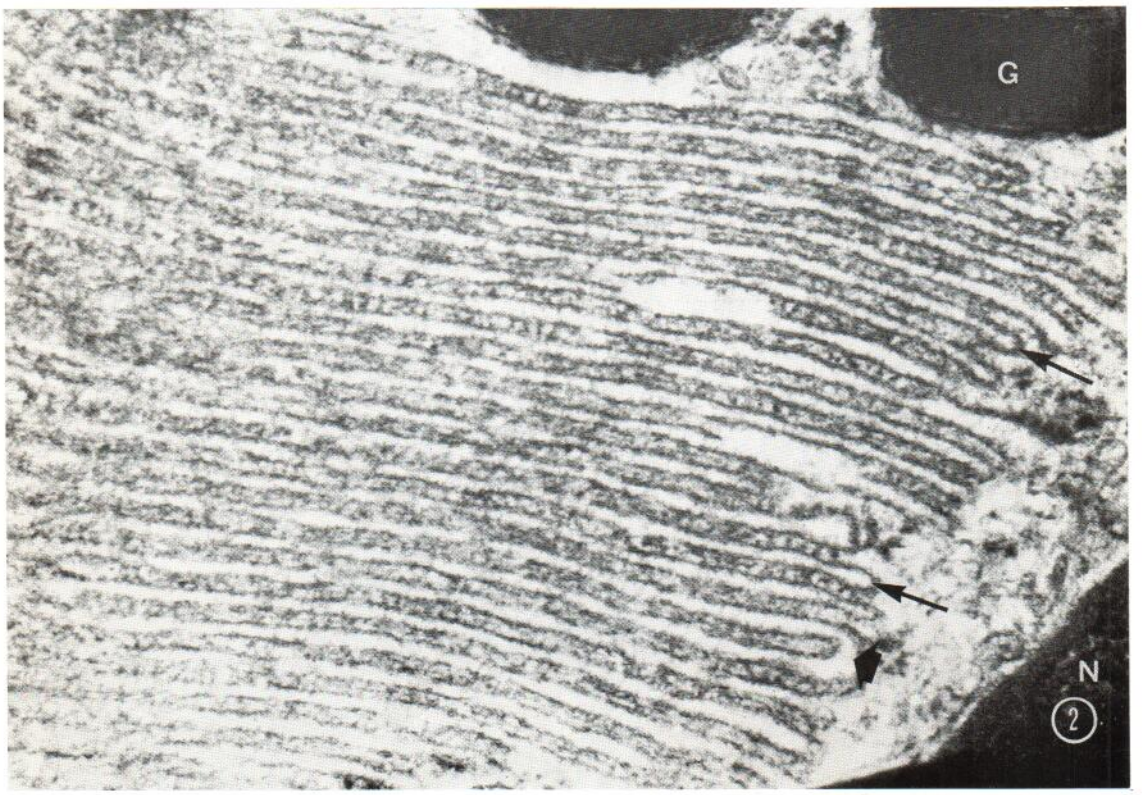
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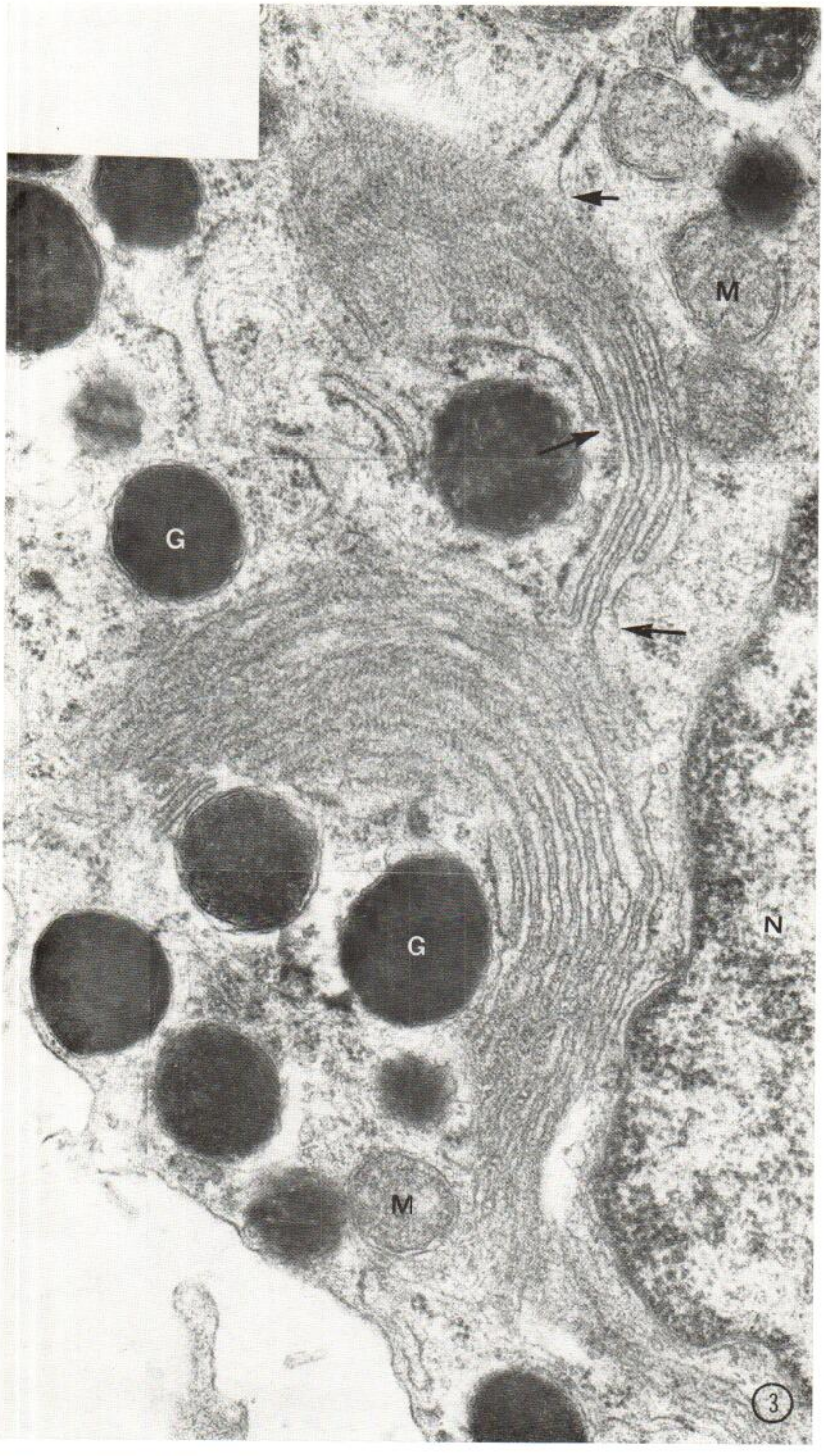
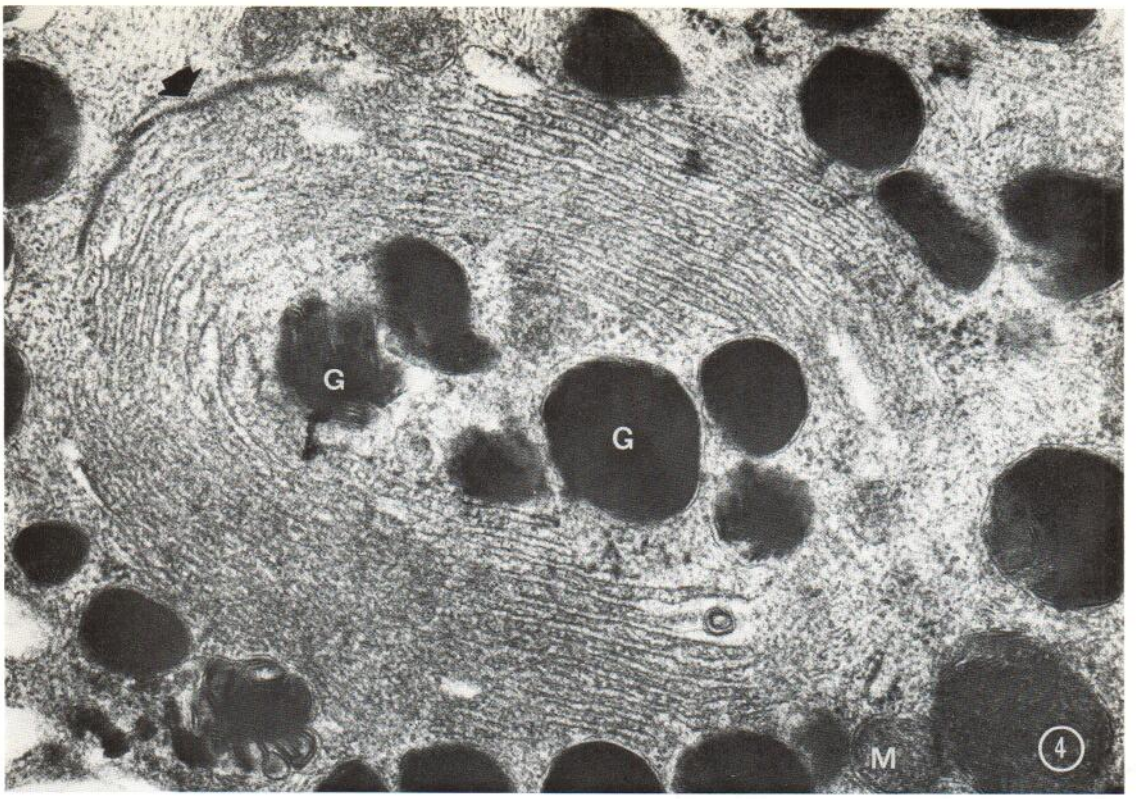
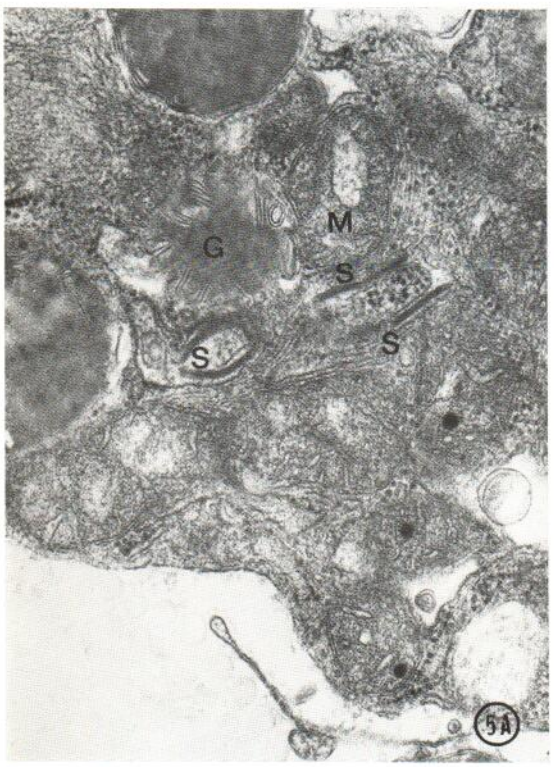


Fig. 3. Lucent bands in strata showing bent forms. Arrows indicate continuities between granular endoplasmic reticulum and the lucent bands. *G*, indicates mast-cell granules; *N*, nucleus; *M*, mitochondria. × 40,000.





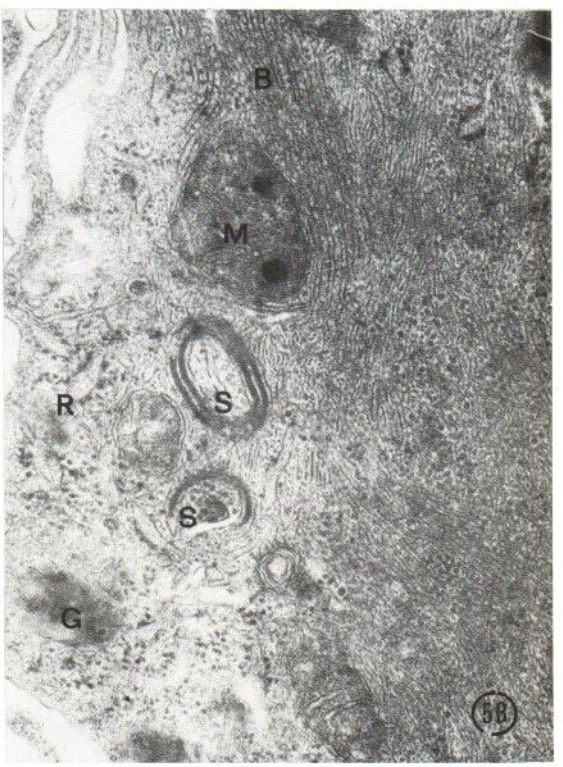


Fig. 4. Circular lucent bands in strata surrounded by mitochondria (*M*) and mast-cell granules (*G*). An arrow indicates a dense strip with periodical banding. × 44,000.

Fig. 5 A, B. Dense strips (*S*) in spaces bounded by a membrane and showing various forms. *B*, lucent band array; *R*, granular endoplasmic reticulum; *M*, mitochondria; *G*, mast-cell granules. × 44,000.

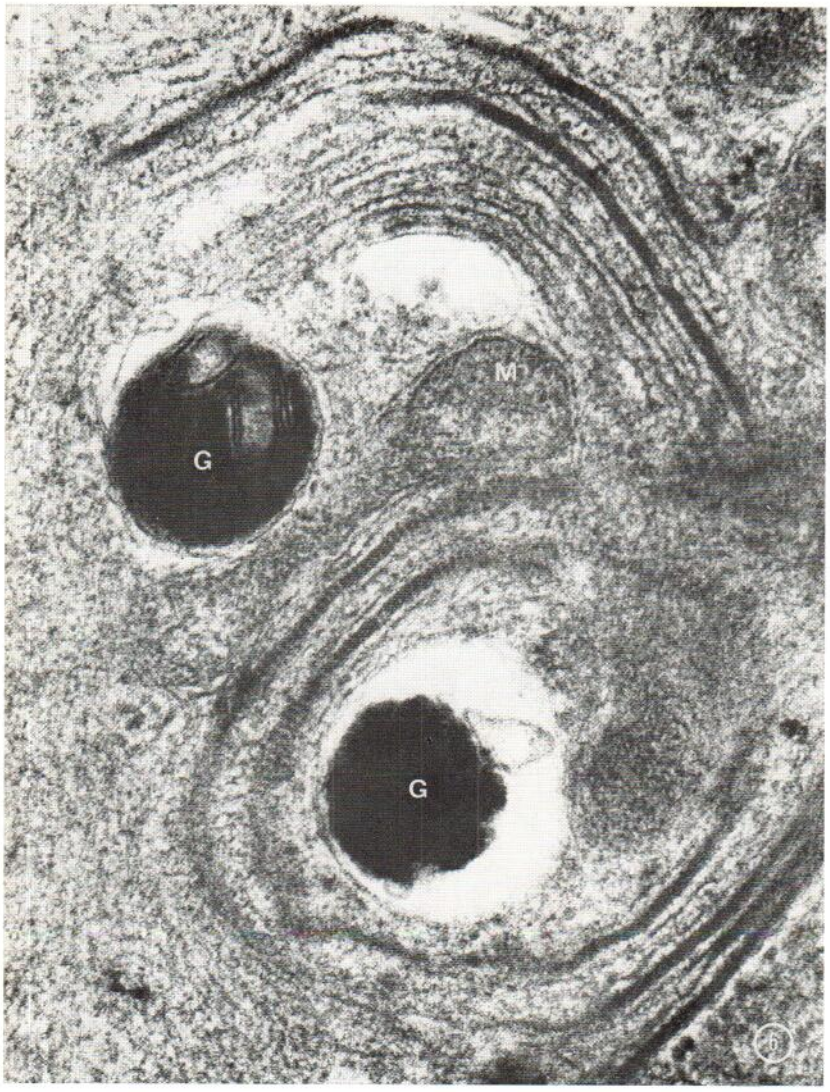


Fig. 6. Dense strips in the area of lucent bands. The strips show periodical banding. The lucent areas containing dense strips are wider than those without strips. *G*, granules; *M*, mitochondrion. × 104,000.

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-

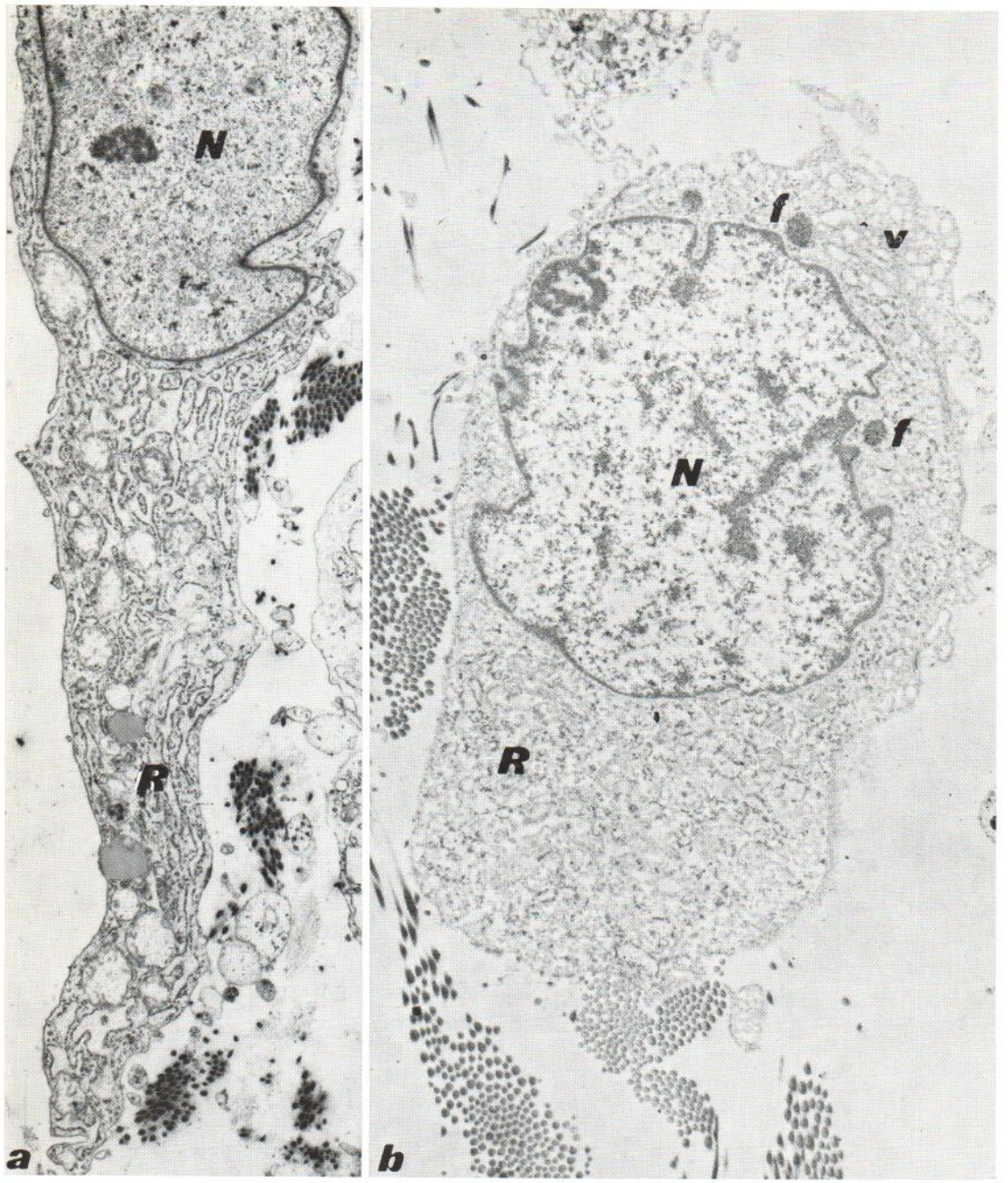


Fig. 1. (*a*) An elongated dermal fibroblast from normal skin. The cytoplasm is filled with a number of sinusoidal arrays of rough-surfaced endoplasmic reticulum (*R*) × *N*-nucleus. × 8500. (*b*) A shrunken fibroblast from the petechial lesion. Rough-surfaced endoplasmic reticulum (*R*) is markedly 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-

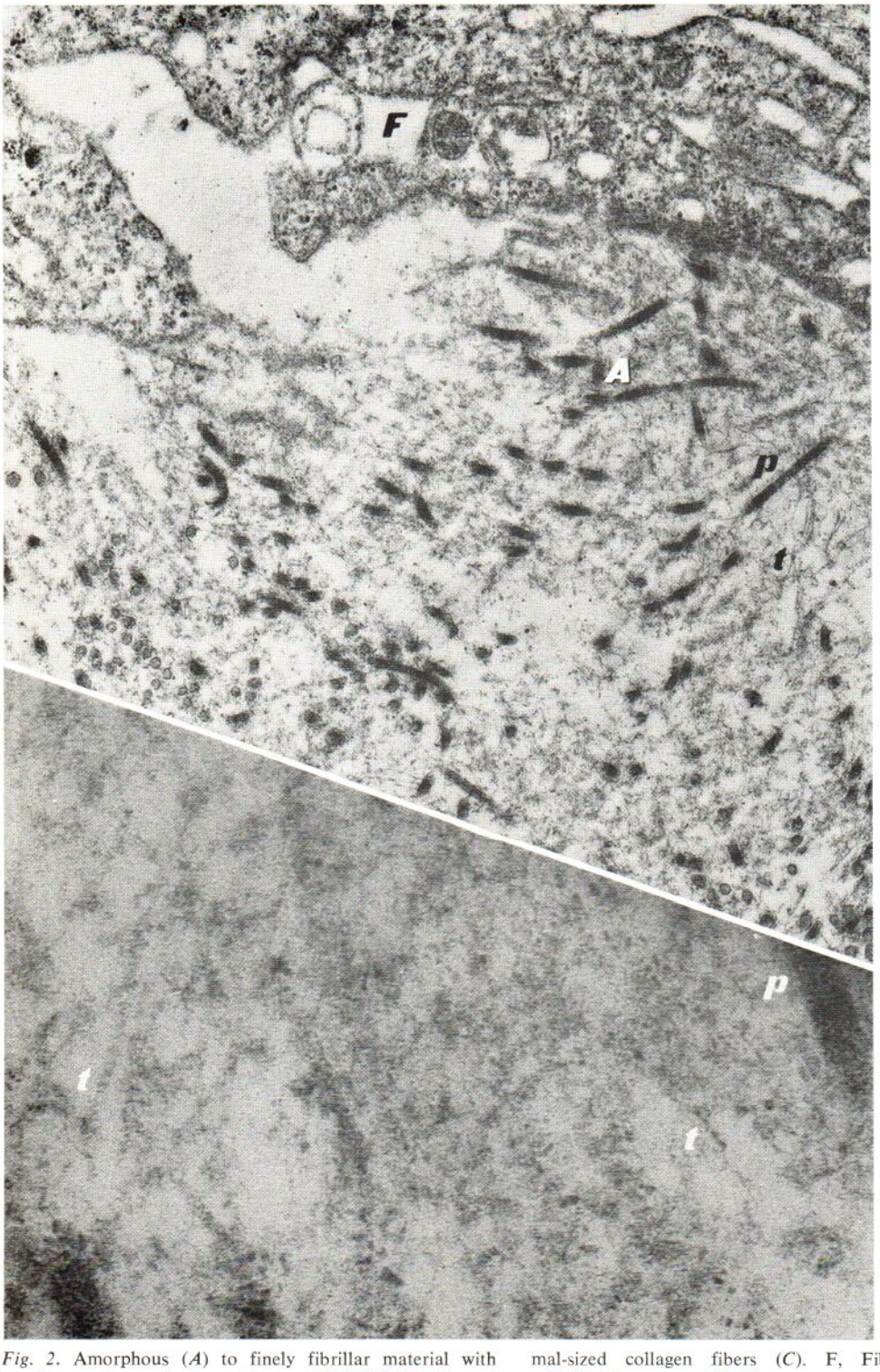


Fig. 2. Amorphous (*A*) to finely fibrillar material with beading pattern of tropocollagen (120–150 A) (*t*) and precollagen (200–550 A) (*p*) fills the spaces between normal-sized collagen fibers (*C*). F, Fibroblast. Upper: × 27,000. Lower: × 260,400.

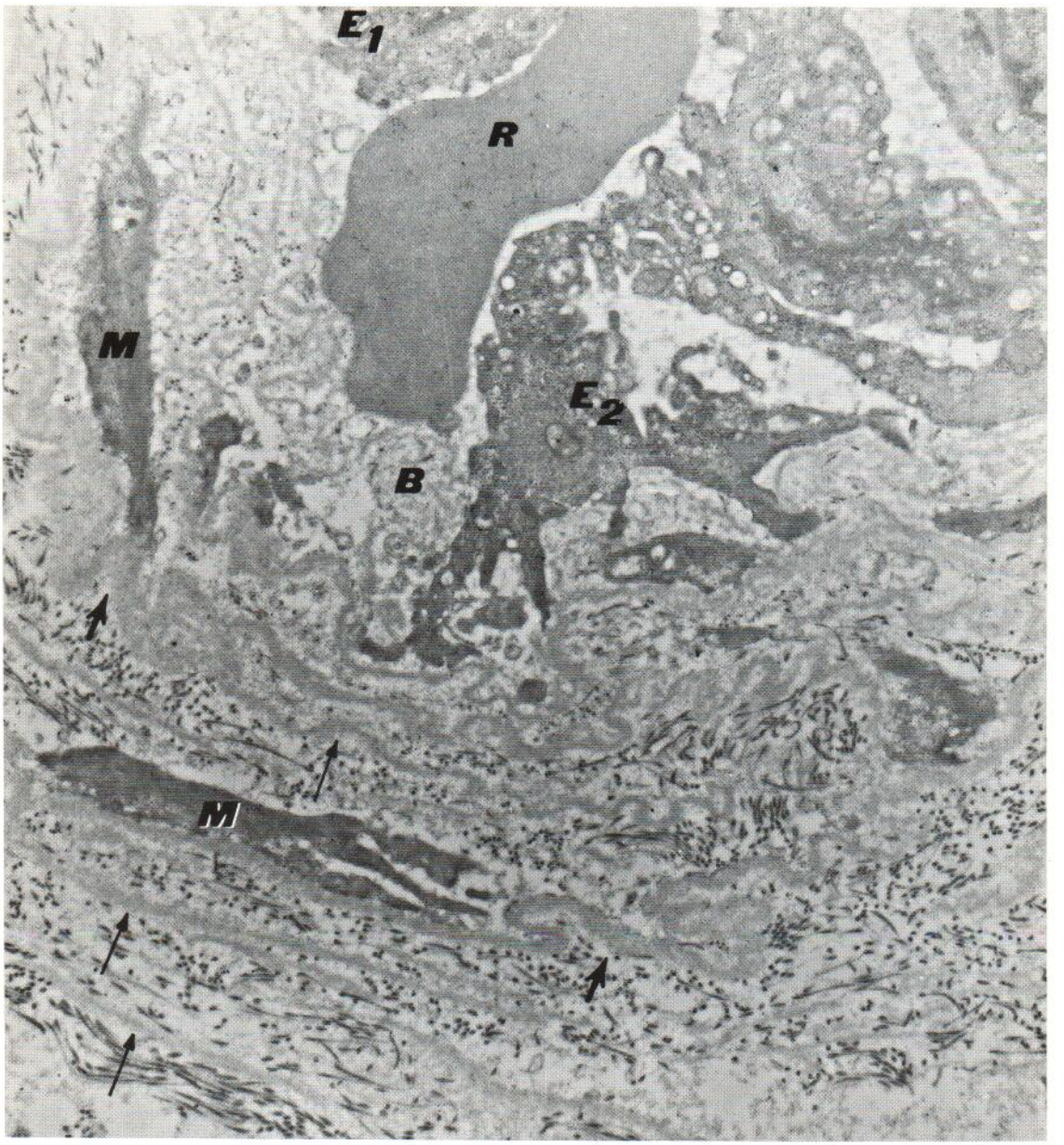


Fig. 3. An erythrocyte (*R*) is extravasating through the separation of two endothelial cells (E_1, E_2). It touches swollen basal lamina-collagen complex (*B*) which exists between endothelium (intima) (E_1, E_2) and muscle cells (media) (*M*). Note multiplication of basal laminae (arrows) and increased 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

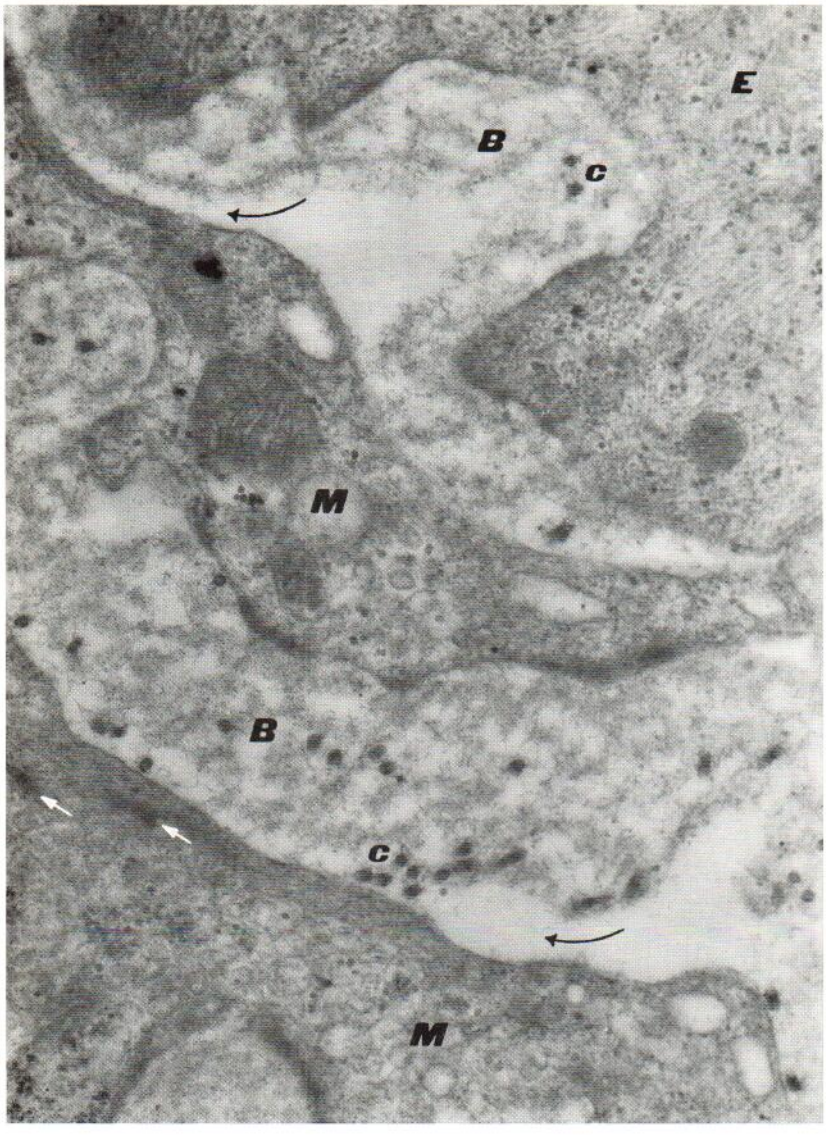


Fig. 4. A high-magnification view of swollen basal lamina-collagen complex (*B*) which fills the spaces between endothelial cells (*E*) and smooth muscle cells (*M*) of a medium-sized vessel. *c*, Collagen fiber; *M* smooth muscle cells; *E*, endothelial cell; White arrows, characteristic black speck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

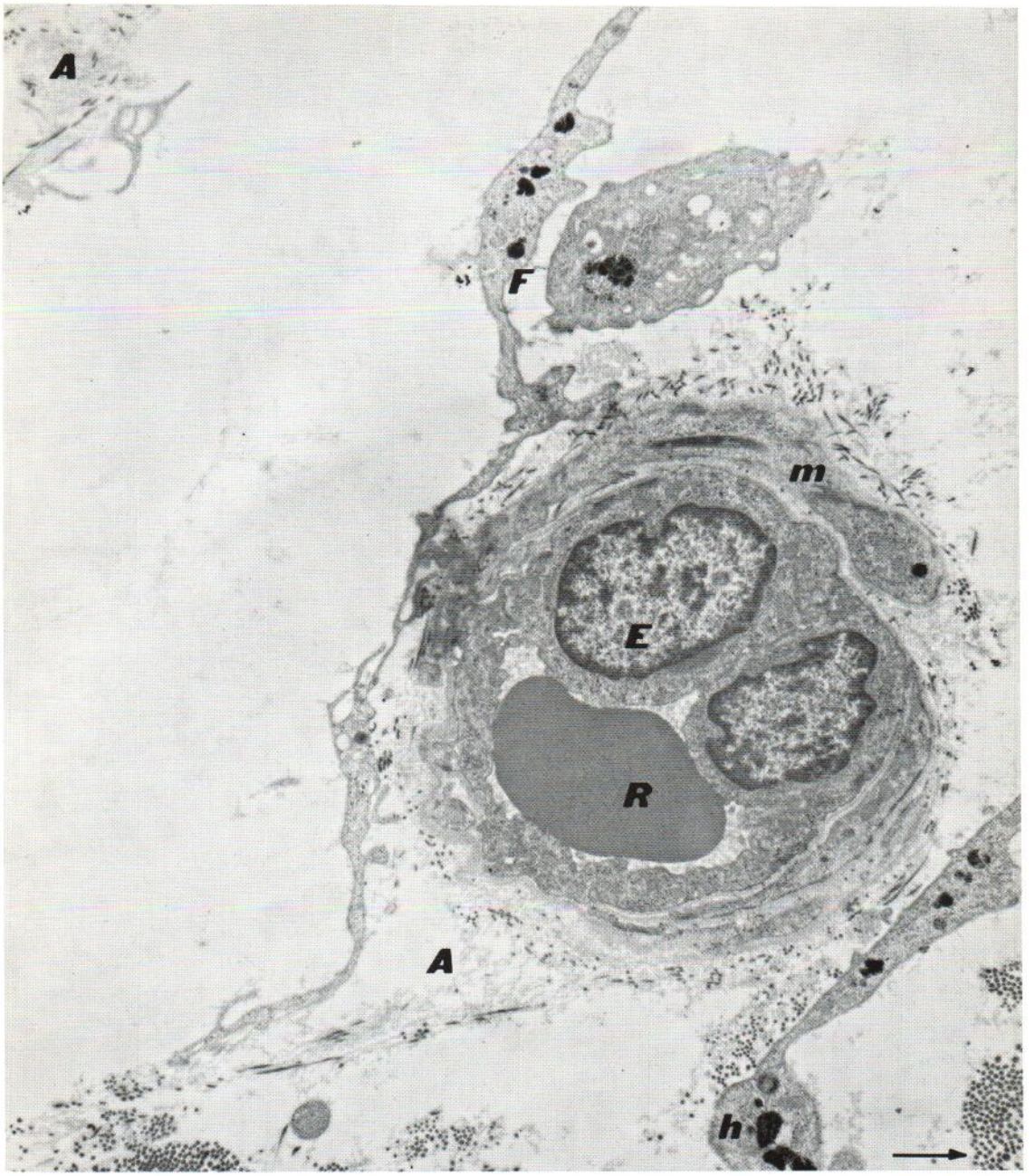


Fig. 5. An arteriole in the mid-dermis appears floating in an empty space which apparently was produced by dissolution of perivascular collagenous tissue. The remaining collagen fibers are extremely variable in size (arrow). Amorphous material (*A*) is increased. *E*, Endothelial cell; *F*, Phagocyte; *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).

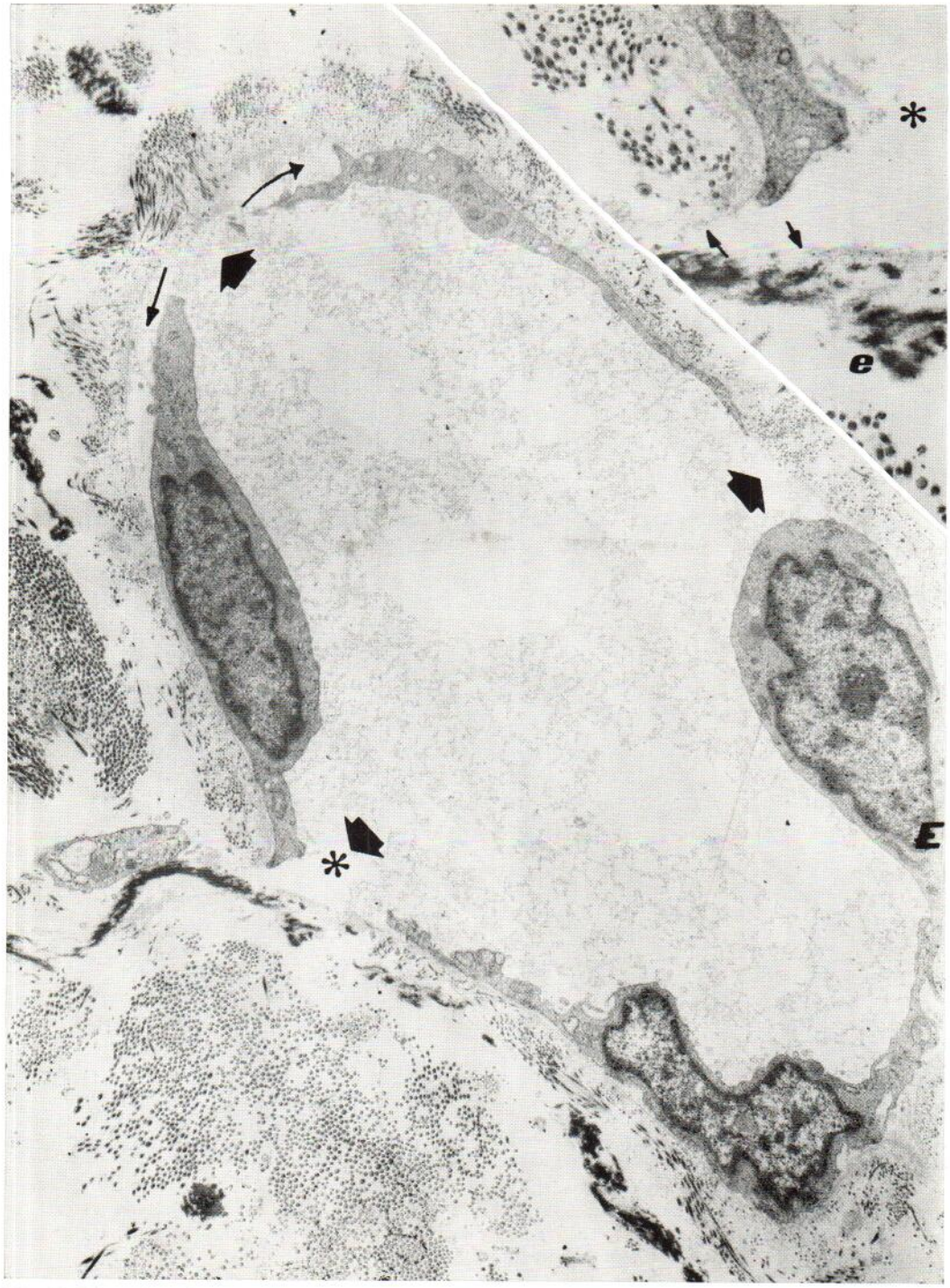


Fig. 6. Disruption of endothelial lining (large arrows) by separation of endothelial cells (*E*) and detachment of some of these from underlying basal lamina (thin arrows) are seen. This vessel is regarded as a venule of the smallest caliber (approximately 20 μ) and not a lymphatic because of the well-developed 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.

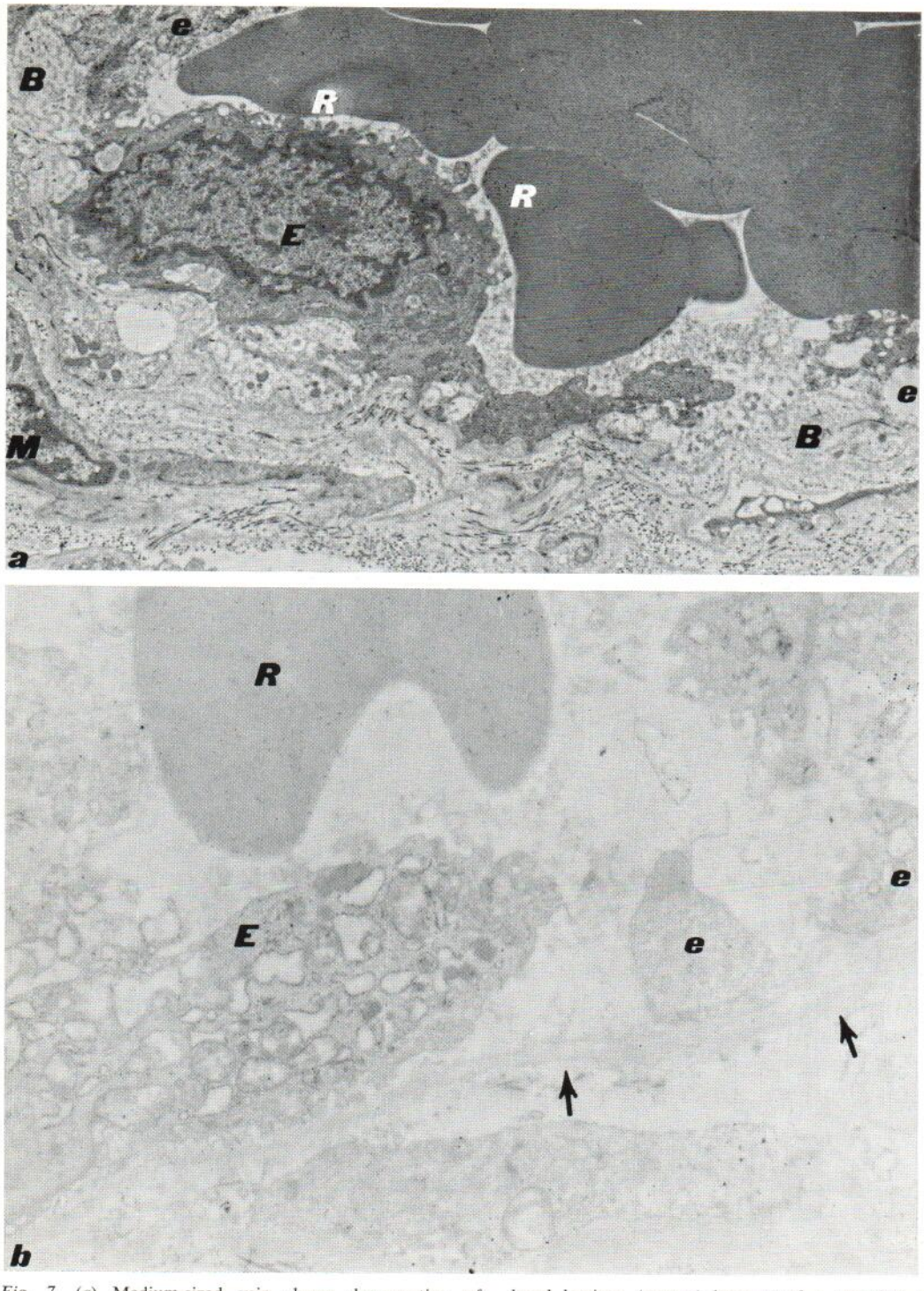


Fig. 7. (*a*) Medium-sized vein shows degeneration of endothelial cells (*e*) and swelling of basal lamina-collagen complex (*B*). *E*, Intact endothelial cell; *M*, smooth muscle cell; *R*, erythrocyte. × 7750. (*b*) The integrity of this small vein is maintained by only a few layers of basal laminae (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.

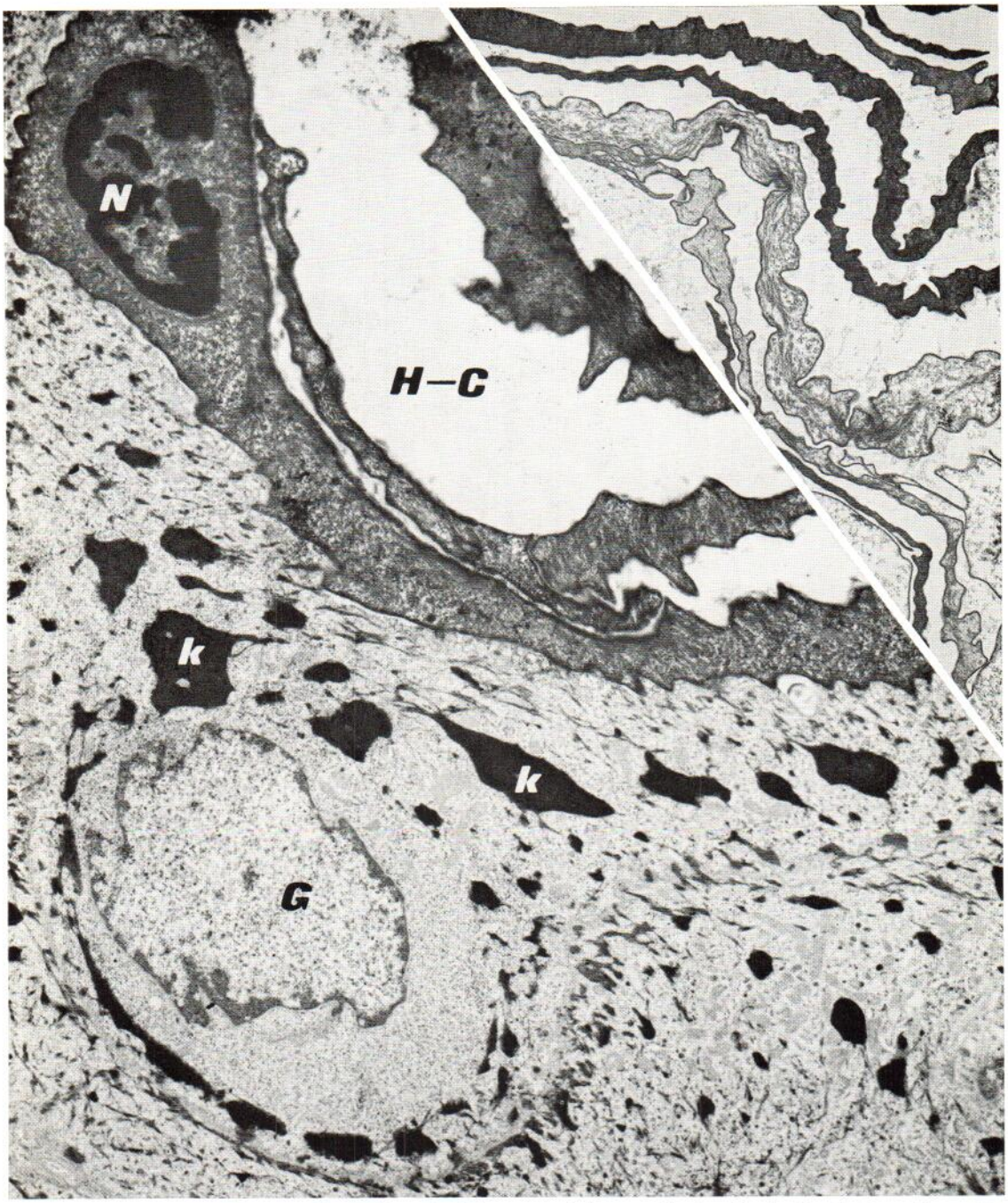


Fig. 8. A nucleus-retaining horny cell (*N*) lines the hair canal (*H–C*) which is plugged with shed, loosely connected horny cells (see inset). *G*, Nucleus of granular cell; *K*, keratohyaline granules. × 5750. Inset: × 2580.

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-

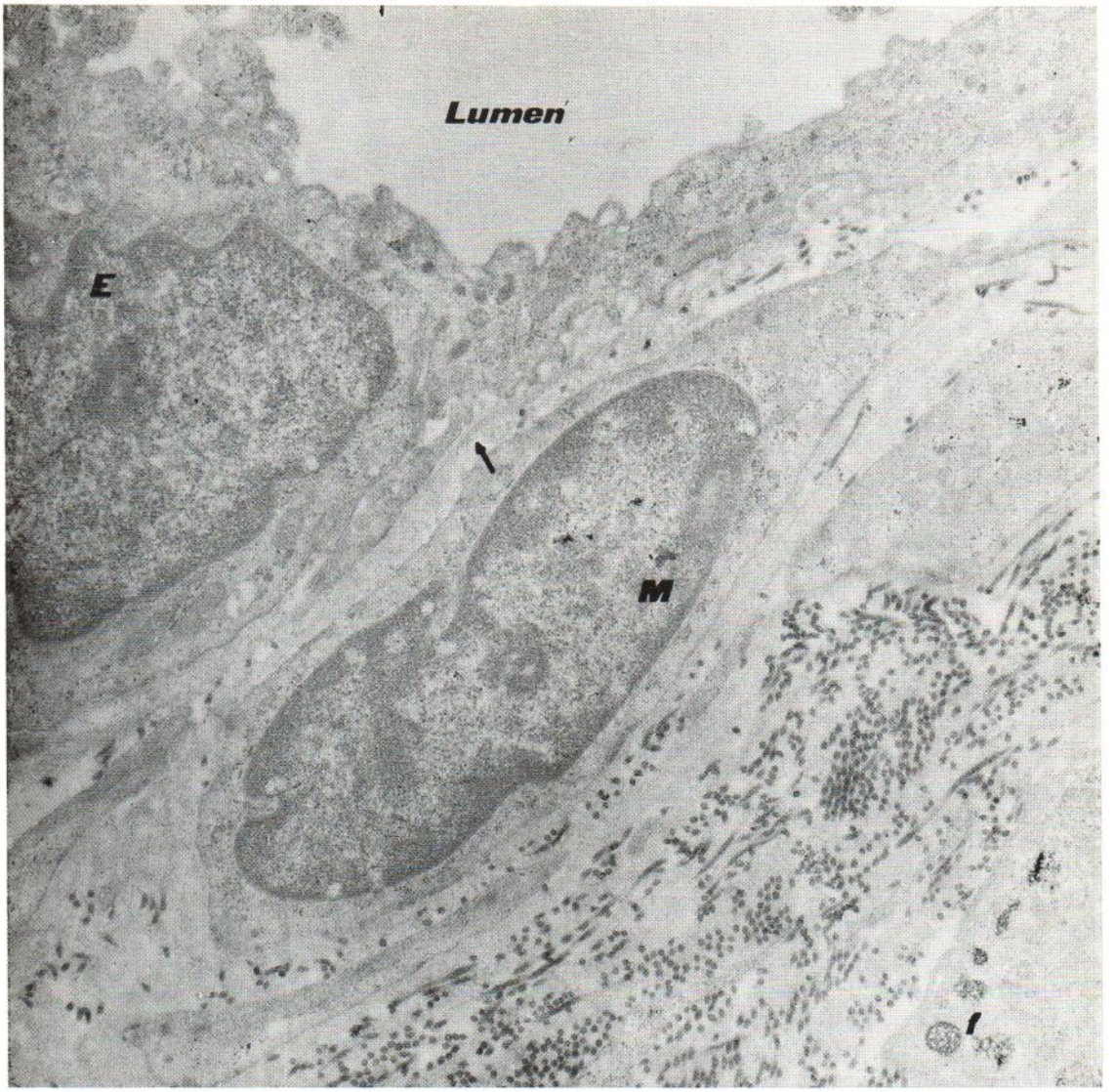


Fig. 9. Biopsy specimen after recovery. A medium-sized venule shows a complete regeneration of the endothelial cells (*E*), absence of the swollen basal lamina-collagen complex and only one layer of the basal lamina (arrow). See Figs. 3, 4, 7 *a* and *b* for comparison. *f*, Ferritin particles in lysosomes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

ACKNOWLEDGEMENTS

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ELASTOLYTIC ENZYMES IN SURFACE WASHINGS OF HUMAN SKIN

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Abstract. The hypothesis that the enzymes of the elastase complex play a role in the metabolism of elastin and in maintenance and/or degeneration of the skin elastic tissue depends on the assumption that these pancreatic enzymes pass into the skin via the circulation. Since the presence of these enzymes can be demonstrated in blood and in the vessel wall, experiments were started to investigate if they could be detected in human skin. The results of the study clearly show that small amounts of elastoproteinase, elastomucase and the elastoproteinase inhibitor are sometimes present in the washings of the human arm.

Balo (1) has pointed out that the elastolytic enzymes of the elastase complex may be decisive in the decomposition of elastic fibers as well as in their synthesis and, moreover, that they are essential for the maintenance of the intact elastic tissue as a whole. Most of these investigations have been carried out with ligamental elastin, aortic elastin and lung tissue, but little attention has been paid to the skin elastic tissue. The susceptibility of skin elastin to elastase digestion and changes in the concentration of the serum elastoproteinase inhibitor have been studied in pseudoxanthema elasticum, senile and solar elastosis, nevus elasticus, striae distensae, lax skin, Ehlers-Danlos Syndrome and some other dermal diseases (2, 5–7, 10–11, 16, 22, 23).

Until recently, the enzymes of the elastase complex could only be isolated and purified from pancreatic powder and were found in the pancreatic juice and in urine. In recent years, however, the presence of these enzymes and enzyme inhibitors were demonstrated in the circulation, in some tissue cultures and in bovine aortic wall (13). The presence of collagenase in rat skin (9, 18) and collagenolytic activity in tissue culture specimens of human skin (3, 19) also prompted investigations into elastolytic enzymes in human skin.

MATERIAL AND METHODS

In an investigation of the effect of alkaline soaps on the skin it was found that concentrated washings of the arms of volunteers sometimes contained small amounts of proteins. One of these proteins could be located in the paper electrophoretogram in exactly the same place as the proteolytic elastase component. This technique has been used in our experiments.

The hand and arm of a volunteer was washed thoroughly with soap and water and then soaked for 30 min in a 2 l graduated glass cylinder filled with a Teorell-Stenhagen buffer of pH 11, heated to 37°C. Hand and arm were frequently rotated in the cylinder. The temperature of the buffer decreased during this period to about 30°C. The skin extract was then neutralized to pH 6–7, rapidly cooled to 4°C, filtered on a Buchner and concentrated in a rotating vacuo-evaporator (Rotavapor, Buchli, Switzerland). The concentrated extract was dialyzed against distilled water in the cold room for three days with several changes of the water and finally lyophilized. In some cases a precipitate was formed during dialysis. This precipitate was collected and dried with acetone. Care was taken that the arm and hand of the volunteer did not show any healing wounds or other damaged area.

The buffer used consisted of 0.004 *M* citric acid, 0.006 *M* phosphoric acid, 0.006 *M* boric acid, 0.029 *N* sodium hydroxide and a sufficient amount of hydrochloric acid to adjust the pH of the buffer to 11.

The dried extracts were investigated for enzyme and inhibitor activity on alkali-treated elastin at pH 8.7 and on acid-treated elastin at pH 7.2. These two substrates are routinely used in our laboratory for the determination of the activity of elastoproteinase, elastomucase (the mucolytic elastase component), and the elastoproteinase inhibitor (12). The enzyme system for measuring elastoproteinase activity in skin extracts consisted of 50 mg elastin homogenized in 10 ml of a borate buffer ($\Gamma/2 =$

0.135; pH 8.7 or 7.2) and x mg dried extract. For the determination of the activity of elastomucase and elastoproteinase inhibitor, 0.05 or 0.1 mg of a highly purified elastoproteinase preparation was also added to the enzyme system (the presence of elastomucase in the skin extract enhances the activity of elastoproteinase; the concentration of the inhibitor can be calculated from the reduced enzyme activity). The enzyme system was incubated for 3 hours at 37°C. The amount of elastin solubilized was measured by the biuret method (12).

The elastoproteinase activity is expressed in enzyme units as follows: E.U. = amount of enzyme solubilizing 50% of the total amount of elastin in the reaction mixture under the experimental conditions used (E.U./mg = elastoproteinase units per mg dried skin washing).

The elastomucase activity is expressed in Em.U. (amount of elastomucase enhancing the activity of 0.05–0.1 mg of a highly purified elastoproteinase by 50% under the experimental conditions used; Em.U./mg = elastomucase units per mg dried extract).

The elastoproteinase inhibitor activity is expressed in I.U. (amount of inhibitor reducing the activity of 0.1 mg of highly purified elastoproteinase by 50% under the standard conditions; I.U./mg = inhibitor units per mg dried skin washing).

One of the disadvantages of heterogenous enzyme systems is the fact that the enzyme reactions depend largely on the particle size of the substrate and subsequently on the stability of the elastin suspension between inversions of the reaction tubes. The accuracy of the measurements of the various enzyme activities has been studied in a large number of experiments. The standard deviation calculated from triplicate determinations of the activity of elastoproteinase is about 5% and that of the activity of elastomucase about 10%. Under the experimental conditions used in the experiments with skin extracts an elastoproteinase activity of 0.02 E.U./mg, an elastomucase activity of 0.05 Em.U./mg and an elastoproteinase inhibitor activity of 0.04 I.U./mg can be detected reliably.

From most of the skin washings paper electrophoretograms were performed with the Beckman paper electrophoresis apparatus. (Djurrum type; Veronal buffer of $\Gamma/2 = 0.075$ and pH 8.6; Whatman 3MM paper strips: constant current of 14 mA; mean voltage of 160 V; running period 11 hours). The paper strips were dried at 120°C and stained with Amido Black 10 B. In this way the bands of 60 μg of enzyme give a just-visible staining.

RESULTS

Table I and Fig. 1 are examples of the results obtained with this technique. The yield of washings varied widely. Sometimes a large amount of material completely insoluble in veronal or borate buffers was present (see the dark colored protein band on the line of application in the paper electrophoretograms). In some cases only this electrophoretically immobile material could be detected

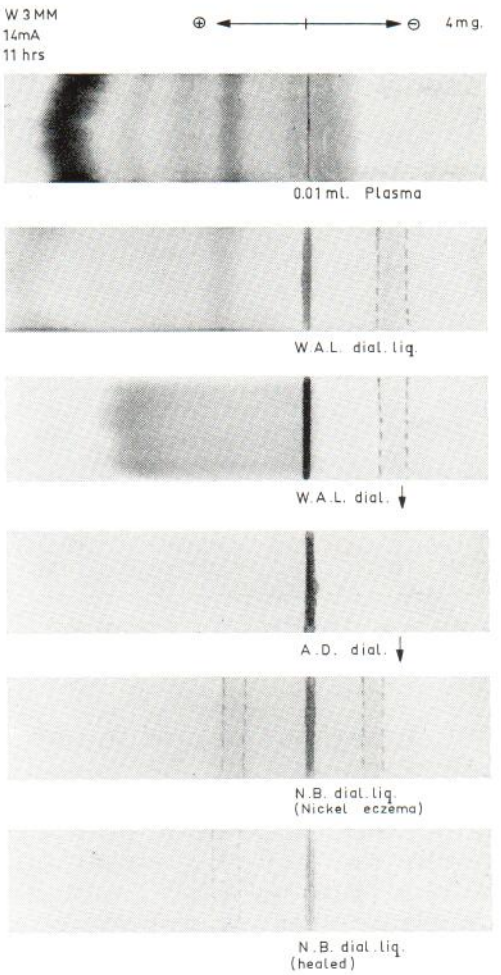

Fig. 1. Paper electrophoretograms of alkaline skin extracts of human arms, sometimes showing the protein band of the enzyme elastoproteinase (band between the dotted lines at the cathodic side of the line of application). For technical data see text.

in the skin washings. In other cases the elastoproteinase band on the cathodic side of the paper strip and some kinds of β- and/or α_2-globulin-like proteins on the anodic side were present (as compared with the paper electrophoretogram of human serum run under the same experimental conditions).

Table I shows that in the skin washings of some volunteers, measurable amounts of elastoproteinase could be detected in spite of the large amount of inert protein material. This material influenced the exact weight of the soluble protein (enzyme activity expressed in units per mg of

Table I. *Elastolytic activities in washings of the human arm (Teorell–Stenhagen buffer of pH 11)*

Name	Sex	Age	Yield	Alkali-treated elastin pH 8.7			Acid-treated elastin pH 7.2		
				E.U./mg	Em.U./mg	I.U./mg	E.U./mg	Em.U./mg	I.U./mg
C. P. O.	♀	21	52 mg	0.04	0.02	—	0.03	0.09	—
A. D.	♀	?	19 mg	0.09	0.10	—	0.07	0.08	—
P. L.	♀	33	160 mg	0.01	—	0.14	0.01	—	0.49
W. A. L.	♂	41	186 mg	0.39	0.04	—	0.29	—	0.04
			dial. ↓						
			17 mg	0.41	0.05	—			
B. J.	♂	43	194 mg	0.13	0.06	—	0.08	0.03	—
			dial. ↓						
			7 mg	0.03	—	—			
G. V. M.	♂	49	54 mg	0.46	0.07	—	0.34	0.06	—
G. G.	♂	58	207 mg	0.00	—	0.24	0.00	—	0.51
J. L. D.	♂	63	63 mg	0.11	0.05	—	0.03	0.08	—
			dial. ↓						
			8 mg	0.02	0.12	—			
A. S. J.	♀	24	39 mg	0.01	—	0.12	0.02	—	0.21
N. B.[a]	♀	55	97 mg	0.27	0.03	—	0.13	0.41	—
N. B.[b]	♀	55	107 mg	0.01	—	0.32	0.00	—	0.70

[a] Patient with so-called nickel eczema on hand and arm.
[b] Patient after healing of the eczema.

total dried skin washings) but could also intervene in the enzymic reaction.

The elastomucase activity is always extremely small. This is due, in part, to the denaturation of the active enzyme by the alkaline buffer and in part also to the fact that if both enzymes are simultaneously present in such small amounts in the reaction mixture, the elastolytic activity measured in the blank is already the combined effect of elastoproteinase and elastomucase. In other words: what will be measured is the excess of elastomucase. On the other hand, if an excess of enzyme inhibitor is present in the skin washings, no enzyme activity can be measured at all (see Table I: P. L., G. G. and N. B.).

The patient identified as N. B. is noteworthy. After healing of the eczema on her arms, the washings did not contain active enzyme but, on the contrary, showed a high elastoproteinase inhibitor activity. In this connection reference is also made to the data obtained from a patient with a so-called dishydrotic eczema. The paper electrophoretogram of the blister fluid shows the protein band of the elastoproteinase. The fluid itself appears to contain 0.45 EU./ml blister fluid (Fig. 2). In two other cases of this eczema these results could not be reproduced, probably due to the fact that the blister fluid was contaminated with large amounts of serum protein (enzyme activity completely covered by the serum enzyme inhibitor activity).

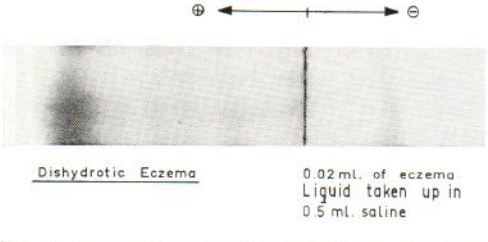

Fig. 2. Paper electrophoretogram of the blister fluid of a dishydrotic eczema diluted with saline. For technical data see text.

DISCUSSION

Elastin is usually held to be an inert protein with a very low metabolic turnover. However, the results of various experiments permit the conclusion that it is more metabolically active than believed hitherto, possibly by reconstitution in situ by binding to acid mucopolysaccharides and other ground substances (21). Subtle molecular events and macromolecular heterogeneity may be responsible for the degenerative reactions in skin connective tissue (4, 25). The concept that the en-

zymes of the elastase complex may to some extent be involved in the maintenance and/or degeneration of skin elastic tissue depends on the assumption that these pancreatic enzymes pass into the skin via the circulation.

The finding of these enzymes in skin washings with alkaline buffers is an indication of their presence in skin itself. Taking into account the high efficiency of the epidermal barrier it is more likely that the enzymes are of epidermal rather than of dermal origin. If the latter is the case, the surface treatment with the pH 11 buffer must have altered drastically the barrier properties of the epidermis. Recently, Yamada & Ofuji (24) demonstrated histochemical evidence of the presence of proteolytic enzymes in human epidermis.

Another explanation of the presence of these enzymes in skin extracts might be their excretion together with sweat secretion. However, the only indication in this direction is the impression that much less material can be extracted from skin with alkaline buffer at 20°C than at 37°C. In any case, the next step will be, identification of elastolytic enzymes in the subepidermal connective tissue.

A bacterial or fungal origin of the elastolytic enzymes can be excluded for the following reasons: (*a*) arms and hands of none of the volunteers showed any inflammatory type of infection (except patient N. B.); no "elastase-producing" dermatophytes or pathogenic fungi were thus present (20); (*b*) the characteristics of two other bacterial elastases (Pseudomonas aeruginosa and Flavobacterium elastolyticum) largely differ from those of pancreatic elastase, e.g. in respect of their place in the paper electrophoretogram, and in regard to pH and temperature optima (14, 15, 17). The fact that the enzyme activities in the skin washings are much less marked than expected from work with pancreatic enzymes is probably not only due to the high pH of the buffer and the temperature during the soaking period of the arm, but can also be explained by the observations of Hall (8) who demonstrated that elastoproteinase exists in two forms: a monomer form (the free enzyme molecule) and the dimer form (two molecules cross-linked by calcium). These two forms of the enzyme act on elastin in a different way and also differ from each other in some other characteristics (e.g. heat lability). Since the Teorell–Stenhagen buffer contains calcium-chelating agents, this may not only have affected the extraction of the enzymes from the skin, but may also inhibit the elastoproteinase activity (in the same way as citric acid and EDTA).

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PLETHYSMOGRAPHIC RECORDINGS OF SKIN PULSES WITH PARTICULAR REFERENCE TO THE PIEZOELECTRIC METHOD

I. *Preliminary Report*

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Abstract. A piezoelectric plethysmograph measuring skin pulses is described. The piezoelectric method may be applied on extremities, normal skin of the forehead and on hyperaemic diseased skin. Some results of the registrations are reported. Advantages and drawbacks compared with the photoelectric method are briefly outlined. The recording of skin pulses gives objective information on vasoconstriction and vasodilatation of the cutaneous vessels. This study suggests that recordings of this type may have great value in investigations of the vasoconstrictor potency of some topical drugs, for instance corticosteroids.

This report deals with a new method for pulse registration in normal and diseased skin by means of a piezoelectric plethysmograph (piezo = pressure). The method may have several applications in dermatology (1, 2, 3, 8). The preliminary results are described of a study of vasoconstrictor effects of corticosteroid ointments in normal and psoriatic skin, and of pulse registrations from extremities.

METHOD

The piezoelectric applicator is shown in Fig. 1. Fundamentally a piezoelectric crystal transforms pressure in the crystal into an electric charge proportional to the force applied. The piezoelectric crystal is hermetically sealed in a small microphone capsule ($18 \times 18 \times 6$ mm) used in the transducer. A sensitive bar (sensor) which transforms the skin pulses is glued to the centre of the crystal. During registration the transducer rests freely on a wing-formed plate (Fig. 1 *a*). In investigations of the extremities the plate is replaced by a hollow capsule which encloses the pulpa of the finger or the toe (Fig. 1 *b*). The part of the sensor which touches the skin measures 3 mm^2 and exerts a pressure of approximately 2 mm Hg against the skin surface. This pressure may be regulated by turning the plate or the capsule, but is considered to be constant during registration. The transducer is sensitive to pressure variations of 0.5 mm Hg minimum. The pulse waves were recorded on an electrocardiograph with a speed of 50 mm/sec. The pre-amplifier circuit is shown in Fig. 2. In order to obtain equal external conditions and diminish the vasoconstrictive effects of low temperatures, all registrations were performed at a room temperature of 25°C (5, 10). In pa-

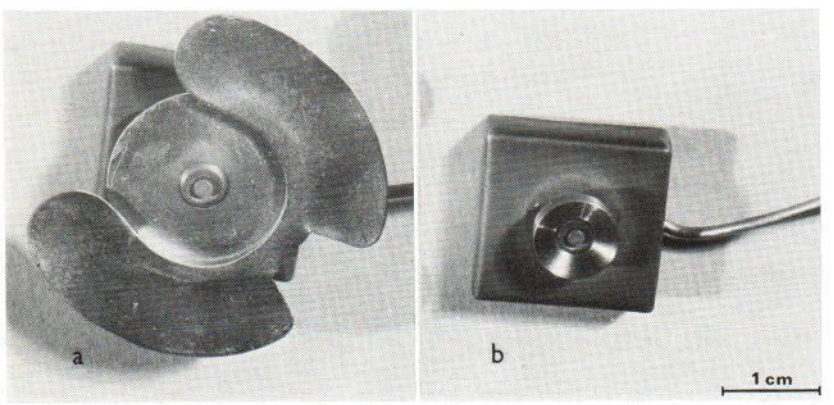

Fig. 1. Close-up of transducer showing applicator for skin regions (*a*), and for extremities (*b*).

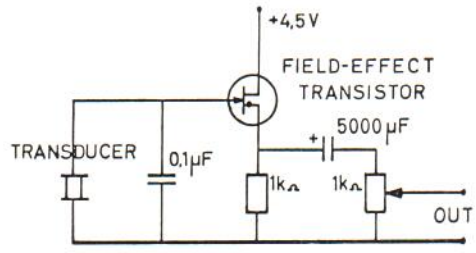

Fig. 2. The pre-amplifier circuit.

tients with psoriasis, lesions on the upper trunk and patella were used for the recordings. The forehead was the site in subjects with normal skin. Recordings from the extremities were performed on the third finger and second toe respectively. Further details of the method will be reported subsequently.

RESULTS

Fig. 3 shows registrations from normal skin of the forehead before and after the application of betamethasone-17-valerate, 0.1 % in an ointment base under plastic occlusion. Large pulse waves (Fig. 3 *a*) are seen with an ascending and a descending branch. On the latter, a rebound, *E*, is observed which is the positive wave produced by the closure of the aortic valves. After 24 hours occlusion the pulsations are reduced (Fig. 3 *b*), and after 48 hours the pulse waves have disappeared (Fig. 3 *c*). The skin pulses reappeared within 24 hours of removal of the occlusive bandage (Fig. 3 *d*). Corresponding registrations were performed on psoriatic skin (Fig. 4). These showed more flattened pulse curves without rebound. After 24 and 48 hours' application of betamethasone-17-valerate under plastic occlusion the pulse amplitudes were successively reduced (Fig. 4 *b* and *c*) and disappeared after 72 hours (Fig. 4 *d*). At that time the eruptions appeared pale and less infiltrated.

However, within 24 hours of conclusion of the treatment the pulse waves reappeared (Fig. 4 *e*). As control the same experiments were performed with the ointment base only and on the same sub-

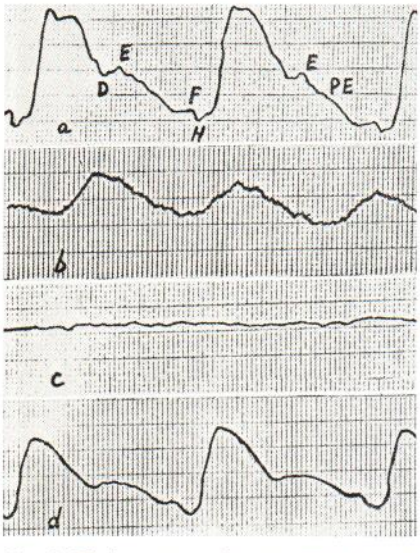

Fig. 3. Plethysmogram from normal skin of the forehead. Before treatment (*a*), after 24 and 48 hours of treatment (*b*) and (*c*). 24 hours after cessation of treatment (*d*).

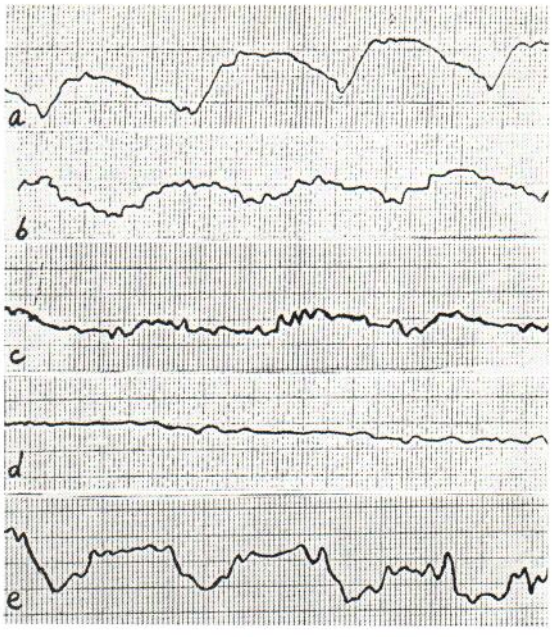

Fig. 4. Plethysmogram from psoriatic skin. Before treatment (*a*), after 24, 48 and 72 hours of treatment (*b*), (*c*) and (*d*). Reappearance of pulse waves 24 hours after cessation of treatment (*e*).

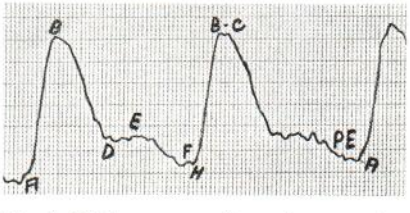

Fig. 5. Plethysmogram from the second toe in a patient with venous ulcer.

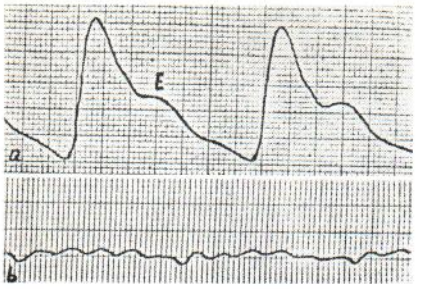

Fig. 6. Normal subject. Plethysmogram from the third finger (*a*). After immersion in cold water (15°C) producing vasoconstriction (*b*).

jects investigated with betamethasone-17-valerate. No changes of the pulse curves were observed in psoriatic or normal subjects during these control experiments.

Fig. 5 shows pulse waves from the second toe of a patient with venous leg ulcer. According to Jacquet (7), the pulse waves and the small waves superimposed upon these indicate the following: *A* for the foot of the systolic, ascending branch, *B* and *C* for the beginning and ending of the systolic plateau, *D* for the catacrotic incisure, *E* for the top of the dicrotic wave or rebound, *PE* for the part of the descending limb distally to *E*, *F* for the positive wave due to the contraction of the auricles and *H* for the negative wave due to the isometric contraction of the heart chambers. The second part of the top of the curve and the descending branch—the angiologic part of the pulse curve—represent the microcirculation.

Registration from the third finger of a normal subject is shown in Fig. 6. After immersion in cold water, 15°C (6), the pulse waves disappeared.

DISCUSSION

The method used in this investigation measures pulsations of blood in the small skin vessels. An increase in pulsation indicates vasodilatation and vice versa. In 1938 Hertzman (4) was the first to describe a photoelectric method recording skin pulses in regions other than the extremities. Later (5) he made the following statement: "The estimation of the rates of cutaneous blood flows from the photoelectric recordings of the skin volume pulses is sufficiently correct to have value in the study of vascular reactions in the skin." Hertzman calibrated the method and found maximal flows in facial skin including the forehead, and in fingers and toes, implying a corresponding larger vascular bed. His method of recording the skin pulses was not applied in dermatological investigations until recently (9). It is emphasized, however, that according to Winsor (13) the instrument gives qualitative but not quantitative information relative to skin flow. At the skin clinic of the Ullevaal Hospital the piezoelectric method has been used for two years, and appears to give valuable information concerning the vasoconstrictor potency of topical drugs, e.g. corticosteroids. Recordings of the skin pulses which give objective evidence of vasoconstriction and vasodilatation, as well as the microcirculation, have also proved to be of value in the study of various dermatoses.

Comparing the piezoelectric and photoelectric methods, the latter has the following limitations: it measures only the variations of the optic density (7, 11), which depends on the various tissues traversed by the light. Furthermore, the pulsations may be reduced by venous stasis. These facts make the photoelectric method less convenient than the piezoelectric one for comparative investigations performed on the extremities (11). However, the method is well suited for studies performed on the skin in other regions (5).

The use of the piezoelectric method is restricted to the regions referred to in this paper. In hyperemic states and in dermatoses accompanied by vasodilatation it is very useful. Concerning registration from the extremities it is superior to the photoelectric method (7, 11).

The piezoelectric method can be calibrated in terms of pressure. In applying the capsule which encloses the pulpa of the finger and the toe, the apparatus is registering tissue volume changes during the systole. The method is thus a plethysmograph, according to Jacquet (7). The curves are distinguished by their reproducibility and the great sensitivity of the apparatus makes it possible to observe even the smallest oscillations. The latter are important in the study of the microcirculation, e.g. in venous leg ulcers (8, 11, 12). The possibility of applying the apparatus on extremities as well as other areas makes it suitable in dermatological investigations.

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EFFECTS OF ANTIHISTAMINES, ACETYLSALICYLIC ACID AND PREDNISONE ON CUTANEOUS REACTIONS TO KALLIKREIN AND PROSTAGLANDIN E_1

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Abstract. The effects of antihistamines, acetylsalicylic acid and prednisone on the reactions to intradermally injected kallikrein, prostaglandin E_1, bradykinin and histamine were studied in patients with various minor skin disorders and in patients with chronic urticaria.

Systemic treatment with mepyramine reduced the size of the histamine-induced weal and both mepyramine and cyproheptadine strongly inhibited the axon-reflex mediated flare. None of them significantly lessened the weal produced by bradykinin. Nor did acetylsalicylic acid or prednisone change the response to histamine or bradykinin.

The antihistamines slightly reduced the reactions to kallikrein in patients with a normal kallikrein sensitivity. A more pronounced inhibition of the kallikrein reactions was seen in patients with chronic urticaria who initially showed strong reactions to kallikrein. An inhibition of the kallikrein reaction was also seen after treatment with prednisone and, to some extent, after acetylsalicylic acid.

A slight, but inconsistent, decrease of the erythematous reaction to prostaglandin E_1 was observed during treatment with antihistamines and prednisone while acetylsalicylic acid did not influence the reaction.

The vascular reactivity in human skin to intracutaneously injected kallikrein and prostaglandins E has recently been studied (9, 10). Abnormally increased reactions to both of these substances were found in patients with chronic urticaria. These findings actualized the need of further knowledge about the possibilities of altering the effects of kallikrein and prostaglandins E. It was considered logical to begin with a study of the effects of antihistamines and corticosteroids, as they are the drugs mainly used for symptomatic treatment of chronic urticaria. Since it is possible that the kallikrein-kinin system and the prostaglandins are involved as mediators of inflammatory reactions, the influence of an antiphlogistic, acetyl-salicylic acid was also studied.

MATERIAL AND METHODS

Patients. The study was carried out on seventy-one patients (thirty men and forty-one women). Their ages varied between twenty and sixty-three years. Sixty of them were older than thirty years. Fifteen patients had chronic urticaria and the remainder had various minor dermatoses, such as localized eczematous dermatitis (thirty-four patients), rosacea (five patients) and varicose leg ulcer (four patients). None of the patients had atopic dermatitis. The patients had no treatment with any drugs other than those administered for this study.

Substances used for intradermal tests. 1) Kallikrein (Padutin®, Bayer AG, Leverkusen, Germany). The dry powder containing kallikrein 40 U, thiomersal sodium 0.02 mg and sodium chloride 3.44 mg was dissolved in one or two milliliters of saline, giving a concentration of 40 or 20 U/ml. 2) Prostaglandin E_1, (PGE_1) (kindly supplied by Professor Sune Bergström, Stockholm, Sweden). A stock solution containing 50 μg/ml was diluted in saline to concentrations of 5μg and 1μg/ml. 3) Histamine hydrochloride, 0.1 mg/ml. 4) Synthetic bradykinin (BRS, Bradykinin, kindly supplied by Sandoz, Stockholm, Sweden), 0.1 mg/ml.

Procedure. On the first day the patients were given a primary intradermal test on the volar sides of the forearms with kallikrein, 4 U and/or 2 U; PGE_1, 0.5 and/or 0.1 μg; bradykinin, 0.01 mg; histamine, 0,01 mg; all in a volume of 0.1 ml. Often only 2 U of kallikrein were used in patients with chronic urticaria because of their pronounced sensitivity to kallikrein. On the second and third day the patients were given one of the following tablets: mepyramine (Anthisan®, Pharma Rodia, Birkeröd, Denmark), 100 mg three times a day; cyproheptadine (Periactin®, Merck, Sharp & Dohme, Int., New York, USA), 4 mg three times a day; acetylsalicylic acid, 1 g five times a day; prednisone, 50 mg before breakfast. On the third day, two hours after the patients had taken their morning dose of the drug, injections were given at corresponding sites on the other arm. A single dose of 50 mg prednisone or 1 g of acetylsalicylic acid was given to another series of patients.

The test reactions were measured and estimated as previously described in detail (9, 10).

Table I. *Influence of antihistamines, acetylsalicylic acid and prednisone on cutaneous reaction to intradermally injected kallikrein*

Drugs	No. of patients	Mean values ± S.E. of the mean of individual ratios for size of kallikrein reaction after/before treatment			
		0.3 h	1 h	2 h	5 h
		Kallikrein 4 U			
Mepyramine	16	0.91±0.15	0.74±0.14	0.75±0.14	0.64±0.11[c]
Cyproheptadine	11	1.18±0.28	0.84±0.15	0.74±0.16	0.52±0.17[b]
Acetylsalicylic acid	11	1.19±0.07	0.88±0.15	0.78±0.13	0.86±0.13
Prednisone (repeated doses)	11	1.21±0.17	0.82±0.16	0.81±0.14	0.56±0.10[d]
Prednisone (single dose)	15	1.27±0.16	1.30±0.19	0.95±0.18	0.76±0.13
		Kallikrein 2 U			
Mepyramine	17	0.64±0.07[d]	0.75±0.10[a]	0.68±0.12[b]	0.60±0.12[c]
Cyproheptadine	11	0.80±0.13	0.66±0.14[a]	0.70±0.21	0.54±0.10[d]
Acetylsalicylic acid	11	1.30±0.19	0.82±0.06[b]	0.64±0.11[c]	0.49±0.08[d]
Prednisone (repeated doses)	15	1.05±0.14	0.86±0.13	0.86±0.12	0.55±0.10[d]

p = the probability that the differences between the ratios and 1.00 are caused by random factors.
[a] $p<0.05$. [b] $p<0.02$. [c] $p<0.01$. [d] $p<0.001$.

Reproducibility of intradermal reactions. Intradermal tests were carried out for determination of the reproducibility of the test reaction in twenty-five patients with chronic urticaria and with various other skin disorders.

Kallikrein (4 U) and PGE_1 (0.5 μg) were injected at symmetrical sites on the left and right arms either at the same time or on two consecutive days. The mean ratio at 5 hours of the reaction in the left to that in the right arm for kallikrein tests performed at the same time was 1.01±0.07 and 1.06±0.14 for tests performed on two consecutive days. The mean ratio at 1 hour of the PGE_1 tests performed at the same time was 0.99±0.08 and for tests on two consecutive days the ratio was 0.98±0.06. The values obtained at other times after the injection, as well as those obtained with lower doses of kallikrein (1 U) and PGE_1 (0.1 μg), did not differ from those given here.

RESULTS

Effect of antihistamines. The edematous kallikrein infiltration present 5 hours after the injection was reduced by both mepyramine and cyproheptadine, as seen from Table I, where the mean ratios from all patients are given. Patients with chronic urticaria had abnormally increased reactions to kallikrein. In this group the inhibition caused by antihistamines was highly significant at all times of observation (Table II). In the other patients the inhibition after antihistamines was less significant, but the tendency to inhibition was obvious.

The influence of antihistamines on the erythematous reaction induced by 0.1 and 0.5 μg PGE_1 was inconsistent. A slight decrease of the erythematous reaction was seen 20 min after the injection (Table III). The erythema produced at 1 hour by 0.5 μg of PGE_1 was reduced after cyproheptadine, but not after mepyramine. The erythema induced by 0.1 μg of PGE_1 was, however, reduced by mepyramine. The influence on the reaction in eleven patients with chronic urticaria tested with PGE_1 did not deviate from that seen in the twenty-one other patients.

Both mepyramine and cyproheptadine strongly inhibited the axon-reflex mediated flare seen after intradermal injection of histamine (Table IV). After mepyramine the histamine weal was also significantly reduced ($p<0.01$). Cyproheptadine did not influence the size of the wheal. Neither mepyramine nor cyproheptadine changed the wheal seen after bradykinin. Whether or not they influenced the erythema is uncertain, but a marked inhibition of the flare was seen in those patients who developed an axon-reflex mediated flare ($p<0.001$).

Effect of acetylsalicylic acid. The infiltrations induced by 4 U of kallikrein were not significantly reduced by aspirin. The reactions to 2 U of kallikrein were, however, markedly decreased (Table I). Only one patient with chronic urticaria was given the full dose of aspirin. His urticaria worsened on the second day of aspirin consump-

Table II. *Influence of antihistamines and prednisone on the reaction to kallikrein in (a) patients with chronic urticaria; (b) patients with various other dermatoses*

Drugs		No. of patients	Mean values ± S.E. of the mean of individual ratios for size of kallikrein reaction after/before treatment Kallikrein 2 U			
			0.3 h	1 h	2 h	5 h
Mepyramine + Cyproheptadine	(*a*)	11	0.54 ± 0.05^{d}	0.51 ± 0.13^{d}	0.46 ± 0.12^{d}	0.40 ± 0.06^{d}
	(*b*)	18	0.76 ± 0.09^{b}	0.81 ± 0.09	0.85 ± 0.14	0.70 ± 0.11^{b}
Prednisone (repeated doses)	(*a*)	7	0.99 ± 0.21	0.70 ± 0.15	0.74 ± 0.20	0.42 ± 0.13^{c}
	(*b*)	8	1.12 ± 0.15	0.99 ± 0.18	0.89 ± 0.16	0.68 ± 0.12^{a}

[a] $p < 0.05$. [b] $p < 0.02$. [c] $p < 0.01$. [d] $p < 0.001$.

tion, but his reaction to kallikrein remained the same. Four patients with chronic urticaria were given a single dose of 1 g of aspirin without any obvious influence on the reactivity to kallikrein.

The reactions to PGE_1, histamine and bradykinin were not influenced by treatment with aspirin (Tables III and IV).

Effect of prednisone. After repeated doses of 50 mg of prednisone, the kallikrein infiltrations present 5 hours after injection were diminished. The patients with chronic urticaria showed a more pronounced reduction of their kallikrein reactivity than the other patients (Table II). A single dose of 50 mg prednisone caused no significant change of the intradermal reaction to kallikrein; but none of the patients given this dose of prednisone had chronic urticaria.

No effect on the response to histamine or bradykinin was detected after treatment with prednisone (Table IV) and the response to PGE_1 was only slightly diminished ($p < 0.05$) (Table III).

DISCUSSION

Effects on reactions to kallikrein. There are few substances available with a specific inhibitory effect on the kallikrein-kinin system that are also suitable for therapeutic use. A kallikrein inhibitor purified from cattle parotid gland and lung (Trasylol®, Bayer AG, Leverkusen, Germany) normalized the reactions to kallikrein in patients with chronic urticaria. This cannot be recommended for repeated use at present, however, as there seems to be a risk of serious, allergic side-effects (11). No specific antagonists to kinins are available, although some antihistamines are, to some extent, also anti-bradykinins. From animal and in vitro experiments, inconsistent results have been reported about the effects of antihistamines, acetyl-salicylic acid and corticosteroids on the kallikrein-kinin system (6). It is difficult, therefore, to draw conclusions on the effect of these drugs on the kallikrein-kinin system that are valid for humans. These difficulties are also partly de-

Table III. *Influence of antihistamines, acetylsalicylic acid and prednisone on cutaneous reaction to intradermally injected prostaglandin E_1*

Drugs	No. of patients	Mean values ± S.E. of the mean of individual ratios for size of reaction to PGE, after/before treatment			
		PGE_1 0.1 μg		PGE_1 0.5 μg	
		0.3 h	1 h	0.3 h	1 h
Mepyramine	20	0.84 ± 0.08	0.81 ± 0.07^{b}	0.85 ± 0.08	1.00 ± 0.08
Cyproheptadine	12	0.84 ± 0.09	0.85 ± 0.09	0.89 ± 0.03^{c}	0.80 ± 0.05^{c}
Acetylsalicylic acid	13	1.00 ± 0.11	0.99 ± 0.08	1.16 ± 0.13	1.08 ± 0.12
Prednisone (repeated doses)	13	1.03 ± 0.14	0.74 ± 0.10^{a}	1.04 ± 0.12	0.89 ± 0.09
Prednisone (single dose)	13	—	—	1.14 ± 0.15	0.99 ± 0.08

[a] $p < 0.05$. [b] $p < 0.02$. [c] $p < 0.01$.

Table IV. *Influence of antihistamines, acetylsalicylic acid and prednisone on cutaneous reactions to histamine and bradykinin*

Drug	No. of patients	Mean values ± S.E. of the mean of individual ratios for size of reaction to histamine and bradykinin after/before treatment		
		Histamine		Bradykinin
		Weal 0.3 h	Flare 0.5 h	Weal 0.3 h
Mepyramine	20	0.78 ± 0.07^a	0.60 ± 0.06^b	1.07 ± 0.20
Cyproheptadine	10	0.91 ± 0.24	0.37 ± 0.09^b	1.20 ± 0.18
Acetylsalicylic acid	13	1.17 ± 0.14	0.91 ± 0.08	1.21 ± 0.15
Prednisone (repeated doses)	13	1.33 ± 0.19	0.78 ± 0.21	1.10 ± 0.17

[a] $p < 0.01$. [b] $p < 0.001$.

pendent upon the incompleteness of our knowledge about the factors involved in the activation of kallikrein in man. For instance, the significance of histamine and histamine release for this process is not clear. In dogs injected intra-arterially with histamine or the histamine releasing compound 48/80 an increased kinin-forming activity was detected in lymph (4). Our findings of delayed whealing after intradermal injection of histamine in patients with increased reactions to kallikrein also indicate that kallikrein may be activated by histamine (9). On the other hand, simultaneous injections of histamine and kallikrein produced the same reaction as kallikrein alone in patients with normal sensitivity to kallikrein; nor did pretreatment with compound 48/80 influence the reaction to kallikrein (15). A reduction of the kallikrein-induced edema was, however, obtained after treatment with both mepyramine and cyproheptadine. Mepyramine is known to be one of the more specific antihistamines and is probably devoid of an antibradykinin effect whereas cyproheptadine can, in vitro, inhibit both histamine and bradykinin (19). None of these antihistamines influenced the wealing reaction to bradykinin. The reduction of the response to kallikrein after antihistamines might indicate either that a histamine release occurs parallel with the formation of kinins or that the antihistamines have other effects, not associated with their antihistamine properties, which result in subsequent reduction of the kallikrein-induced edema. The probability of kinins being able to release histamine and vice versa has been discussed by Melmon & Cline (13). The presence of an axon-reflex mediated flare after the injection of bradykinin in many normal subjects might also indicate that kinins could induce a histamine release (9).

The strong reaction to kallikrein found in most patients with chronic urticaria still was increased after antihistamines although it was markedly less when compared with that before antihistamines. The therapeutic effect of antihistamine in chronic urticaria was, however, usually poor (14). In view of the inhibition of the kallikrein reaction, a better effect of antihistamines on the clinical symptoms should, perhaps, have been anticipated. A possible explanation for this discrepancy might be that the role of histamine in the activation of kallikrein or formation of kinins is not dominant, or that the kinins formed are different from those produced by injection of kallikrein.

The effect of acetylsalicylic acid on experimentally induced inflammatory states has been reported by several authors. Administration of salicylate was found to suppress the inflammatory edema produced by thermal injury in rats, and rubefacients in man and guinea pigs (6). In these tests antihistamines had no inhibitory effect. Northover & Subramanian observed an inhibitory action by salicylates on the activities of kallikrein in vitro (17). They also noted that a marked inhibition of the local reaction to intradermally injected kallikrein in rabbits occurred on systemic administration of analgesic-antipyretic drugs. Their findings could not be confirmed by Hebborn & Shaw (7) or Lewis (12). They obtained no effect with acetylsalicylic acid on the activity of kallikrein, but nearly complete inhibition after administration of Trasylol®. Davies et al. also demonstrated that aspirin and various other anti-inflammatory drugs had no effect on kinin forma-

tion in vitro (2). They could not preclude an in vivo effect from these drugs, however, either from metabolites or through inhibition of some other factor relevant for activation of kallikrein.

In the current investigation aspirin was found to reduce the effects of kallikrein in patients without urticaria, but did not change the reactions to histamine or bradykinin. The mechanism of this inhibitory action is not clear. There is evidence that salicylates exert their anti-inflammatory action by inhibiting venular constriction and by decreasing capillary and especially venular permeability (18). It has also been proposed that salicylates act by releasing corticosteroids, by blocking the destruction of the steroids or by acting synergistically with steroids (3). Another factor of importance for the inhibitory action of both aspirin and corticosteroids on the size of the test might be that they inhibit the spreading of the injected kallikrein.

A marked inhibition of the kallikrein reaction was observed during treatment with prednisone, and this was especially pronounced in patients with chronic urticaria. Opinions on the effects of corticosteroids on the kallikrein-kinin system differ. Cline & Melmon found that cortisol prevented release of kinin from substrate by granulocytes or contact with glass (1). Whether the steroid influenced the substrate or the kallikrein activation was not clear. They also discussed the probability of interference with the action of the Hageman factor. Their findings could not be confirmed by Eisen et al. (5). They found little, if any, inhibition of kinin formation by corticosteroids. The results of the present study indicated, however, that the reaction to kallikrein in vivo was inhibited by prednisone, especially in patients with chronic urticaria, all of whom had abnormally increased reactions to kallikrein.

Effects on reactions to Prostaglandin E_1. The prostaglandins E_1 and E_2 are potent local vasodilators of the small arteries when injected intradermally. The effect on the skin seems to be similar to the reaction described as antidromic vasodilatation and it was therefore suggested that the prostaglandins E might be mediators of this reaction (10). It is possible that part of the vasodilatation occurring in inflammatory reactions might be of this antidromic type (20).

Little is known about the influence of various pharmacologic agents and drugs on the cutaneous reactions to PGE. In a previous study it was found that large doses of epinephrine were needed to blanch or prevent an erythematous reaction to PGE (10). It was also found that treatment with antihistamines had no inhibitory effect on the production of the PGE erythema, whereas in the present study a slight inhibition could be observed during antihistamine treatment. Different antihistamines, doses and ways of administration were used and the dose of PGE was larger (5 μg) than in the present study. It was also previously shown that pretreatment of the skin with the compound 48/80 did not influence the response to 0.1 and 1 μg of PGE. This, as well as the slight influence of antihistamines, may indicate that the role of histamine is not essential for the formation of an erythematous response to PGE_1. The slight inhibition obtained from antihistamines on the PGE_1 erythema in this study might possibly be due to an inhibition of a concomitant axon-reflex mediated flare. It could not, however, be a main component of the erythema as the histamine-induced flare—in contrast to the PGE erythema—was strongly inhibited by antihistamines.

After the administration of salicylates the reaction to PGE_1 was essentially the same as before treatment. The effect on the hyperalgesia experienced at the site of PGE_1 erythema was difficult to evaluate. The lack of effect on the erythematous response might be explained if it is correct that salicylates have no vasoconstrictor ability, but rather inhibit inflammatory reactions by decreasing capillary and venular permeability and by inhibiting venous constriction (18). The decrease in the reaction to PGE_1 after repeated doses of prednisone is not of the same magnitude as that seen after local treatment with fluocinolone acetonide. The latter produced blanching of the skin and inhibition of the axon-reflex mediated flare (8) and diminished the reaction to PGE_1 both initially and one to two hours after injection (10). The differences obtained between systemic and local treatment might be explained by the strong vasoconstrictive properties of fluocinolone. It is also likely that the local concentration of active steroid is higher after fluocinolone treatment.

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DECREASED CUTANEOUS REACTIONS TO KALLIKREIN IN PATIENTS WITH ATOPIC DERMATITIS AND PSORIASIS

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Abstract. Reactivity to intradermally injected kallikrein, histamine and bradykinin was studied in patients with various dermatoses. In patients with atopic dermatitis, reaction to kallikrein 5 to 24 hours after injection was strongly diminished. As a rule, these patients had no axon-reflex mediated flare after histamine and bradykinin; when the bradykinin induced weal disappeared, blanching was present at the site of the weal. In a group of patients with "healed" atopic dermatitis and normal levels of serum IgE, the reactions to kallikrein and histamine were normal. Patients with psoriasis had a lowered reactivity to kallikrein compared with that seen in healthy controls. The reactions to histamine and bradykinin were about the same as in the control group. The cause of the diminished reactivity to kallikrein is discussed. It may be associated with the low incidence of eczema and a diminished tendency to develop delayed hypersensitivity reactions observed in patients with psoriasis. A few patients with acne vulgaris also showed small reactions to kallikrein, but usually the reactions were within normal limits. Patients with eczematous dermatitis and a group of patients with various skin disorders also showed normal reactions.

Increased vascular reactions to kallikrein were found in patients with chronic urticaria (9). Preliminary results indicated a lowered reactivity in patients with atopic dermatitis and psoriasis and, occasionally, also in patients with acne vulgaris. The cutaneous reactions to kallikrein were therefore studied further in these and other dermatoses.

MATERIAL AND METHODS

Patients

Atopic dermatitis. (*a*) Twenty patients (8 men, 12 women), most of them between 18 and 30 years of age, with typical atopic skin changes which had usually been present for a long time. The skin was usually dry and slightly lichenified on the volar surfaces of the forearms. Raised levels of IgE (840 to 31,000 ng/ml) were present in ten of the patients, while six patients had levels within normal limits. Three patients had eosiniphilia, 9 to 18%; in one of them the IgE level was normal.

(*b*) Six patients (2 men, 4 women) between the ages of 15 and 30 years. They had previously suffered from atopic dermatitis, but were now free from dermatitic changes. Their IgE levels were normal.

Psoriasis. Forty-four patients (27 men, 17 women) with psoriasis of varying severity and duration. Fourteen of the patients were younger than 30 years of age. The areas used for tests were free from psoriatic lesions.

Acne vulgaris. Forty-two patients (16 men, 26 women) between 14 and 22 years of age. The acne was classified as Grade I to II in twenty-seven patients according to the criteria used by Pillsbury et al. (13); the remainder had Grade III to IV.

Eczematous dermatitis. Thirty patients (17 men, 13 women), twenty-six of whom were older than 30 years of age. They had eczematous dermatitis of varying severity, but the areas used for intradermal tests were free from dermatitic changes. Four of the patients had a hypostatic eczema, four had a nummular eczema, four had an allergic contact eczema, and the cause was unknown in the remainder. Positive patch test reactions were present in thirteen patients.

Various skin disorders. This group comprised thirty-one patients (8 men, 23 women), all of them older than 30 years of age and eight of them older than 60 years. Some of the diagnoses were: pemphigus vulgaris (1), purpura (1), rosacea (6), erythema nodosum (3), discoid lupus erythematousus (5), pruritus (2) hypertrichosis (1), mycosis fungoides (1), varicose leg ulcer (5), dermatitis herpetiformis (1).

Controls. Forty-five apparently healthy subjects (28 men, 17 women), mostly hospital personnel between the ages of 22 and 56 years. Twenty-five of these forty-five subjects had also been used as controls in a previous study (9), and twenty subjects were added subsequently. Two of the controls in the original group had suffered from acne and seborrhea between the ages of 13 and 17 years, but had not had active acne lesions in the most recent years.

Substances used for intradermal tests

Kallikrein (Padutin®, Bayer AG., Leverkusen, Germany). The dry powder containing kallikrein 40 U, thiomersal-

Table I. *Reaction to intradermal injection of kallikrein*

Diagnoses	No. of patients	Mean area of infiltration in mm² ± S.E.M. 0.3 h	1 h	2 h	5 h	24 h
Controls < 30 years	31	131±15	136±26	143±38	448±104	1109±140
Controls ⩾ 30 years	14	121±14	162±21	246±41	413±63	567±133
Atopic dermatitis < 30 years	20	145±22	117±23	131±26	106±27[c]	41±15[d]
Eczematous dermatitis > 30 years	30	145±11	201±19	254±31	470±47	422±74
Psoriasis < 30 years	14	122±16	201±45	192±33	196±76	191±84[d]
Psoriasis > 30 years	30	141±18	166±19	188±22	222±36[b]	225±85[a]
Acne vulgaris < 30 years	42	127±13	206±38	189±38	231±62	1094±126

p = probability that the difference between controls and others is caused by random factors.
[a] $p<0.05$. [b] $p<0.02$. [c] $p<0.01$. [d] $p<0.001$.

sodium 0.02 mg and sodium chloride 3.44 mg was dissolved in one milliliter of saline, giving a concentration of 40 U/ml. Histamine hydrochloride, 0.1 mg/ml. Synthetic bradykinin (BRS, bradykinin, Sandoz, kindly supplied by Sandoz, Stockholm, Sweden), 0.1 mg/ml.

Procedure

Injections of 4 U kallikrein, 0.01 mg histamine, and 0.01 mg bradykinin were given intradermally on the volar side of the forearm. The injected volume was 0.1 ml. The size of the reaction was measured 20 min and 1, 2, 5 and 24 hours after the injection and the area calculated as described previously (9).

RESULTS

In Table I the mean areas of the reactions to kallikrein are listed for the different groups of dermatoses as well as for two groups of controls.

Atopic dermatitis

(*a*) In the first two hours there were no significant changes in reactivity to kallikrein when compared with the controls, but at 5 and 24 hours the edematous infiltration was significantly smaller than that found in the control group. The erythema was usually slight or absent and the tenderness to pressure was also less pronounced than in other subjects.

The weals induced by histamine and bradykinin were not significantly smaller than in controls; however, the erythema was weak in most patients and the axon-reflex mediated flare was usually absent. When the weal was disappearing, the skin at the site of the bradykinin weal usually showed blanching 30 to 60 min after the injection. As a rule, there was also a blanching tendency after histamine.

(*b*) The reactions to kallikrein and histamine in six patients with a "healed" atopic dermatitis were normal (range of area of kallikrein infiltration at 24 hours: 835 to 1920 mm²).

Psoriasis

The reaction to kallikrein in patients with psoriasis was decreased at 5 to 24 hours, compared with healthy controls. In patients younger than 30 years, the decrease was best seen at 24 hours, which is the time for maximal reaction in healthy subjects of this age group. In the older patients the differences were most pronounced at 5 hours when the control subjects of this age group usually had their maximal reaction. The size of the reactions was not correlated to the severity or duration of the psoriasis. The weal and flare reactions to bradykinin and to histamine did not differ significantly from those of the control group.

Acne vulgaris

These patients showed wide variations in their sensitivity to kallikrein. Nine of the forty-two patients had remarkably small infiltrates (⩽100 mm²) or showed no infiltration either at 5 or 24 hours. Four of the nine patients with small reactions had a Grade III to IV acne, the others a Grade I to II. In most patients, however, the reactivity to kallikrein did not differ from that observed in controls, and the mean area of the kallikrein reaction for the whole group of acne patients did not differ significantly from that of the control group. As far as could be seen from this limited number of patients, the reactivity did not seem to be correlated to age, sex, severity of acne or to the ABO blood groups. The reactions to hista-

mine and bradykinin did not differ from those of the controls.

Eczematous dermatitis

The reactivity to kallikrein in this group of patients did not differ from that found in a control group. Also the reactions to histamine and bradykinin were the same as in the controls.

Various disorders

No obvious deviations from ordinary reactivity to kallikrein were observed in any of the patients included in this group.

Controls

In the control groups of the current study, the mean area of infiltration present 5 and 24 hours after the injection of kallikrein was somewhat larger than that found in the original control groups. In our earlier control groups, four subjects had minor reactions to kallikrein. Two of them had a moderate seborrhea and, between the ages of 13 and 17 years, they had suffered from acne. None of the controls who were added subsequently had a history of acne. They all had fairly large, but superficial and non-voluminous, reactions to kallikrein.

DISCUSSION

Atopic dermatitis

The presence of abnormal vascular reactions in atopic dermatitis is well known. They may be manifested as white dermographism, by delayed blanching after an intradermal injection of acetylcholine, by the absence of an axon-reflex mediated flare after an intradermal injection of histamine or by blanching after topical application of furfuryl-nicotinate. The blanching seems most likely to be due to vasoconstriction of the superficial vessels in the skin. A lowered threshold to epinephrine-induced blanching in patients with atopic dermatitis (8) and differences in the storage of catecholamines (18) might be possible mechanisms for the increased vasoconstriction, but the basic cause of this abnormal reactivity is unknown. An increased tendency to edema formation has also been claimed to be present in atopic skin and believed to cause or contribute to the blanching (2).

In the present study the reaction to local injection of kallikrein was markedly decreased compared with that seen in healthy subjects. Normally, an edematous infiltration of the skin is present 5 to 24 hours after the injection. In patients with atopic dermatitis this infiltration was usually insignificant. Intradermally injected bradykinin produced no or only a slight erythema and there was no axon-reflex mediated flare as seen in healthy controls of the same age. The initial reaction was usually followed by blanching after 30 to 60 min. The cause of these deviations from the normal response to kallikrein and bradykinin is not known, but it seems likely that it is connected with the tendency to vasoconstriction present in this disorder. A catecholamine release has been demonstrated in various experimental conditions after injection of both histamine and bradykinin (15). One possible explanation for the decreased reactions found in patients with atopic dermatitis might be that bradykinin and kallikrein induce a release of catecholamines which is more pronounced than in normal skin. The increased sensitivity to catecholamines found in the atopic skin may be a contributory factor. To some extent the kallikrein edema in these patients might also, in fact, be "hidden" in the dry or lichenified skin.

Nothing is yet known about the presence of IgE in atopic skin or the role of this immunoglobulin as a responsible factor for the vascular abnormalities of atopic dermatitis. It is known, however, that the serum levels of IgE tend to become normalized in patients with a "healed" atopic dermatitis (7). The six patients with a "healed" atopic dermatitis included in this study had normal levels of IgE and they also showed normal reactions to kallikrein and to histamine. The possibility might therefore be speculated upon that the presence of an increased amount of reaginic antibodies (IgE) may dispose to the abnormal vascular reactivity. The finding of abnormal vascular reactions in some patients with normal IgE would seem, however, to speak against such a possibility.

Psoriasis

One of the characteristics of psoriasis is the presence of tortuous, dilated and stretched capillaries (6, 17). They are best seen in the center of the plaques, but are, to some extent, also present in

non-involved skin (16). Whether the abnormal vessels are of primary significance for development of the condition or secondary to the psoriatic lesions is not known. The rate of the blood flow is increased in the psoriatic plaques (4). Little is known, however, about vascular reactions or about the response to vasoactive drugs in psoriatic skin. Holti observed a slight decrease in response to histamine and Trafuril® in the normal appearing skin both in psoriatic patients and their relatives (5), and Millberg found a delay in the development of reactive hyperemia (11).

In the present study, patients with psoriasis did not differ from healthy subjects in their reactivity to histamine and bradykinin, whereas the reactivity to kallikrein was markedly diminished. The mechanism of decreased reactivity to kallikrein in psoriasis is not known. Low levels of polypeptides and a blockade of dipeptidase activity in psoriatic skin have been reported by Paschoud et al. (12); this is a finding which might be connected with the presence of a reduction in kallikrein reactions in psoriasis. The possibility of an increased rate of blood flow also in the normal appearing skin, inducing a wash-away effect, might also be considered.

The diminished kallikrein reactions in psoriasis contrast with the highly increased reactions to this enzyme found in chronic urticaria (9). Clinically also these two conditions seem to be opposite to one another. Four patients out of a total of eight hundred and eighteen with psoriasis observed in the past two years also had a diagnosis of chronic urticaria, but the disorders were not active at the same time. Thus, during periods of urticaria, the psoriasis healed or improved markedly and, when their urticaria disappeared, the psoriasis returned.

There is also evidence that patients with psoriasis differ from healthy subjects in their immune response as well as in their ability to develop delayed inflammatory reactions (3). Eczematous reactions are not common in patients with psoriasis (1). Only five of the eight hundred and eighteen patients with psoriasis visiting this clinic in the past two years were found to have an eczematous dermatitis as well; three of these patients had a positive patch test. Patients with psoriasis are not easily sensitized with dinitrochlorobenzene (DNCB) and paranitrosodimethylaniline (NSMA) compared with normal subjects (3), and they have also been found to show a decreased immune response to plaque antigen (3). Epstein et al. therefore suggested that "psoriatic patients suffered a relative block in the immunologic system governing delayed hypersensitivity".

Since patients with abnormally increased sensitivity to kallikrein showed very strong delayed reactions to PPD tuberculin, it was previously suggested that the kallikrein-kinin system might be involved as mediator not only of urticaria formation, but also of delayed hypersensitivity reactions (10). In the current study the patients with eczematous dermatitis and a contact allergy had normal reactions to kallikrein. A diminished reactivity to kallikrein, together with a decreased tendency to develop delayed allergic reactions, found in psoriasis, may further strengthen the hypothesis that the kallikrein-kinin system might be of importance for these allergic reactions.

Acne vulgaris

In most patients with acne the reactions to kallikrein were within normal limits and the mean values did not differ significantly from those of a control group. However, among the forty-two patients with acne there were nine with definitely diminished reactions. Among the controls four subjects also had small-sized reactions. Two of them had acne and seborrhea in their adolescence. In view of the relationship claimed to exist between the vascular alterations in seborrheic conditions and psoriasis (14), it is interesting to find a lowered reactivity to kallikrein not only in psoriasis, but occasionally also in acne vulgaris.

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GASTROINTESTINAL DYSFUNCTION IN DERMATITIS HERPETIFORMIS

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Abstract. Nine patients with dermatitis herpetiformis were investigated for intestinal dysfunction. In six patients small intestinal mucosa showed "disappearance" of villi. The disaccharidase activities of the mucosa were abnormal in five of eight patients studied and the dipeptidase activities in six.

Dermatitis herpetiformis is often associated with disturbed function of the small bowel (9, 10, 16, 17, 23, 25). Severe histopathological changes of the small intestine with disappearance of the villi have also been described (2, 9, 10, 16, 17, 23, 25). The stereomicroscopical and histological appearance of the jejunal mucosa in the enteropathy of dermatitis herpetiformis is similar to that of the coeliac syndrome, but the malabsorption is less pronounced (23).

In untreated coeliac disease the disaccharidase activity (1, 6) as well as the dipeptidase activity (15) of the small intestine is decreased. Remission of the disease, usually in response to gluten-free diet, results in an increase of the enzyme activities in the mucosa (15, 20). Fraser et al. (9) also found disaccharidase activity (maltase, lactase and sucrase) to be related to the histologic appearance of the small intestine in dermatitis herpetiformis. No investigations of the dipeptidases of the intestinal mucosa in dermatitis herpetiformis have been published.

In the present investigation of patients with dermatitis herpetiformis, routine gastrointestinal studies were performed as well as measurements of disaccharidase activity and dipeptidase activity in small-intestinal mucosal biopsies.

CLINICAL MATERIAL AND METHODS

Nine patients, 8 men and 1 woman, age 25 to 73 years, were examined. They had had dermatitis herpetiformis for 8 to 28 years. All of them had been treated with diaminodiphenylsulfone (Avlosulfon, ICI) for 3 to 11 years and were receiving such treatment at the time of the investigation. The sulfone dose varied between 0.05 and 0.4 g and was usually 0.1 g daily. Doses of more than 0.2 g had been taken against doctors' advice when the symptoms were very severe. All nine patients were hospitalized during the investigation. They had been admitted either because the skin symptoms were difficult to control or because anemia had developed during treatment. The patients were specifically questioned regarding previous or recent gastrointestinal symptoms and in all of them radiological examination of the gastrointestinal tract by barium contrast was performed.

Hemoglobin, red blood cells, reticulocytes, serum iron, total iron binding capacity, serum haptoglobin and sternal marrow were examined by conventional methods.

The serum B_{12} content was measured by an isotope technique (24). Values between 150 and 900 pg/ml were regarded as normal.

Folic acid in whole blood and in the serum was assessed with *Lactobacillus casei* at the Central Chemical Laboratory, Sahlgren's Hospital, Gothenburg (12). Serum values between 2.8 and 3.5 ng/ml and whole blood values between 40 and 150 ng/ml were regarded as normal.

Formiminoglutaminic acid (FIGLU) in the urine was estimated after loading with 15 g of histidine per os. Excretion of more than 20 μM/hour was regarded as abnormal.

Vitamin B_{12} absorption was studied with the Schilling test, values above 10% being regarded as normal.

D-xylose absorption was measured by analysis of the amount excreted in the urine within 5 hours after an oral dose of 5 g D-xylose. Less than 1.4 g was regarded as abnormal.

Faecal fat excretion was determined as the average per day after collection for 3 days with the patient on a normal ward diet (14). More than 5 g fat per day was considered abnormal.

Peroral mucosal biopsy of the small intestine was performed with a Crosby capsule at the duodeno-jejunal flexure under fluoroscopic control. In one patient (no. 1), operated upon according to Billroth I, the biopsy specimen was obtained 40 cm from the gastroenterostomy, and in another (no. 2), operated upon according to Bill-

Table I. *Clinical and laboratory findings and appearance of small intestinal mucosa in patients with dermatitis herpetiformis*

Pat. no.	Sex	Age (y.)	DH duration (y.)	Height (cm)	Weight (kg)	Gastro-intest. sympt.	Faecal-fat excret (g/day)	5 h urinary xylose (g)	Haemo-globin (g per 100 ml)	Serum folate (ng per ml)	Whole blood folate (ng per ml)	Serum B_{12} (pg per ml)	FIGLU in urine (μM per hour)	Small intestinal mucosal appearance
1	♂	73	18	164	58	+	2.7	1.9	9.9	1.4	163	190	—[a]	Villous
2	♂	56	8	166	63	+	1.8	1.9	11.6	—	—	330	6	Villous
3	♂	42	11	166	71	0	4.3	0.8	13.4	1.9	83	450	10	Flat
4[b]	♂	45	8	178	72	0	10.3	2.2	12.0	1.7	46	290	24	Villous Flat Flat
5	♂	59	28	170	58	+	3.3	1.9	12.8	3.3	65	155	—	Convoluted
6	♂	50	21	173	86	0	6.6	1.9	12.2	1.7	75	460	19	Flat
7	♂	65	20	173	68	0	6.5	1.1	7.6	2.3	56	200	20	Villous
8	♀	25	13	178	53	0	9.6	1.7	11.6	2.0	56	—	16	Partly flat, partly convoluted
9	♂	68	17	174	61	+	14.0	0.8	8.8	1.4	—	860	39	Flat
Normal values							< 5.0	> 1.3		2.8–3.5	40–150	150–900	< 20	

[a] —= not analysed. [b] before diet.

roth II, from the upper part of the jejunum (the efferent loop).

The biopsy specimens were divided in three pieces. One was mounted on a plastic net and immersed in 4% formaldehyde for histologic examination. The other two pieces were immediately frozen in dry ice for measurements of disaccharidase and dipeptidase activities. Disaccharidases were determined according to Dahlqvist (5) and dipeptidase activities according to Josefsson & Lindberg (13) and Lindberg et al. (15). Unless otherwise stated each of the patients was examined by all methods used.

RESULTS

History and clinical observations (Table I). Two patients had undergone gastric resection. One of them (no. 1), who had been operated 20 years previously because of duodenal and gastric ulcers, complained of flatulence and mucus in the stools. The other (no. 2) had 9 years previously been operated because of duodenal ulcer. Since then he had had no symptoms related to the digestive tract. One patient (no. 5) had 15 years earlier had a long spell of diarrhoea. A fourth (no. 9) was admitted in a poor general condition after having been troubled for several months by vomiting and loose stools. Five of the patients had no gastrointestinal symptoms.

One patient (no. 8) was underweight and one (no. 6) was overweight. The others did not deviate appreciably from ideal weight (7).

X-ray examination of the stomach and intestine showed a normal postoperative appearance in cases 1 and 2 and only one patient (no. 9) showed pathological changes of the small bowel with alternating strictures and dilatations of the intestinal lumen. Superior mesenteric arteriography was done in four of the patients (nos. 2, 6, 7 and 9). The results of these examinations will be the subject of a separate paper (4).

Hematological findings. All patients except no. 3 had a hemoglobin value of < 13.0 g/100 ml; in five the anemia was mild, and in three (nos. 1, 7 and 9) more pronounced with hemoglobin values of 9.9, 7.6 and 8.8 g/100 ml. The serum iron was between 25 and 75 μg/100 ml in these three patients and in no. 4. Three of the patients with sideropenia showed an increased TIBC while in one (no. 9) TIBC was only 200 μg/100 ml. This patient had pronounced hypoproteinemia with a serum albumin of 1.8 g/100 ml.

The sternal marrow was examined in all patients except no. 3. An increased erythroid myeloid ratio was noted in three patients (nos. 2, 6 and 8). No megaloblasts were seen in any of the cases.

All patients showed signs of hemolysis. In seven the haptoglobin was decreased, 2–24 mg/100 ml. Reticulocytes were in all patients at least on one occasion more than 1.6%.

Table II. *Histologic appearance and disaccharidase activities (units per gram protein) of small-intestinal mucosal biopsy specimens from patients with dermatitis herpetiformis*

Patient no.	Appearance	Maltase	Isomaltase	Saccharase	Trehalase	Lactase
1	Villous	293	73	67	17	42
2	Villous	143	43	51	11	14
3	Flat	0	0	0	0	0
	Villous	263	92	75	24	3.3
4[b]	Flat	18	4.6	5.0	0.8	0.8
	Flat	62	18	15	3.0	2.2
5	Convoluted	—[a]	—	—	—	—
6	Flat	25	8.6	6.4	1.4	0.6
7	Villous	170	39	41	20	40
8	Partly flat, partly convoluted	67	18	18	3.5	7.4
9	Flat	108	30	28	2.3	7.6
Controls, mean value ($n=9$)		322	69.5	63.7	23.0	23.2
(range)		(126–446)	(31.4–142)	(33.6–148)	(10.9–36.3)	(8.7–36.3)

[a] — = not studied. [b] before diet.

Serum B_{12} was normal in all of the eight patients examined. The serum folate was between 1.4 and 2.3 ng/ml in six patients. The remaining two had normal values. FIGLU was analysed in eight patients and was increased in two of them: no. 4 excreted 24 μM FIGLU/hour and no. 9 excreted 39 μM/hour.

Absorption studies. All subjects showed a normal Schilling test. D-xylose absorption was abnormal in three patients (Table I).

Faecal fat excretion was increased in five patients (Table I). It was normal in the two patients who had undergone gastric resection.

Peroral biopsy of small-intestinal mucosa (Tables I, II and III). In seven of the patients only one mucosal biopsy was performed. In patient no. 3 two biopsies were taken with an interval of two months with the patient on a normal diet and the same medicamental treatment on both occasions. In patient no. 4 four biopsies were performed, two before, one after four months and the fourth after nine months of treatment with gluten-free diet.

In Table I the mucosal changes are classified morphologically in accordance with Shuster et al. (23). Severe enteropathy corresponding to what

Table III. *Histologic appearance and dipeptidase activities (units per mg nitrogen) of small-intestinal mucosal biopsy specimens from patients with dermatitis herpetiformis*

Patient no.	Appearance	Glycyl-L-leucine	L-Alanyl-L-glutamic acid	L-Glutamyl-L-valine	L-Alanyl-L-proline	Glycyl-L-glutamine[a]
1	Villous	304	56.6	30.9	17.4	—[b]
2	Villous	233	42.7	22.2	8.2	—
3	Flat	—	—	—	—	—
	Villous	170	23.5	9.7	7.7	50.3
4[c]	Flat	41	7.5	0	1.0	16.3
	Flat	136	26.2	7.7	1.2	49.7
5	Convoluted	—	—	—	—	—
6	Flat	72	19.8	0.4	0.6	32.8
7	Villous	—	35.0	8.3	5.0	22.6
8	Partly flat, partly convoluted	132	34.5	11.3	5.2	52.8
9	Flat	82	23.4	4.8	1.6	28.7
Controls, mean value ($n=9$)		216	37.5	22.2	10.0	65.4
(range)		(182–328)	(26.3–52.5)	(16.6–35.3)	(8.0–16.0)	(37.4–110)

[a] 0.04 *M* aqueous solution was used as substrate. pH optimum 7.7 (Lindberg, unpublished observations).
[b] — = not studied. [c] before diet.

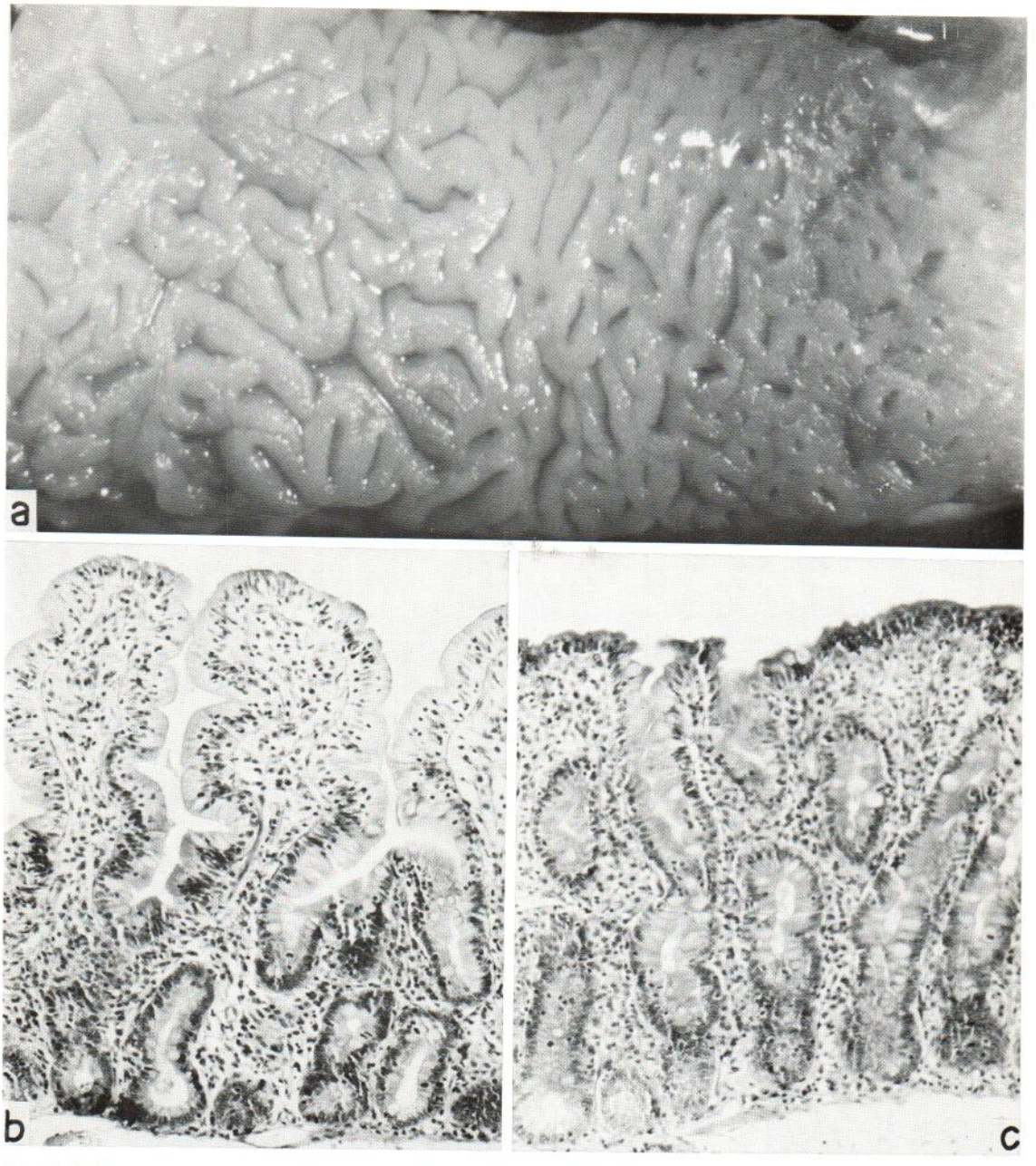

Fig. 1. Patient no. 8. The heterogenous picture of the mucosa from the duodeno-jejunal flexure is illustrated: (*a*) Appearance under the dissecting microscope with convoluted pattern to the left and flat mucosa to the right. (*b*) Low-power view of the convoluted part of the biopsy with distinct villous pattern and high columnar surface epithelium. (*c*) Low-power view of the flat mucosa. Note elongated crypts and low irregular surface epithelium.

is generally called partial or subtotal villous atrophy was seen in patients nos. 5, 6 and 9, in one of the biopsy specimens from patient no. 3 and initially in patient no. 4. Patient no. 8 showed a heterogeneous picture with severe changes in some areas and more preserved mucosa in others (Fig. 1). Two biopsy specimens (patients nos. 2 and 7) showed non-specific changes in the intestinal mucosa with atrophy of the type described by Foroozan & Trier (8). The biopsy from

patient no. 1 and the second biopsy from patient no. 3 were normal. All disaccharidases were abnormally low in the mucosal samples from patients nos. 6 and 8, the initial two biopsies from patient no. 4 and the first biopsy from patient no. 3 (Table II). In the second specimen from patient no. 3 only lactase deficiency was observed. In patient no. 9 all five enzyme activities were low but only trehalase and lactase were definitely abnormal. Disaccharidase activities were normal in patients nos. 1, 2 and 7.

Five dipeptidase activities were studied and the results are given in Table III. All five were definitely low in the first biopsy specimen from patient no. 4 and in nos. 6 and 9. In patient no. 7 the activity against L-alanyl-L-glutamic acid was normal while the activities against the other three dipeptides studied were decreased. The second biopsy specimen from case 4 and the one from patient no. 8 had both low activities against L-glutamyl-L-valine and L-alanyl-L-proline, slightly decreased activity against glycyl-L-leucine but normal activities against the remaining two dipeptides. The first biopsy specimen from patient no. 3 was not studied but the second had low activities against L-glutamyl-L-valine and slightly decreased activity against L-alanyl-L-glutamic acid. Normal dipeptidase activities were found in patients nos. 1 and 2.

In patient no. 5 the mucosal sample was too small to permit enzyme activity analysis.

When patient no. 4 had been on a gluten-free diet for four months the changes in the intestinal mucosa showed distinct regression with single plump, leaf-shaped villi but still abundant cellular infiltration. All five disaccharidases were normalized. After the patient had been on a gluten-free diet for 9 months the histological changes had regressed even more. The dipeptidase activities were, however, still significantly decreased (Table IV).

Table IV. *Histologic appearance and disaccharidase and dipeptidase activities of the small intestinal mucosa in patient no. 4 before and after treatment with gluten-free diet*

	Appearance	Maltase	Isomaltase	Saccharase	Trehalase	Lactase	Glycyl-L-leucine	L-Alanyl-L-glutamic acid	L-Glutamyl-L-valine	L-Alanyl-L-proline	Glycyl-L-glutamine[a]
Before diet	Flat	17.5	4.6	5.0	0.8	0.8	41	7.5	0	1.0	16.3
Before diet	Flat	61.5	18.2	15.2	3.0	2.2	136	26.2	7.7	1.2	49.7
After 4 months diet	Convoluted-villous	213	56.4	64.2	15.5	17.9	—[b]	—	—	—	—
After 9 months' diet	Villous	137	44.4	41.2	9.6	10.0	77	12.8	0	1.2	21.1
Controls, mean value	($n=9$) (range)	232 (126–446)	69.5 (31.4–142)	63.7 (33.6–148)	23.0 (10.9–36.3)	23.2 (8.7–36.3)	216 (182–328)	37.5 (26.3–52.5)	22.2 (16.6–35.3)	10.0 (8.0–16.0)	65.4 (37.4–110)

[a] 0.04 *M* aqueous solution was used as substrate. pH optimum 7.7 (Lindberg, unpublished observations).
[b] — = not studied.

DISCUSSION

Several authors have shown that intestinal dysfunction of the type seen in coeliac disease is common in dermatitis herpetiformis (9, 10, 16, 17, 23, 25). In a compilation of personal cases and other materials Shuster et al. found subtotal or partial "villous atrophy" in 50 of 83 patients with dermatitis herpetiformis (23). Such "disappear-

ance" of the villi was observed in six of our nine patients (Table I).

Subnormal disaccharidase activities were found in 7 out of 12 cases of dermatitis herpetiformis studied by Fraser et al. (9). In our group of eight patients studied, five showed subnormal disaccharidase values (Table II).

Hitherto no studies of the intestinal dipeptidases in dermatitis herpetiformis have been performed. In three of our patients all five dipeptidase activities studied were low. In three patients low activities were found against at least two of the dipeptides (Table III).

Thus, in our group of eight patients studied, only two were completely normal. These two had both undergone gastric resection.

Of the six biopsies with low dipeptidase activities four showed "disappearance" of the villi. Five of them had abnormal disaccharidase activities. Thus determination of the didpeptidase activity seems to be a sensitive test for small intestinal mucosal dysfunction in dermatitis herpetiformis as in gluten-induced enteropathy (15).

It is known that intestinal biopsy specimens obtained simultaneously from one and the same individual can show different pictures (21). In one of our patients some areas of the specimen showed severe enteropathy, while others had a more normal appearance (Fig. 1). In another patient biopsies with an interval of two months showed completely different histological pictures in spite of the fact that the diet or the therapeutic measures had not been altered. The findings in these two cases indicate that the bowel lesions may be patchy.

In all patients with steatorrhea the condition was only mild, an observation noted also in previous series of patients with dermatitis herpetiformis. It is therefore remarkable that when enzyme changes occurred in the intestinal mucosa they were as severe as in coeliac disease with its more pronounced symptoms of malabsorption. It is possible that the reason for milder symptoms in the enteropathy of dermatitis herpetiformis is that the intestinal changes are circumscribed. In coeliac disease the severity of the illness is related to the length of bowel injured (22).

In all of our nine patients there was some sign of gastrointestinal abnormality. In this conjunction it should, however, be pointed out that our cases were selected: only patients with dermatitis herpetiformis difficult to control or with anemia were examined.

In two patients who had undergone gastric resection dermatitis herpetiformis appeared 1 and 2 years after the operation. In one of the patients no intestinal changes at all were seen, while the other showed slight histological alterations. The enzyme activities in these two patients were normal. However, it should be pointed out that the biopsies were taken from the upper jejunum, where the enzyme activities normally are somewhat higher in comparison with those in the mucosa of the duodeno-jejunal flexure (3, 18).

Investigations of relatives of patients with dermatitis herpetiformis have revealed signs of enteropathy with or without symptoms (17, 23). A brother of our patient no. 5 had coeliac disease with typical intestinal biopsies but no dermatitis.

Treatment with gluten-free diet can improve the intestinal symptoms of coeliac disease both in patients with and without associated dermatitis herpetiformis (11, 19, 23). Fry et al. (11) registered improvement of the skin symptoms as well in patients with dermatitis herpetiformis and enteropathy, while Shuster et al. (23) found no evidence for gluten-free diet having an effect on the dermatosis.

Patient no. 4 was studied after he had strictly avoided gluten for 9 months. Already after 4 months a certain histological normalization was noted and the disaccharidases had become normal. Yet after 9 months of treatment with gluten-free diet the dipeptidases were still subnormal (Table IV). While on diet the patient had put on 5 kg and he felt much better. He had never before succeeded in gaining weight. His skin symptoms had, however, not been affected by the diet and still required sulfone treatment as before.

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