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VIRUS PARTICLES IN LUPUS ERYTHEMATOSUS

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Abstract. Uninvolved and involved skin from 10 patients with systemic lupus erythematosus was studied by electron microscopy. In all patients, filiform inclusions were found in the granular endoplasmic reticulum of vascular endothelial cells in the corium. Similar inclusions were also located in the reticulum of fibroblasts and monocytic cells of the corium and in the ground cytoplasm of epithelial cells of the epidermis. Extracellular filiform inclusions enclosed by a membrane were found in the interfibrillar space and in vascular lumina. These extracellular particles probably originate from disintegrated cells. Absence of ribosomal grains and a broken limiting membrane of the cisternae containing filiform inclusions suggest that nucleocapsid synthesis occurs immediately under the limiting membrane. On comparison with previous experimental results of studies of known paramyxovirus, the presence of *budding figures* of vascular endothelial cells, basal epidermal cells and dermal fibroblasts of involved lupus erythematosus skin, and virus-like particles in the dermo-epidermal junction and the upper dermis as well as in vascular intima favour a virus etiology of lupus erythematosus. Intranuclear bodies and double-membrane bounded bodies in lysosomes and endocytic vesicles may be reactive products of the cells.

Recently, several investigators have described inclusion bodies in granular endoplasmic reticulum of endothelial cells of glomerular capillaries in lupus nephritis (12, 13, 14, 15, 21, 22, 29). Their shapes closely resembled ribonucleoprotein strands (nucleocapsids) of paramyxovirus, and, logically, suspicion of a viral etiology of lupus erythematosus has arisen. Later on, inclusions have been noted in skin cells of both discoid and systemic lupus erythematosus, located in vascular endothelial cells, fibroblasts and endothelial cells (17, 18, 29). Similar inclusions have been found in lymphoid cells of lymph nodes (16). To obtain further information on the viral etiology of lupus erythematosus, detailed ultrastructural studies of skin were carried out and the results compared with previous electron microscopical studies of known paramyxovirus in experimental infection (7, 8, 19, 20, 24, 25, 26, 30, 31, 32).

MATERIAL AND METHODS

Biopsies of systemic lupus erythematosus were removed from involved skin of ten and uninvolved skin of two patients. The specimens were fixed in 6% glutaraldehyde in Veronal acetate buffer, pH 7.2, with 7.5% sucrose. After osmification, the specimens were washed, dehydrated in a series of alcohols of increasing concentration, and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate and examined by a Siemens electron microscope (Elmiskop IA) at 80 kV with a double condensor system.

OBSERVATIONS

Filiform inclusions were mostly seen as balls of threads in various cells of mesenchymal as well as ectodermal origin. *Endothelial cells* of dermal vessels in both involved and uninvolved skin of all patients examined, contained such inclusions in the granular endoplasmic reticulum and in the nuclear envelopes (Figs. 1, 2). Granular endoplasmic reticulum containing filiform inclusions was often dilated and in parts of its walls there were no ribosomal grains and an indistinct, broken, limiting membrane. A lucent zone separated the balls from the ground cytoplasm (Fig. 2). *Fibroblasts* of involved skin also contained inclusions in the granular endoplasmic reticulum. *Monocytic cells* of the corium, and the basal and Malpighian cells of the *epidermis,* held inclusions free in their ground cytoplasm. Occasionally, balls were found in the space *between collagen fibrils* (Fig. 4) and in *vascular lumina* of involved skin (Fig. 3). The extracellular balls were about 80 ×

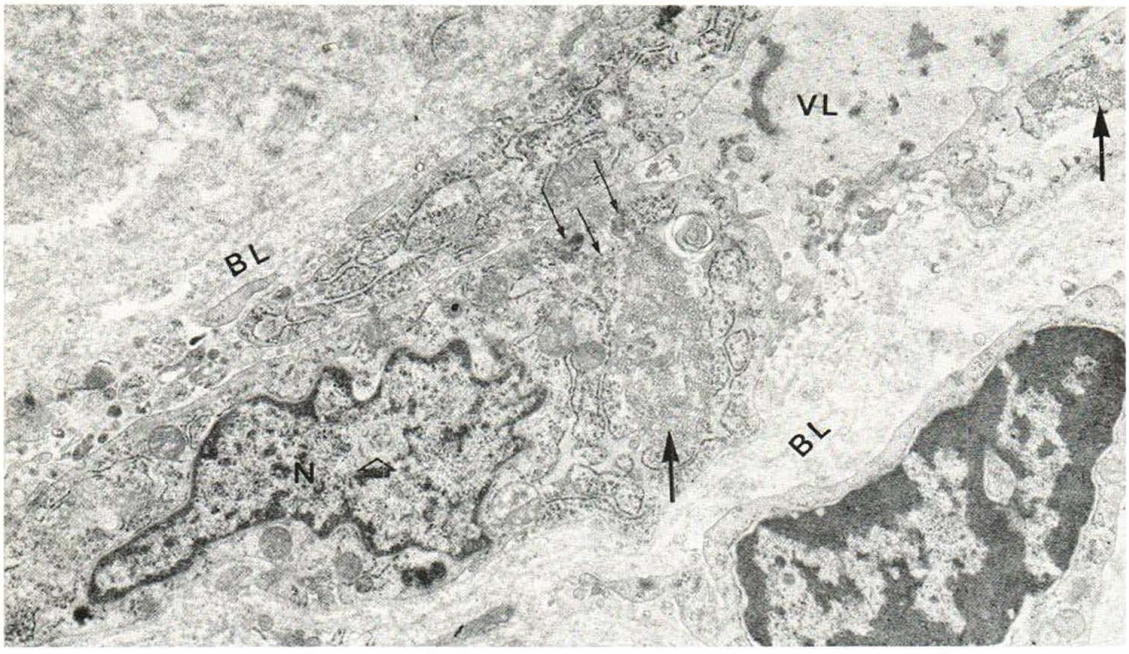

Fig. 1. Vascular endothelial cells of involved skin, containing filiform inclusions in granular endoplasmic reticulum (*thick arrow*), double-membrane bounded bodies (*fine arrows*) and an intranuclear inclusion (*framed arrow*). *VL*, Vascular lumen; *BL*, basal lamina; *N*, nucleus of an endothelial cell. × 13 500.

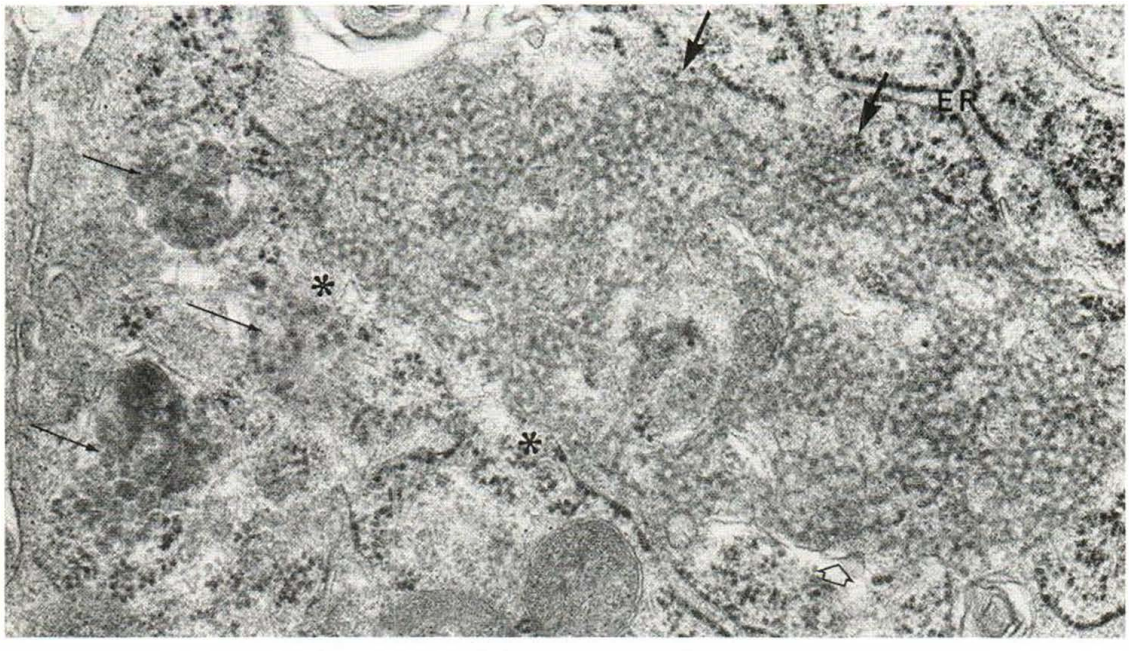

Fig. 2. Part of the endothelial cell shown in Fig. 1. The filiform inclusion is seen as a ball of threads in the granular endoplasmic reticulum. Framed arrow indicates the limiting membrane of the reticulum presenting a few ribosomal grains. Thick arrows indicate a blurred limiting membrane and indistinct ribosomal grains. In the area between asterisks, a space separates the inclusion from the ribosomal grains delineated by a broken limiting membrane. Three arrows show grouped double-membrane bounded bodies. *ER*, Granular endoplasmic reticulum. × 45 000.

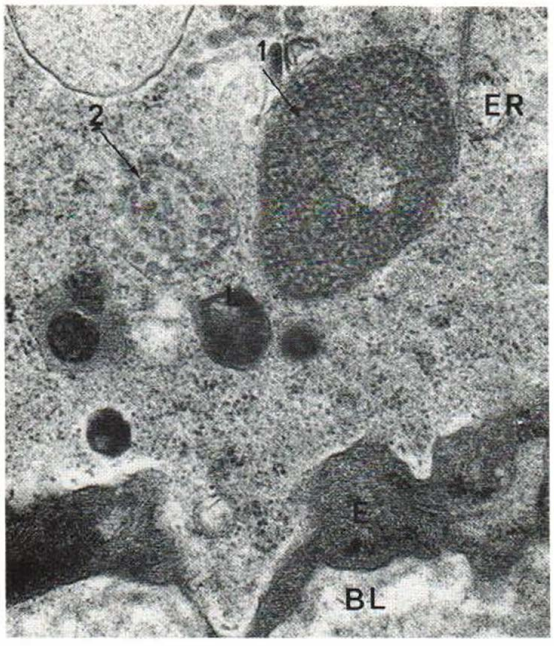

Fig. 3. Vascular lumen in involved dermis with a ball of filiform inclusions enclosed by a single membrane (*arrow 1*). Double-membrane bounded bodies within an enclosing membrane (*arrow 2*). *ER*, Granular endoplasmic reticulum free in the lumen; *L*, lysosome free in the lumen; *E*, an endothelial cell; *BL*, basal lamina. × 30 500.

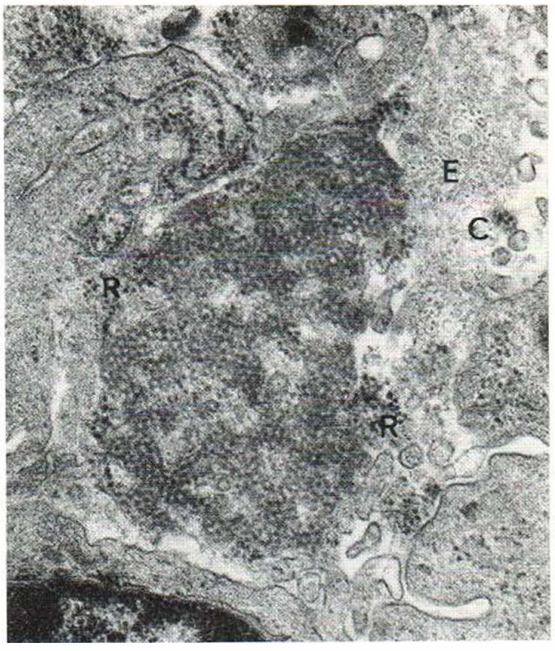

Fig. 4. A ball of filiform inclusions in interfibrillar space. Free ribosomal grains (*R*) are seen around the ball. *C*: Collagen fibrils. *E*, Elastic fibrils. × 30 500.

100 nm and enclosed by a single membrane. Ribosomal grains and lysosome-like particles were seen in the vicinity of the balls. The individual inclusion was about 20 nm thick and more than 100 nm long (Fig. 5). The surface of the thread showed cross-striations, with about 2 nm wide dense bands occurring repeatedly at about 2 nm wide intervals. The cut-surface was round with several dense spots and fine strands, but real tubular figures as described in previous papers were not evident. The threads were connected to each other by fine filaments.

Double-membrane bounded bodies in the cytoplasm. The endothelial and epidermal cells containing filiform inclusions often contained double-membrane bounded bodies as well, mainly in endocytic vesicles, lysosomes and granular endoplasmic reticulum. In endocytic vesicles and lysosomes (Figs. 7, 8) the bodies were about 70 nm in diameter. They had a lucent central core and were enclosed by a double membrane the lamellae of which held a regular distance of about 8 nm. These bodies also occurred in masses, either in

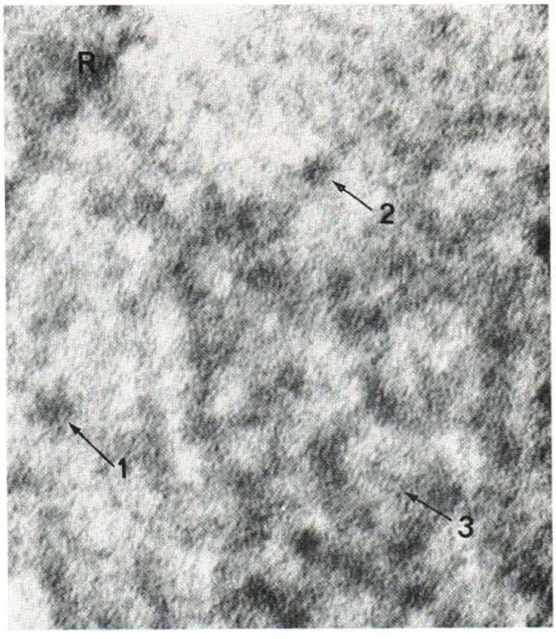

Fig. 5. Section of Fig. 2 (right-hand thick arrow). Arrows 1 and 2 show round cut surfaces and arrow 3 shows cross striations. Threads are connected by very fine filaments. *R*, Ribosomal grain. × 183 000.

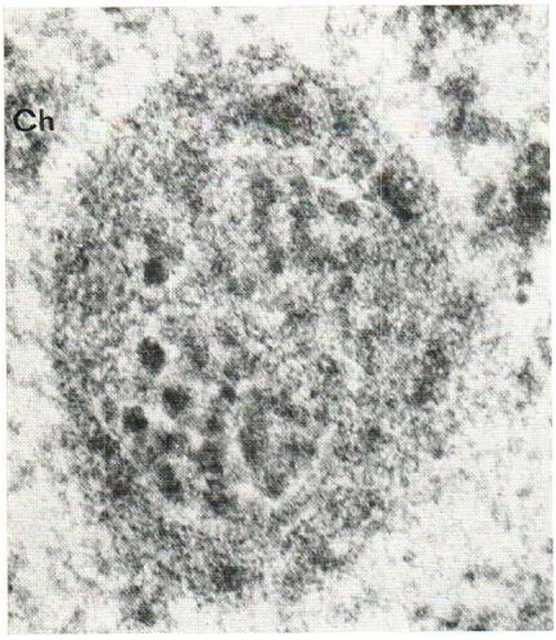

Fig. 6. Inclusion in a chromatin-free area of a nucleus of an endothelial cell. The inclusion shows irregular spots and strands in the centre surrounded by a filamentous zone. *Ch:* Chromatin grains. × 91 500.

single-membrane bounded cytoplasmic areas (Fig. 10) or in cisternae of granular endoplasmic reticulum in which filiform inclusions were contained (Fig. 9). The bodies in these organelles varied in size from 20 to 70 nm. They had a

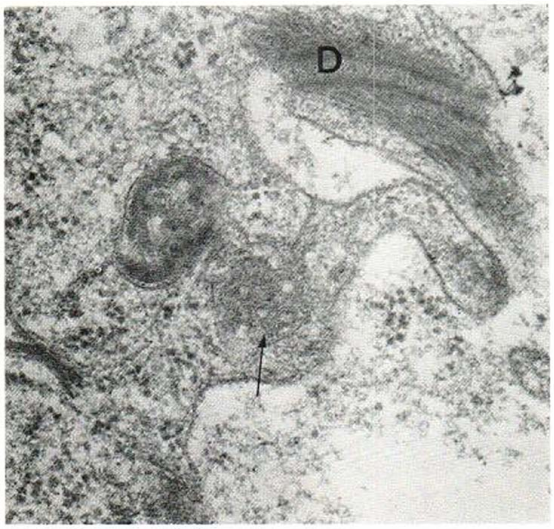

Fig. 7. An epidermal cell shows multivesicular body of lysosome (*arrow*). *D,* Desmosome. × 45 700.

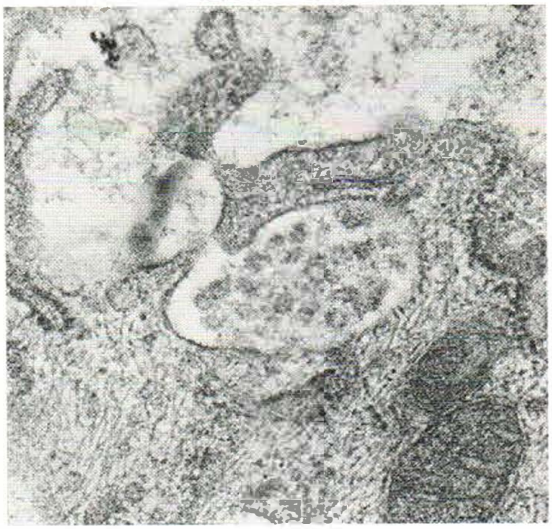

Fig. 8. An endocytic vesicle of a vascular endothelial cell in involved dermis. The vesicle contains double-membrane bounded bodies with lucent centres. × 47 500.

lucent or dense core (Fig. 10), and coexisted occasionally with horseshoe-shaped and straight double-membrane particles (Fig. 9).

Intranuclear bodies. Cell nuclei in involved skin, both epidermal and dermal, contained one or several oval light areas in the dense chromatin pattern (Fig. 1). In these areas, some dense spots and strands, 20 to 40 nm in width, were seen surrounded by a filamentous zone (Fig. 6), the width of which ranged between 20 and 80 nm.

Budding figures. The surfaces of vascular endothelial cells and large fibroblasts as well as basal epidermal cells of involved skin (Figs. 11 *a,* 11 *b,* 13), which cells contained filiform inclusions, occasionally showed spherical swellings and round particles with a long neck. The buds contained indistinct filiform inclusions and were limited by a distinct double membrane. Their diameters ranged from 130 to 200 nm. Free, round particles were also seen in the neighbouring areas (Figs. 11 *a,* 11 *b*).

Membrane-enclosed particles in dermo–epidermal junction and vascular walls. In the subepidermal space (Fig. 13) and the upper corium of involved skin, especially where the basal lamina extended to a great depth of the corium (Figs. 12, 14), numerous round and oval particles were observed. The particles were enclosed by an outer, about 17 nm thick, band and an inner limiting

membrane (Figs. 13, 14, 16). Furthermore, they were encircled partially or completely by basal lamina material. Anchoring fibrils emerged from the basal lamina into the corium (Figs. 13, 14) lending to the structure a character similar to a half-desmosome with matching basal lamina. Some particles outside the basal lamina were enclosed by a double membrane. The contents of the particles were of two different types (Fig. 14). Some contained approx. 20 nm thick filiform inclusions (Figs. 14, 16). The individual thread had cross striations and connections to neighbouring loops, as described above. The diameters of this type of particle measured from 100 to 750 nm. The other particles showed a dense peripheral zone and a lucent centre (Fig. 14). The diameters of this type were 100 to 300 nm. Figures intermediate to both were also seen (Fig. 14). In uninvolved skin, masses sized about 110 × 720 nm and composed of about 8 nm thick, parallel arranged filaments, were observed (Fig. 17). They were rather few, but always located in the subepidermal space of normal dermo–epidermal junction. The outer surface of the mass was covered by an indistinct membrane and separated from a basal epidermal cell by a space, while the dermal surface of the particle was encircled by an inner limiting membrane and an outer, about 17 nm thick, band facing the basal lamina. The arrangement thus simulated normal dermo–epidermal junction, although no distinct anchoring filaments were seen.

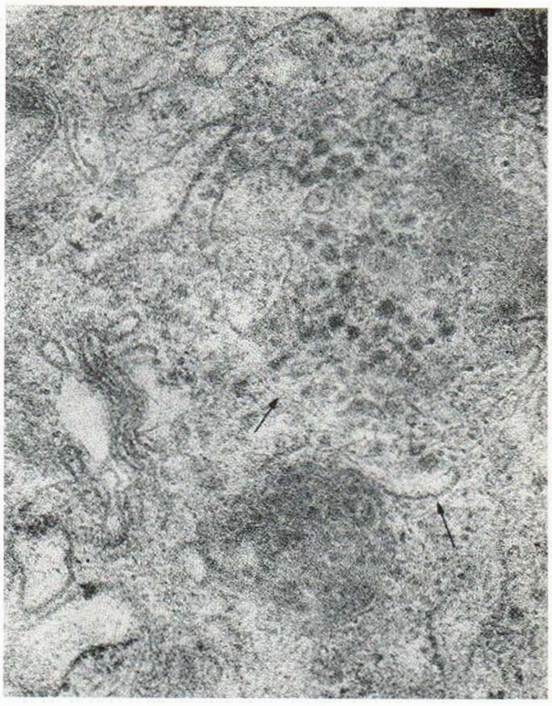

Fig. 10. A large and a small group of double-membrane bounded bodies with dense central cores in single-membrane limited areas. Arrows indicate membrane. × 47 500.

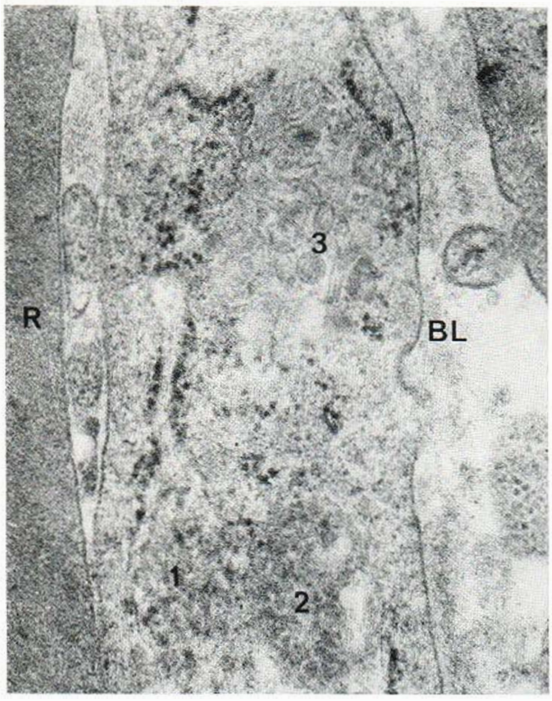

Fig. 9. Endothelial cell. Dilated granular endoplasmic reticulum contains filiform inclusions (marked by *1*) and double-membrane bounded bodies (marked by *2* and *3*). Straight and horseshoe-shaped double membranes are seen in the area marked by 3. *BL*, Basal lamina; *R*, red blood cell in the vascular lumen. × 47 500.

In the vascular walls, round particles were located in the space between the basal laminae of endothelial and muscular cells (Fig. 15). They were similar to the first type found in the dermo–epidermal junction of involved skin. However, their size was only about 100 to 300 nm in diameter. The particles were not encircled by basal lamina, but by an enclosing membrane composed of an inner limiting membrane and a thick outer band.

DISCUSSION

The constant occurrence of filiform inclusions in various tissue cells of lupus erythematosus sug-

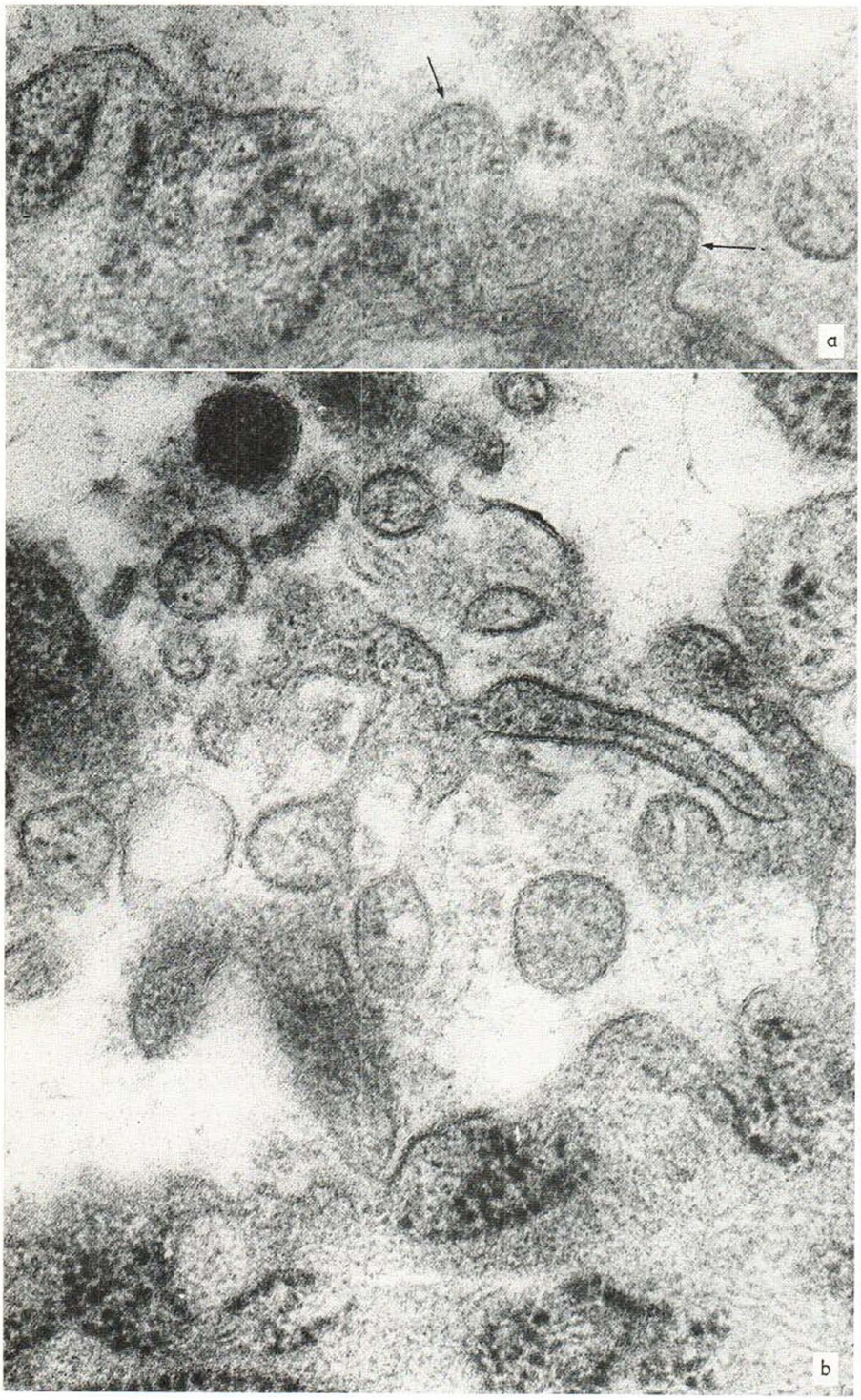

Fig. 11 a and *b*. The surface of a large fibroblast containing filiform inclusions. (*a*) Two buds (*arrows*) show indistinct filiform inclusions and are covered by a distinct double membrane. Three particles are seen free in the extracellular space. (*b*) Particles with a long neck, free particles and spherical swellings on the cell surface are seen. They are covered by a distinct double membrane and contain filiform inclusions. × 89 000.

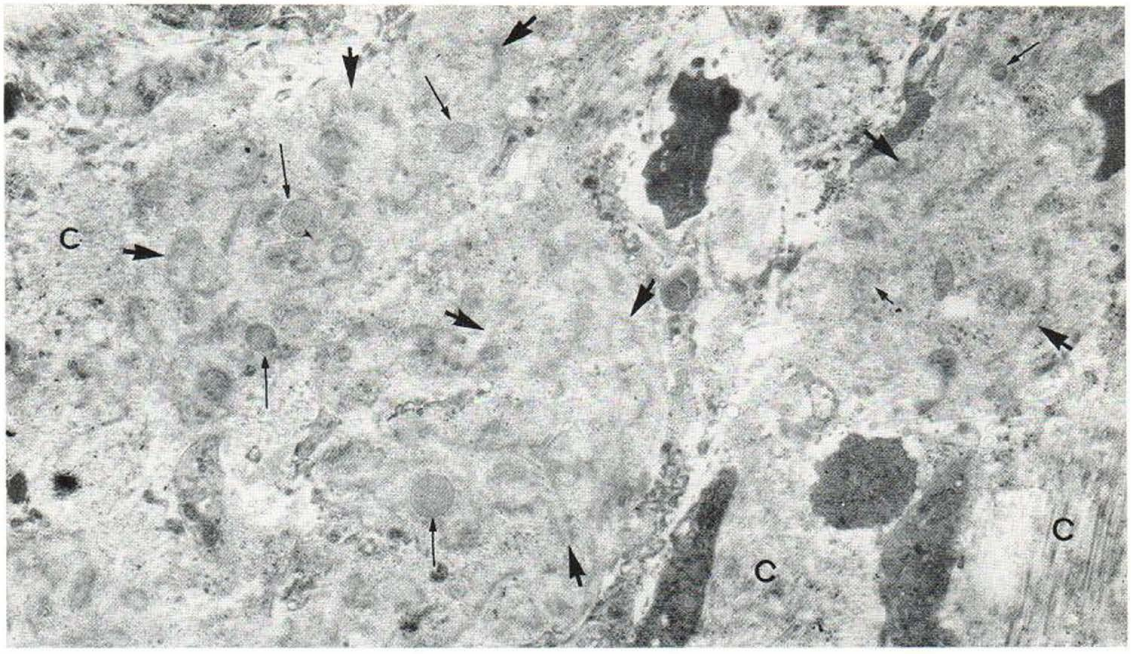

Fig. 12. Basal lamina (arrow-heads) shows a complicated pattern in the upper dermis of involved skin. Arrows indicate membrane-enclosed particles. *C*, Thick collagen bundle. × 8 900.

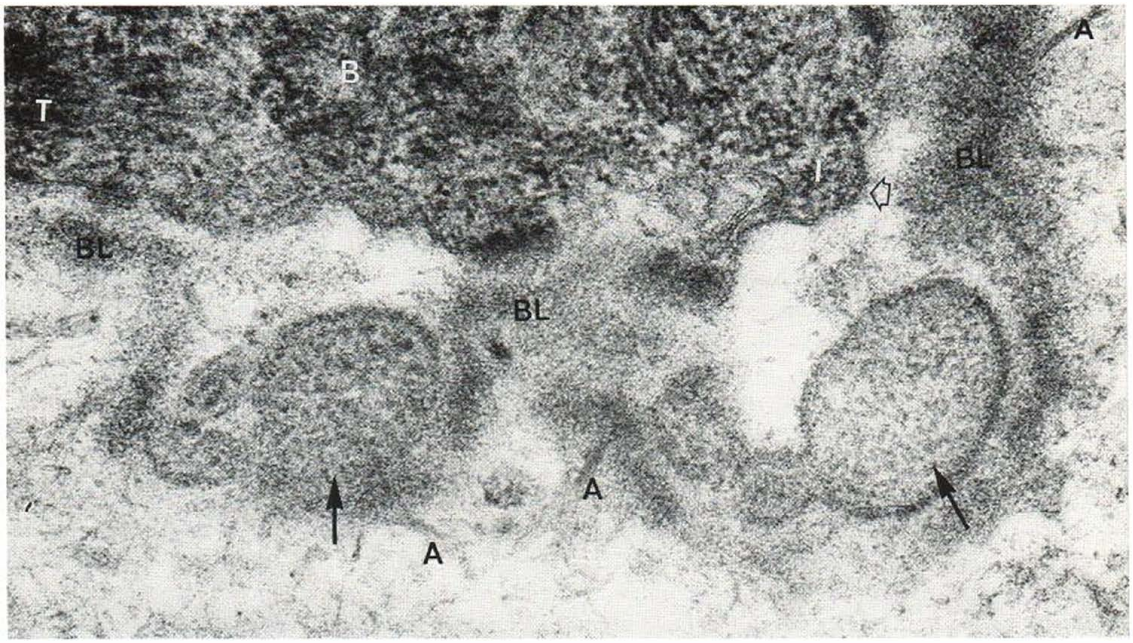

Fig. 13. Two membrane-enclosed particles containing filiform inclusions in the subepidermal space of involved skin (*arrows*). The particles are separated from a basal cell by a space. A framed arrow points to a bud of the basal epidermal cell. *T*, Tonofilaments; *I*, filiform inclusions; *B*, basal epidermal cell; *BL*, basal lamina; *A*, anchoring fibrils. × 89 000.

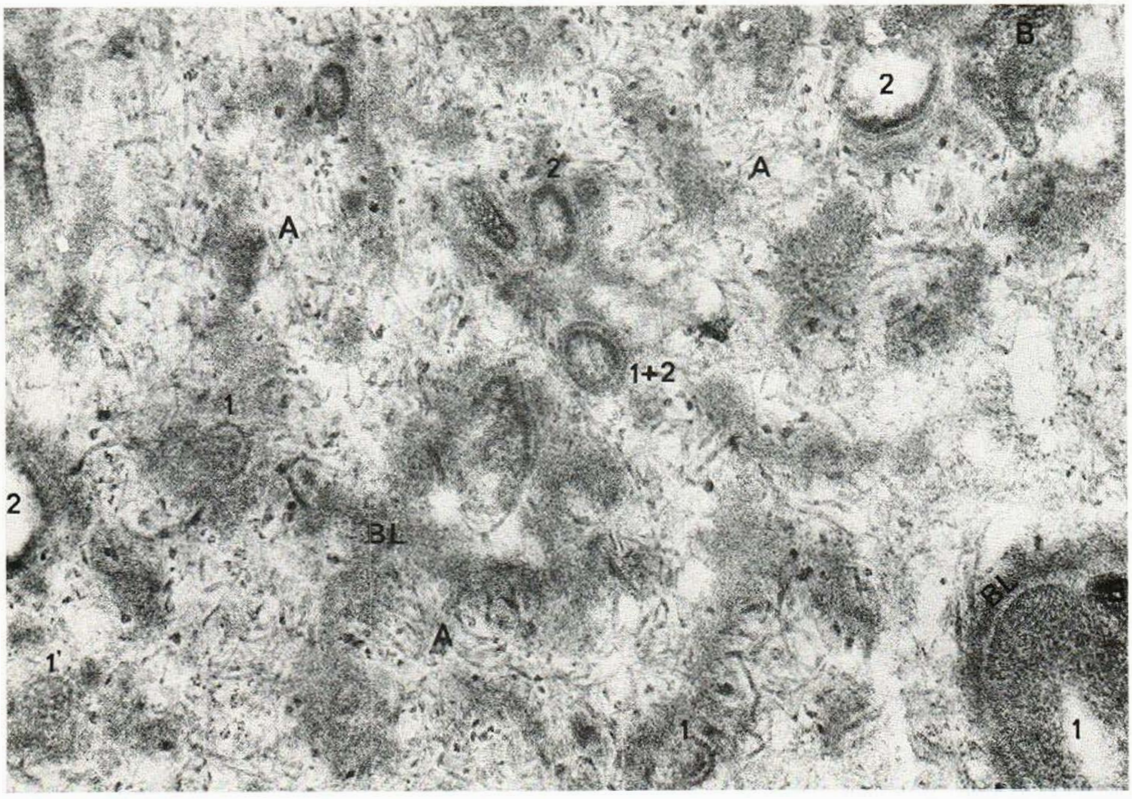

Fig. 14. Upper dermis with irregular basal lamina in involved skin. The particles marked by *1* are of the type shown in Fig. 13. In the lower right-hand corner, a large particle is seen in the subepidermal space. In the lower left-hand corner, a particle (marked *1'*) is enclosed by a distinct double membrane and located outside the basal lamina. The particles marked *2* show a lucent centre and a dense peripheral zone. In the central lucent area, threads may be seen (marked *1+2*). *BL*, Basal lamina; *B*, basal epidermal cell; *A*, anchoring fibrils. × 45 000.

gests that they have a close relationship to the etiology of the disease. They are never found in vascular endothelial cells of the dermis, nor in other tissues of normal individuals. Identical inclusions have been found, however, in some diseases, i.e. skin in dermatomyositis (17, 18), skin and kidney in scleroderma (14, 17, 29), kidney in idiopathic thrombocytopenic purpura (14), kidney in Goodpasture's syndrome (28), skin in the congenital rubella syndrome (17), synovial membrane in rheumatoid arthritis (14) and kidney in lipoid nephritis (9). The filiform inclusion resembles closely the nucleocapsid of paramyxovirus. The virions of parainfluenza, measles and mumps, belonging to the paramyxovirus group, have been isolated and experimental infections studied with the electron microscope (7, 8, 19, 20, 24, 25, 26, 30, 31, 32). These virions are pleomorphic and range in size from 50 to 80 nm in diameter. The individual virion is enveloped by a double membrane and contains ribonucleoprotein strands (nucleocapsid). The nucleocapsid is about 17 to 20 nm in width and about 170 nm in length with a tendency to helical coiling and showing faint, repeating cross striations with intervals of 5 nm. The cut-surface of the nucleocapsid often shows a marginal distinction reminding one of a ring. Some authors have described a central core of 5 to 7 nm in diameter after negative staining. The nucleocapsid appears as a mass, free in the cytoplasm, or surrounded by a granular endoplasmic reticulum. Although the profile of the individual filiform inclusion in lupus erythematosus was identical with the nucleocapsid of paramyxovirus, in contrast to the latter, the inclusion of lupus erythematosus was always located in the reticulum. Hashimoto et al. (17), studying lupus erythematosus, described budding figures of single

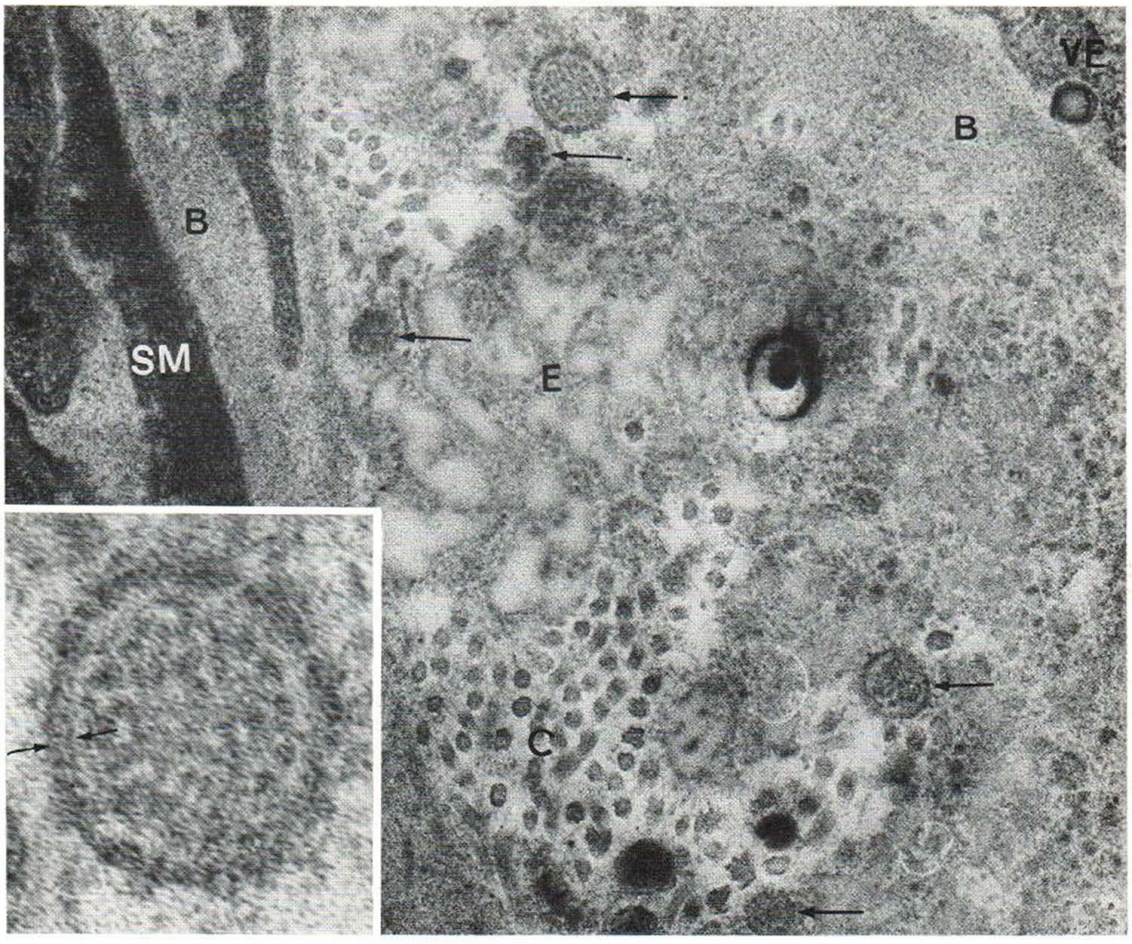

Fig. 15. Membrane-enclosed particles in vascular wall (*arrows*). The particles are similar to that in Fig. 16. *VE:* Vascular endothelial cell. *SM,* Smooth muscle cell; *B,* basal lamina; *E,* elastic fibre. *C,* collagen fibrils. × 56 300. *Inset* shows a membrane-enclosed particle in the wall. The particle contains filiform inclusions and is enclosed by an inner thin and an outer thick membrane (*arrows*). × 225 200.

inclusions on the limiting membrane of the granular endoplasmic reticulum. The present findings of reticulum containing filiform inclusions, the lack of ribosomal grains, indistinct and broken limiting membrane, and dilated cisternae, suggest that the filiform inclusions were not a cellular product secreted in the reticulum, but that synthesis of the inclusion takes place in the cytoplasm close to the reticulum. The inclusions seem to be extruded into the cisternae. Different widths of the cross striations may be caused by different procedures of sectioning and negative staining.

In previous experimental infection studies, virions were observed to bud from the cell surface of host cells (7, 8, 19, 24, 25, 26, 30, 31, 32). Nucleocapsids appeared immediately under the cell membrane, which formed the envelope of the virion. However, the nucleocapsid was not always distinct in the virion or in the bud. The present observations were very similar to these findings. Budding of this type has not been mentioned in previous reports on lupus erythematosus.

Multivesicular bodies are considered to develop by fusion of primary lysosomes with endocytic vesicles containing buds of cell membrane. Biberfeld (1) demonstrated ferritin-labelled unspecific antibody on the buds. Cells of virus-induced lymphoma (10) and dermatomyositis (18) have shown similar figures. The profiles of lysosomes and endocytic vesicles of the present study were

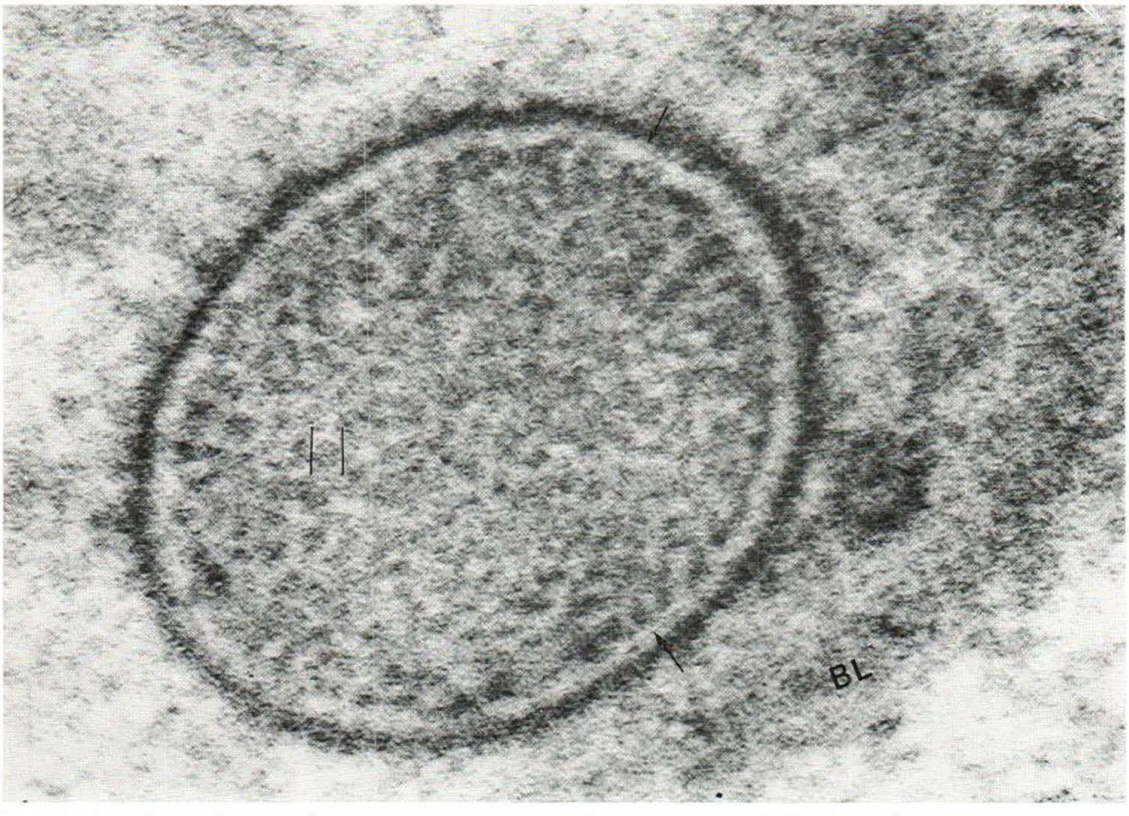

Fig. 16. Membrane-enclosed particle in the dermo-epidermal junction. This is possibly a virion. The particle is enclosed by an outer thick (about 17 nm) membrane. An inner thin membrane appears partially (*thin arrows*). The filiform inclusions inside the particle are 20 nm wide and show cross striations. Double line indicates a cut surface of the thread. *BL*, Basal lamina. × 188 400.

identical with those in Biberfeld's study. The buds seem to become free, as bodies have been found in vascular lumina. The significance of the buds in the lysosomes and the endocytic vesicles is not sufficiently clear. The buds may appear as a reactive product of the cell, inasmuch as a structure identical with that of the present study has been induced in cells by phytohemagglutinin (1). However, the double-membrane bounded bodies in the granular endoplasmic reticulum seen in this study differed from those in the lysosomes and endocytic vesicles in their profiles and their very existence in the reticulum.

Although the bodies presented in Fig. 9 resemble cytomegalovirus particles (30), the lack of these bodies in nuclei does not agree with an assumption of identity. However, the coexistence of the bodies with filiform inclusions in the granular endoplasmic reticulum opens a possibility of a relationship between them.

Intranuclear inclusions have been found in host cells of experimental measles infection (25, 26, 31, 32), while no description is found in the literature of inclusions in host-cell nuclei of experimental infection with parainfluenza and mumps. The intranuclear inclusions in measles are identical with those seen in the cytoplasm and located in chromatin-free areas of nuclei (26, 32), occasionally encircled by a 2–3 nm wide filamentous zone (25, 31). The nuclear inclusions rarely appear together with intracytoplasmic inclusions (25). The intranuclear bodies found in lupus erythematosus in a previous study (36) and in the present one cannot, however, be considered to be an intranuclear appearance of the filiform inclusions seen in measles. The reasons are: the various widths of the central spots and strands in the nuclei (Fig. 6), the constant existence of the enclosing filamentous zone of various widths, and the random occurrence of intranuclear bodies and cytoplasmic

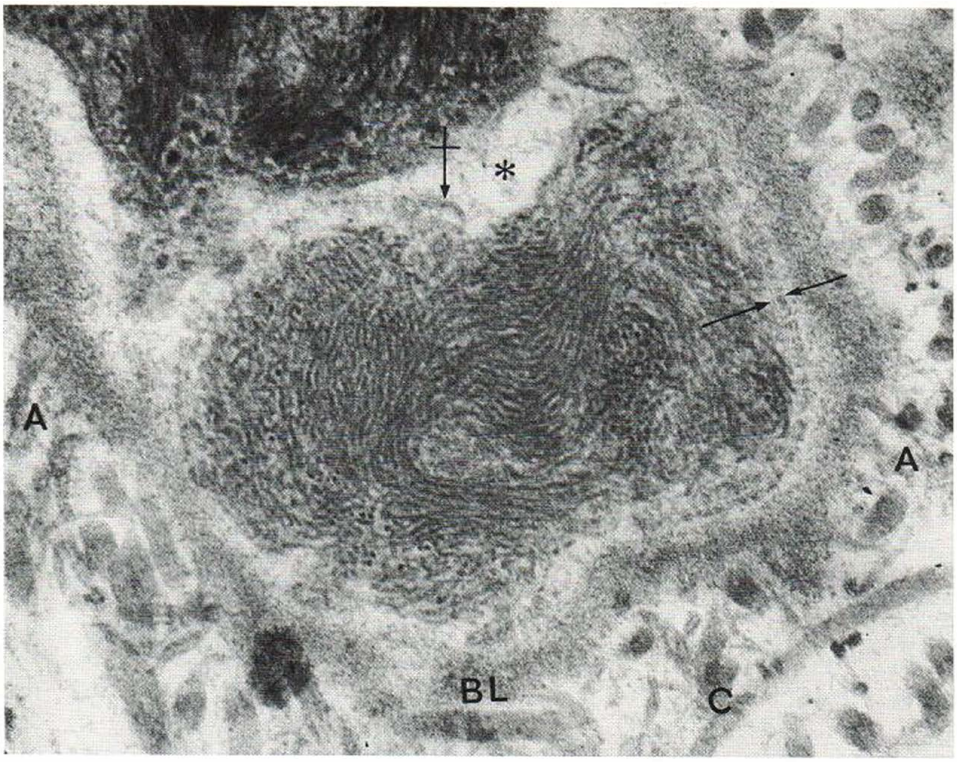

Fig. 17. A mass of filaments in the subepidermal space of uninvolved skin. The individual filament is 8 nm wide. The mass is enclosed by an outer thick and an inner thin membrane (*arrows*) on the dermal side and by an indistinct membrane on the outer side (*arrow with cross*). The particle is separated from the basal cell by a space (*) and faces the basal lamina direct. The arrangement of the mass and the basal lamina simulates normal dermo–epidermal junction. *A*, Anchoring fibril; *C*, collagen fibril; *BL*, basal lamina. × 70 500.

filiform inclusions. On the other hand, intranuclear inclusions similar to those seen in this study have been demonstrated in nuclei of tissue cells in bullous pemphigoid (3), ocular pemphigus (6), herpes zoster (28), psoriasis (4), lichen planus (35), multiple self-healing epithelioma (5), keratoacanthoma (27, 38), basal and squamous cell carcinoma (23), malignant melanoma (28), leishmania infected skin (11), and, occasionally, normal epidermal cells (33, 34). The spots and strands in the centre were not identical in all descriptions despite an identical filamentous peripheral zone. The nuclear bodies in the abovementioned papers have been interpreted as ribosomal material under transport from the nucleolus to the cytoplasm (2, 23). Also, the intranuclear bodies of this study are not considered to be filiform inclusions; they may be a metabolic product of the nucleus.

Extracellular balls of filiform inclusions have not been described previously. They are probably extruded after cell disintegration, since single membrane, ribosomal grains and lysosomes were seen in the vicinity. The single membrane possibly originates from the granular endoplasmic reticulum.

The particles containing filiform inclusions in the dermo–epidermal junction and in the vascular wall may well be virions. The particles having a dense peripheral zone and a lucent centre vary in their appearance. Occasionally, filiform inclusions occur in the lucent centres. The particles in uninvolved skin (Fig. 17) are of unknown nature. They are presumably virions, but their filaments are thinner than the ordinary filiform inclusions of lupus erythematosus. The paramyxovirus particles are enveloped by a double membrane which originates from the cell membrane and produces a desmosome-simulating figure at the point of contact between two virions (8). Thus, half-desmo-

some-like profiles may occur when virions face basal lamina. The thick outer membrane of the particle may arise through interaction of the outer lamella of the enveloping membrane with the basal lamina.

A paramyxovirus etiology of lupus erythematosus is supported by the present study. However, the final proof of a virus etiology requires the isolation of the virus. Immuno-electron microscopical techniques using ferritin-labelled antibody have demonstrated antibody on the vascular and the epithelial basal lamina (37). However, no ferritin grains have hitherto been demonstrated on the above-described virus-like structures.

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