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Morphogenesis of Intracytoplasmic Dense (Inclusion) Bodies in a Recurring Digital Fibrous Tumor of Childhood

Light- and Electron-Microscopic Investigations

D. Stiller and D. Katenkamp

Department of Pathology (Head: Prof. Dr. F. Bolck), Faculty of Medicine, Friedrich-Schiller University, Jena, GDR

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Summary. This study presents the results of light-microscopic, histochemical, and electron-microscopic investigations of dense (inclusion) bodies in a recurring digital fibrous tumor of childhood. At ultrastructural level it was possible to observe several stages of development correlated with changes of ergastoplasmic reticulum. The viral nature of these bodies was refuted and the conclusion was drawn that their formation is associated with a disturbed intracellular metabolism. The defective intracellular transport of collagen precursors followed by abnormal deposition of collagenous proteins, which are also to some extent abnormally aggregated, could be of special importance.

Zusammenfassung. In der vorliegenden Arbeit werden lichtmikroskopische, histochemische und elektronenmikroskopische Untersuchungsergebnisse an sogenannten Einschlußkörperchen bei der von Reye beschriebenen Säuglingsfibromatose der Finger mitgeteilt. Es wurden elektronenmikroskopisch verschiedene Entwicklungsstadien dieser Gebilde in enger Beziehung zu Veränderungen des ergastoplasmatischen Retikulums beobachtet. Die Befunde legen die Folgerung nahe, daß es sich bei diesen Strukturen nicht um Viren oder ihre Produkte, sondern um die abnorme Ablagerung von Proteinen im Rahmen eines gestörten intrazellulären Transportes von Kollagenvorstufen handelt.

Recurring digital fibrous tumors of childhood described first by Reye in 1965 are not frequent in the literature (Allen, 1972). Although the histological picture of a typical fibromatosis was revealed by routine histological methods, this lesion can be easily diagnosed as it is consistently limited to the fingers and toes of infants and contains intracytoplasmic dense (inclusion) bodies often overlooked in HE sections but demonstrable by special stains (Battifora and Hines, 1971).

The nature of these dense bodies is still a matter of controversy. Two opinions predominate in the literature: some authors (Ahlqvist *et al.*, 1967; Pohjanpelto *et al.*, 1967; Burry *et al.*, 1970) support the possibility that the dense (inclusion) bodies are of a viral nature, whereas others (Mehregan *et al.*, 1972) assume that they are abnormal products of a disturbed metabolism of fibroblasts.

We have had the opportunity to examine a case of such a disease by light and electron-microscopic methods. Evaluating numerous ultrathin sections we were able to observe various stages of development of the typical dense (inclusion) bodies indicating the possible nature of them after adding histochemical results.

Material and Methods

A 9-month-old baby girl is reported with a tumor from birth on the third toe of the left foot. The tumor, about the size of a cherry, was operatively removed. The lesion was firm, grayish-white on the cut surface, and was attached to the underlying tissue.

The tissue specimen was fixed in buffered neutral formalin and embedded in paraffin. The following staining reactions were performed: H& E, iron hematoxylin after Heidenhain, cresyl violet, elastica-v. Gieson stain, aldehyde fuchsin, Goldner's trichrome stain, azan stain, Feulgen stain, staining with methyl-green pyronin and tartric acid thionine (Feyrter). For a better characterization of the nature of dense (inclusion) bodies several histochemical methods and digestion experiments were carried out: alloxan-Schiff, coupled diazonium reaction, p-dimethylaminobenzaldehyde condensation after Adams, and rosindole reaction after Glenner for demonstration of tryptophan, coupled tetrazonium reaction, PAS, alcian blue (pH 2.5)-PAS, alcian blue pH 0.5, 1.0, and 2.5, and colloidal iron reaction after Hale. The digestion was done with pepsin, trypsin, and ribonuclease; then the sections were stained with Goldner's trichrome stain and methyl-green pyronin. In addition, an extraction with 1% perchloric acid (1 h at 60° C and 24 hrs at 20° C) was carried out and followed by staining with Goldner and methyl-green pyronin.

From formalin-fixed material, tissue samples were processed for electron-microscopic investigation: postfixation in 3.5% glutaraldehyde and 2% O_8O_4, embedding in Vestopal W, and contrasting with lead citrate.

Results

A. Light Microscopy

The tumor was located directly beneath the epidermis (Fig. 1a) and consisted of moderately cellular interdigitating sheets of proliferating spindle-shaped cells, that resembled fibroblasts, and collagen fibers (Fig. 1b). The growth exhibiting only a few mitoses extended down into the subcutaneous adipose tissue and surrounded and engulfed adnexal structures of skin (Fig. 1c). Most nuclei were oval-shaped and vesicular, usually with one distinct nucleolus. The nuclear membrane was often thickened and occasionally indented by homogeneous round bodies (Fig. 1d). The above-mentioned round bodies, also called inclusion bodies, were typical for the lesion on histological level. They had a diameter of between 6 and 24 μ. They were characteristically situated in the cytoplasm of proliferating fibroblasts, and occasionally close to the nucleus deforming its conture. They were also observed scattered in the extracellular space. In HE and Goldner sections they sometimes exhibited a so-called clear halo (Fig. 1e). These dense round bodies, seen under the light microscope to be without internal structure, stained reddish with hematoxylin and eosin as well as aldehyde fuchsin, red with Goldner's trichrome stain and azan stain, blue with iron hematoxylin; they were fuchsinophilic (v. Gieson method), Feulgen-negative, and not visible after staining with cresyl violet. They showed a reddish-violet hue with methyl-green pyronin.

The round bodies showed the following histochemical results: they were red-violet after use of alloxan-Schiff and coupled diazonium reaction, brown to brownish-violet after the coupled tetrazonium reaction, nonvisible to slightly blue after p-dimethylaminobenzaldehyde and rosindole, and negative after PAS, alcian blue, and colloidal iron method. Likewise, the Feyrter's inclusion method with tartric acid-thionine did not yield a positive result.

Fig. 1. (a) Recurring digital fibrous tumor of childhood grown only exophytically, and covered by an attenuated hyperkeratotic epidermis (H& E, ×6). (b) Typical proliferating spindle-shaped fibroblasts and collagen fibers (H& E, ×320). (c) Fibroblastic tissue engulfing adnexal structures of skin (H& E, ×80). (d) and (e) Dense (inclusion) bodies located in juxtanuclear position (d) or extracellulary (e) (Goldner stain, ×2000)

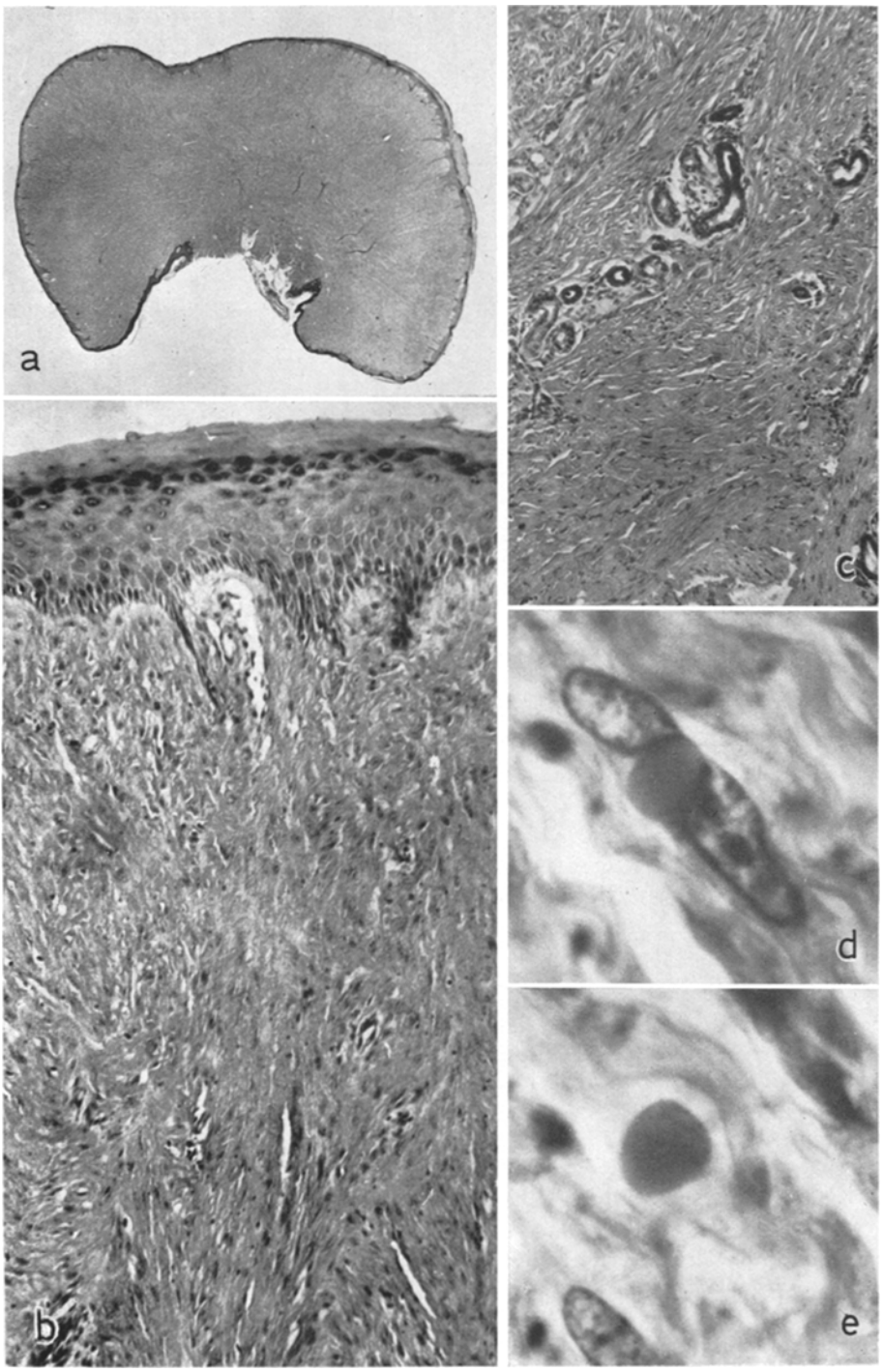

Fig. 1a—e

By means of digestion with various enzymes and following staining with Goldner's trichrome stain and methyl-green pyronin, the prevailing proteinaceous nature of the round bodies was ascertained: they were completely digested by pepsin, the other enzymes did not show any effect, only trypsin somewhat diminished the staining reactions. Unfortunately, pretreatment with collagenase was not possible because of the fixation with formalin (Németh-Csóka and Simor, 1962).

B. Electron Microscopy

Most of the cells were elongated and revealed the appearance of proliferating fibroblasts with abundant and well-developed, rough endoplasmic reticulum which frequently revealed parallel membranes. Furthermore, in the cytoplasm free ribosomes were observed, vesicular structures of varying size and in places fine filaments ranging in diameter between 100 and 120 Å. Mitochondria were present only in a moderate number, but they were not well preserved because of the inadequate fixation.

In several fibroblasts the typical dense (inclusion) bodies could be identified (Fig. 2). They were either in a juxtanuclear position or randomly distributed in the cytoplasm. A perifocal electron-lucent zone could frequently be detected.

The development of these dense (inclusion) bodies obviously was associated closely with certain changes of the rough endoplasmic reticulum (Fig. 3). In the earliest stage of the sequential changes a bellied widening of the ergastoplasmic tube with accumulation of a granular electron-dense material is visible. After reaching an appropriate size, the ergastoplasmic membranes disappear successively, and finally, the content of the ergastoplasmic tube lies free in the cytoplasm and forms the inclusion body.

At higher magnification the bodies consist of granular and fine filamentous material (Fig. 4). The fully developed dense body does not have a limiting membrane; on its periphery, however, fine fibrils with a diameter of about 100 Å can be seen. The interior of these bodies is composed of fibrillar elements measuring from 30 Å to 130 Å in diameter and having sporadically an intimated periodicity of about 240 Å, and of a granular material. Besides, some vesicular structures were included in these dense bodies, which have a diameter ranging from 72 to 120 mμ. The center of these vesicles was either electron-lucent and appeared empty, or it was electron-dense, sometimes with several condensations.

Discussion

It is apparent from the morphological picture that we are dealing with a type of fibromatosis which is unique by reason of its typical dense (inclusion) bodies. On the basis of clinical factors (e.g. occurrence in infants, exclusive predilection for fingers and toes, and tendency to recur, morphological and virological studies) the viral nature of these inclusions was presumed (Reye, 1965; Ahlqvist *et al.*, 1967; Pohjanpelto *et al.*, 1967; Burry *et al.*, 1970). Battifora and Hines (1971) stressed the resemblance to viroplasm seen in cells infected with certain viruses.

The examination of dense (inclusion) bodies in our case verified the preponderance of proteins. The was no evidence of DNA or RNA (see also Shapiro, 1969; Allen, 1972). The positive pyroninophilia in formalin-fixed sections must not be

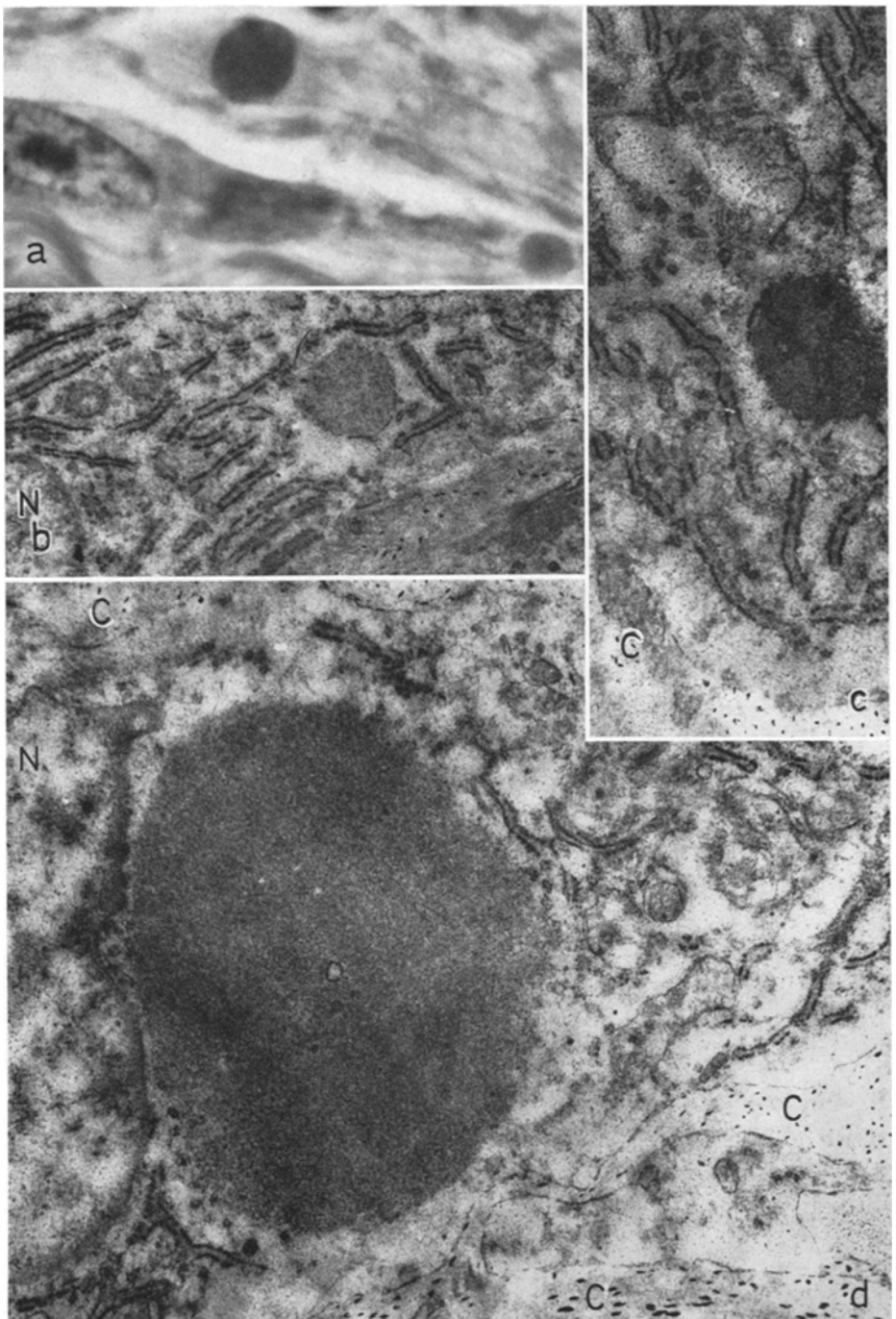

Fig. 2a—d. Varying localization of dense (inclusion) bodies (a, H & E, ×2000). These bodies may be localized amid the ergastoplasmic reticulum (b, ×16400), in the cellular periphery (c, ×20500) and in close proximity to the nucleus (d, ×20500). The clear halo is also discernible electron-microscopically. *N* nucleus, *C* collagen fibers

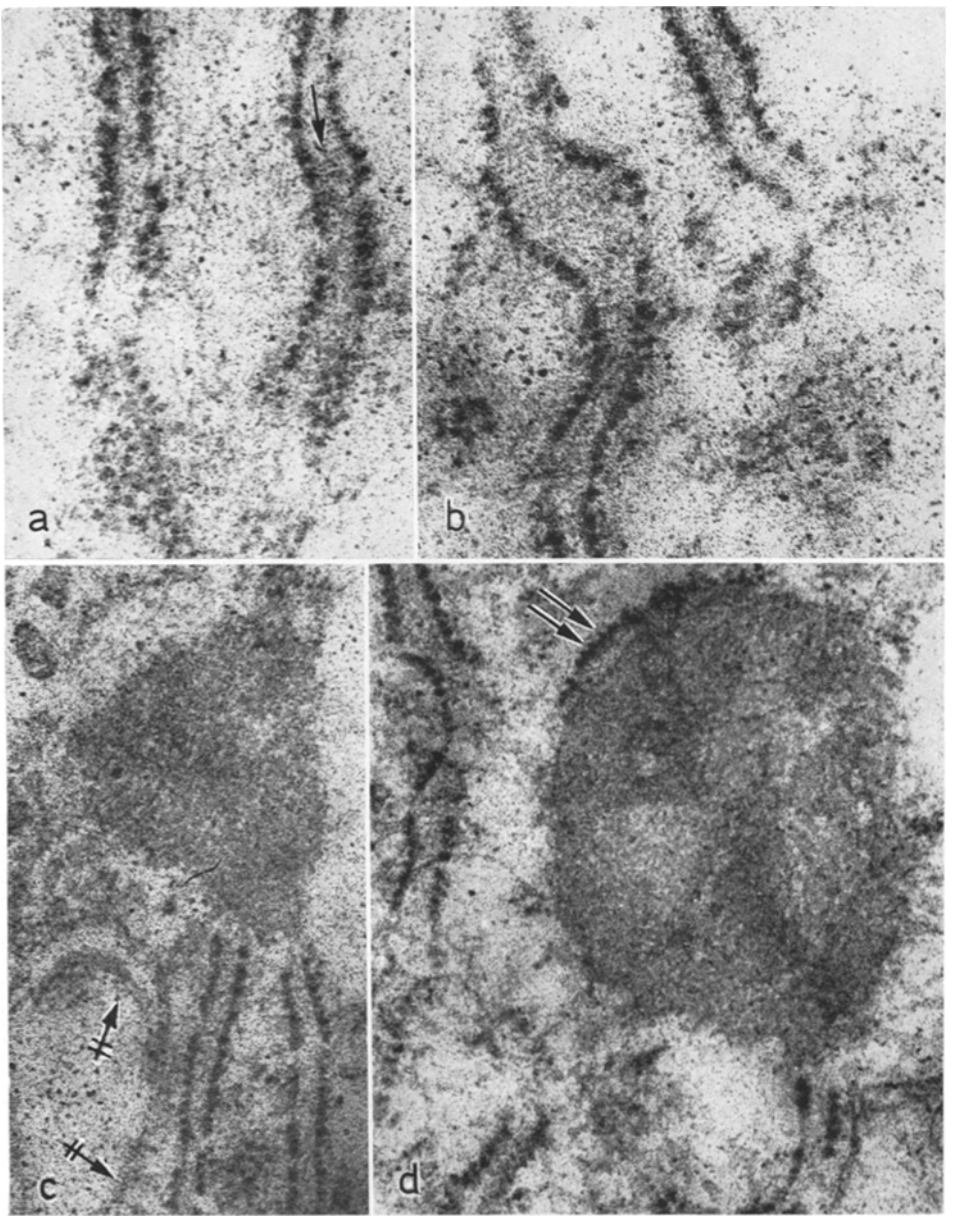

Fig. 3a—d. Stages of development of the dense bodies. (a) Accumulation of a dense granular material in an ergastoplasmic tube. Single beaded structures are visible (→) (×72000). (b) Further accumulation leads to a widening of the ergastoplasmic tube (×72000). (c) and (d) Finally different sized dense bodies can be found exhibiting only a partially limiting ergastoplasmic membrane (⇉). Occasionally intracytoplasmic tubules (‖→) and beaded structures are visible (c, ×54000; d, ×61500)

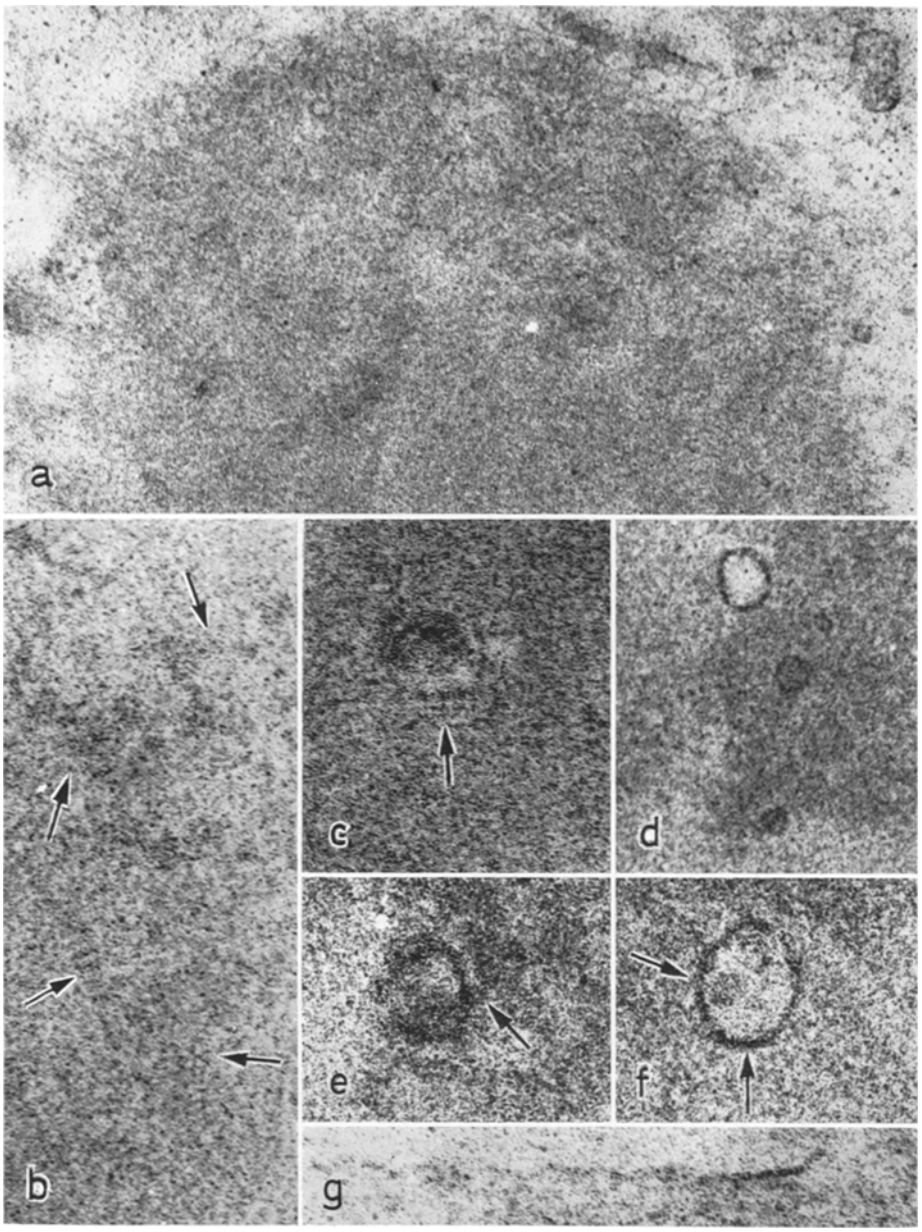

Fig. 4a—e. Structural composition of fully developed dense bodies. (a) Filaments and granular material (×44500). (b) Fibrils with regular periodicity of 240 Å (→) (×108000). (c), (d), (e) and (f) Different contured vesicles with a dense or empty interior surrounded by beaded arciform fibrils (→) (c, ×123000; d, ×61500, e, ×123000, f, ×123000). (g) Example for a long fibril at the periphery of the dense body (×123000)

considered as proof of nucleic acids, this is especially supported in our case by the lack of digestion by ribonuclease and by the ineffeciency of perchloric acid extraction. Therefore, it seems unlikely that these bodies themselves represent virus-like substances.

Local changes of the ergastoplasmic reticulum correlated to the development of dense bodies suggests that they originate within the ergastoplasmic tubes and that destruction of ergastoplasmic membranes follows during the course of their increase. The accumulation of proteinaceous material within cisternas of the ergastoplasmic reticulum is a known cytopathological phenomenon (see Pfeifer and Klinge, 1974). Taking into consideration the digestion by pepsin and the nearly complete resistance to trypsin (Wasserman, 1956) fibers with periodicity suggest a relationship to collagen synthesis. This idea is compatible with the results of staining reactions. However, the typical cross-banding of mature collagen was never seen in dense bodies.

In this connection we must remember that for the normal aggregation of collagen fibrils the so-called microenvironment is of utmost importance (Jackson, 1968). Thus, Gross (1956) could precipitate collagen fibrils with a periodicity of 220 Å from a solution using a peculiar salt concentration.

These findings are supported by observations on an intracytoplasmic collagen synthesis in fibromatoses (Welsh, 1966). However, the association of ergastoplasmic tubes which was not found by Welsh, and the unusual periodicity of 240 Å, suggest a synthesis under abnormal conditions. Perhaps the basic disturbance could be a defective intracellular transport, i.e. a block of the physiological pathway of collagen precursors followed by abnormal deposition and partial and atypical aggregation. The possibility that these dense (inclusion) bodies may consist in part of microfibrils (Haust, 1965) or actin filaments should also be taken into consideration. The significance of the vesicular structures remains open to question, but we believe that they are not akin to viruses.

Thus we interpret the dense (inclusion) bodies as products of an abnormal metabolism of fibroblasts. On this point we are in agreement with Mehregan *et al.* (1972). Grunnet *et al.* (1973) also think that they represent cell constituents rather than viruses. But we are unable to answer the question as to the cause of this abnormal metabolism. Therefore, we must admit to the possibility that the alteration of the metabolism may be caused by a viral infection.

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Doz. Dr. D. Stiller
Pathologisches Institut
der Universität
DDR-69 Jena
Ziegelmühlenweg 1
German Democratic Republic