Modifications of the Fine Structure of the Incisor Pulp of the Guinea Pig during Experimental Scurvy

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A histologic description of dental pulps of scorbutic animals was given in 1916 by Jackson and Moore[1] and in 1919 by Zilva and Wells.[2] Howe,[3] in 1920, described the effect on teeth of a scorbutigenic diet as did Höjer[4] in his well-known studies of 1924. Wolbach and Howe,[5] in 1926, were the first investigators to characterize the condition as something lacking in the intercellular structure that functions in the gelation of the connective tissue. They also concluded, as did Höjer,[4] that there was a failure of the fibroblasts to produce and maintain intracellular material. In studies of the fibroblast in scorbutic animals, Meyer[6] reported vacuolization of the cytoplasm and destruction of the cell membranes. Wolbach, in further studies in 1933,[7] described vacuoles filled with fluid, which he theorized could be a precursor of collagen. The belief that the primary cause of scurvy was impaired cellular secretion was again reported by Ham and Elliot[8] in 1938. Bourne,[9] in 1942, and Danielli,[10] in 1945, came to the conclusion that the deficiency resulted in a decreased rate of conversion of procollagen reticular fibers to mature collagen.

Chen and Postlethwait[11] have concluded that the inability of scorbutic tissues to synthesize collagen is not due to an absence of fibroblasts, which they found to be abundant. There have been several reports stating that the number of fibroblasts is not decreased in scurvy. In fact, Gersh and Catchpole[12] and Barber and Nothacker[13] have reported finding an increase.

Wasserman[14] studied the fibroblasts of scorbutic animals by electron microscopy. He described vesicles containing a precipitate and numerous fibrils of small diameter in the periphery of the cytoplasm. Peach[15] also reported alteration of the ultrastructure of fibroblasts and has suggested that this results in the primary deficiency of connective tissue formation. In recent electron microscope studies, Ross and Benditt[16] have reported that there was little intercellular collagen but that large amounts of poorly structured, dense matter in the extracellular spaces were found within the fibroblast of healing wounds in ascorbic acid-deficient guinea pigs. Aggregates of intracytoplasmic lipid droplets were also frequently found in the cell. They also found that in scorbutic animals proline-H^3 moved from fibroblasts to collagen fibers as in normal animals and to the extracellular fibrillar material[17] The only difference was in the delay in uptake and slower release of the product by the cells in the scorbutic animals.

Biochemical evidence has shown that scorbutic animals are unable to convert proline to hydroxyproline and lysine to hydroxylysine. Moreover, no significant accumulation of proline-rich precursors has been found.[18] The amount of extractable hydroxyproline has been found to parallel the amount of ascorbic acid present in the connective tissue.[11] Recent studies by Ross and Benditt[19] (1964) revealed that fibroblasts from wounds of normal animals had numerous polyribosomes that were thought to be related to continued synthesis of proteins. This configuration was lost in animals with scurvy, although it reappeared as early as four hours after introduction of ascorbic acid to the diet; reorientation was complete after 24 hours. It was suggested that scurvy might alter the messenger RNA, or the ability of the ribosomes to be aggregated by the messenger.

There are other factors concerning the scorbutic condition that are not clear, such

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as the effect of altered ground substance on collagen formation and the function of hyaluronidase in fibrogenesis and maintenance. The role of ascorbic acid in the maintenance of collagen has not been entirely agreed on. Gould[20] (1960) has noted that collagen formed during partial deficiency resorbed extensively after withdrawal of ascorbic acid.

It is the purpose of the present investigation to study the effect of ascorbic acid deficiency on the fibroblast and on the appearance of collagen in the dental pulp of scorbutic guinea pigs. The tooth pulp, especially that of the continually growing rodent incisor, has been shown to be an ideal site for the study of the differentiation, maturation, and regression of the pulp fibroblast. The widely spaced stellate-appearing fibroblasts allow good visualization of the cell surfaces and adjacent extracellular spaces in the study of collagen fibrillogenesis. Furthermore, the guinea pig incisor is the subject of the original light microscope descriptions of scorbutogenesis and thus should be regarded as a good site for ultrastructural studies of the effect of this condition on the fibroblast and fibrillogenesis.

Materials and Methods

Twenty guinea pigs weighing approximately 250 mg. were used. They were pair-fed a scorbutogenic diet* for 30 to 35 days. The control guinea pigs receiving daily oral supplements of 20 mg. of ascorbic acid. At the end of the experimental period, guinea pigs were anesthetized with ether, and their mandibles were rapidly dissected out and bisected along the midline. The body of the mandible was clipped off with shears along the lateral aspect of the base, and the entire incisor, including the growing end, was exposed. The teeth were separated from the mandible and dipped briefly in fixative; the partially calcified dentin from the proximal portion then was removed. The pulp thus exposed was removed from the hard shell of dentin by gentle pulling with forceps.

Small pieces of the pulp were cut and fixed either in 6.5 per cent glutaraldehyde or in 2 per cent osmic acid, both of which were buffered with 10 M phosphate at pH 7.4. Tissues that had been fixed with glutaraldehyde were washed briefly in the buffer and refixed in 2 per cent osmic acid. Sucrose was added to the osmic acid to make a concentration of 4.5 per cent sucrose. After fixation, the tissues were dehydrated through a graduated series of ethanol and embedded in a mixture of epoxy and resin. Sections were made on an ultramicrotome and picked up on grids that were coated with polyvinyl formal resin† reinforced with a thin layer of carbon. Saturated uramyl acetate and 0.1 per cent phosphotungstic acid solutions were used as electron stains. Observations were made in an electron microscope.‡

* General Biochemicals, Chagrin Falls, Ohio.

Observations

A REVIEW OF THE NORMAL PULP STRUCTURE.—The detailed description of normal dental pulp has been given previously.[21–23] The fibroblasts of the dental pulp in control guinea pigs appeared similar to those of connective tissues in other organs. Many of the cells appeared stellate with long cytoplasmic cell processes. Most cells had well-developed organelles with dense, rough-surfaced, endoplasmic reticulum (RER). Some of the RER appeared somewhat dilated; the interior contained flocculant materials that had an electron density similar to that of extracellular substances. In other organelles, the RER was in the form of tight tubules and flattened sacs containing intracisternal materials that were much denser than that of extracellular substances (Fig. 1, 2). The Golgi apparatus occupied a juxtanuclear location and contained an extensively developed stack of membranes and a large number of vesicles and vacuoles. The mitochondria were large, usually ovoid to elongated, and had straight cristae mitochondriales running transversely across the matrix. The ground cytoplasm was dense and contained varying numbers of intracellular fibrils that measured about 60 Å in diameter and were more numerous in cells with the RER of flat profiles. Frequently, a cilium was found near the nucleus; an additional centriole might be located perpendicular to the long axis of the cilium. The nucleus of the fibroblast was ovoid to elongated. The nuclear membrane often had a notch. One or more nucleoli were located in its periphery. Along the surface of the cell, there were numerous vesicles and vacuoles.

† Hitachi HU-11 electron microscope, Perkin Elmer Corp., Norwalk, Conn.

‡ Formvar, Shawinigan Resins Corp., Springfield, Ill.

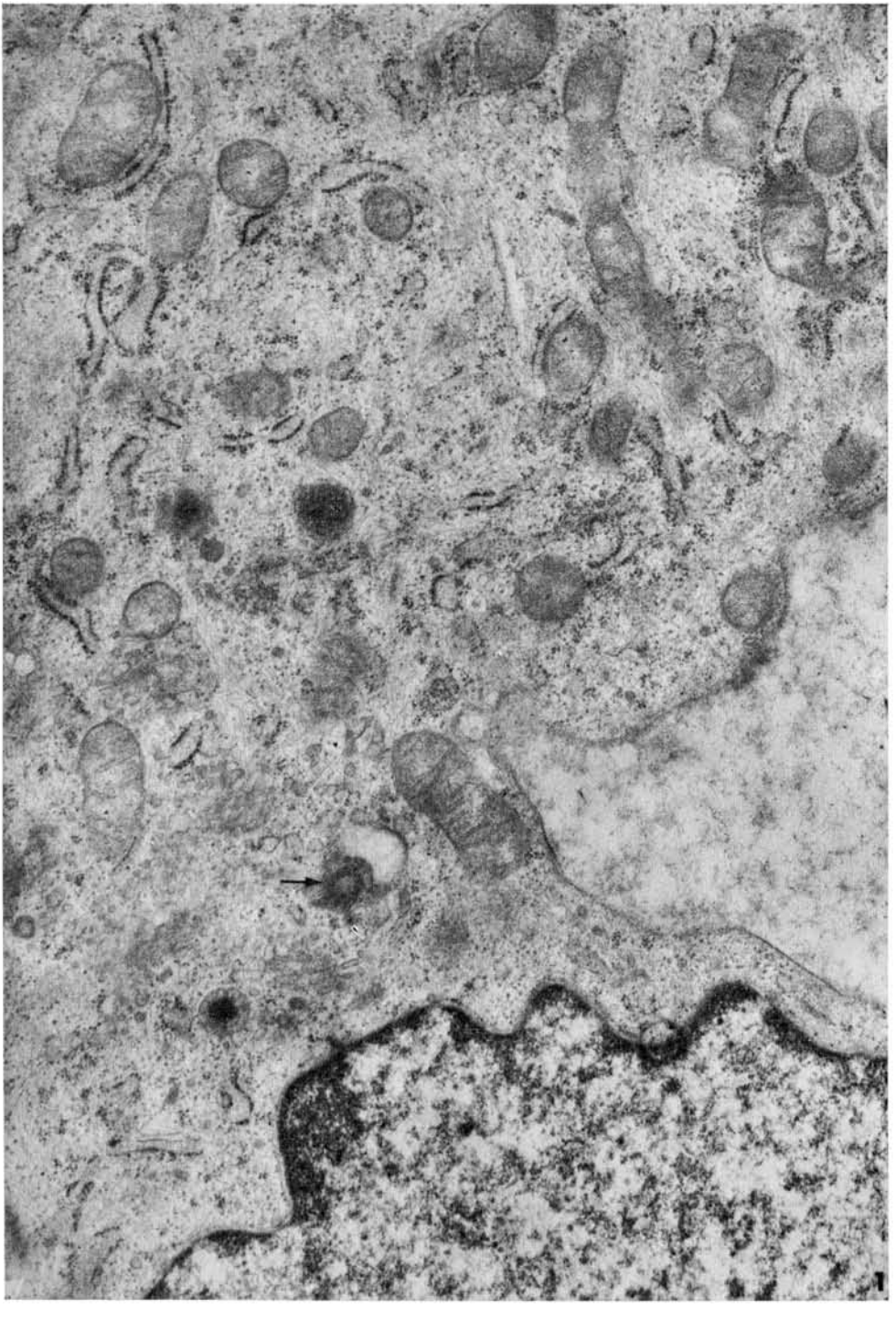

FIG. 1.—A portion of a fibroblast from the incisor pulp of a control guinea pig. The cytoplasmic fine structure contained in this cell conforms to characteristics of fibroblasts found elsewhere in the body. There are fine intracellular fibrils between the profiles of the rough-surfaced endoplasmic reticulum and mitochondria. The content of the reticulum is denser than extracellular substances. A cilium (arrow) is cut through the base, which is surrounded by a semicircular extracellular space produced by invagination of the surface plasma membrane (mag. approx. ×32,000).

Most of the intercellular space was filled with finely granular to fibrillar ground substance that appeared to clump, leaving clear spaces between aggregates. Extracellular fibrils were composed primarily of collagen fibrils with characteristic cross banding and were variable in diameter, ranging from 400 to 700 Å or more (Fig. 3). They were present singly or in small bundles of several to a few dozen fibrils. Where collagen fibrils formed a bundle, the sheaths of ground substance surrounding individual collagen fibrils were fused, and the fibrils were separated only by the ground substances. Fine fibrils located extracellularly were remarkably uniform in diameter, ranging from 100 to 120 Å. In cross section, they appeared to be made up of three or four finer subunits. These fine fibrils sometimes formed a cell covering that was 1μ or more thick.

CHANGES OCCURRING IN THE FIBROBLAST.—The following changes in the ultrastructure characterized the cytoplasm of fibroblasts of scorbutic guinea pigs. The cells were smaller than normal and appeared to have less cytoplasm. All organelles decreased in number and size (Fig. 4). Although occasional cells contained a well-developed RER, many cells were almost devoid of it. When present, the profiles were usually dilated and round (Fig. 5). The number of ribosomes studded on the surface of the RER was small. Cisternae of the RER contained somewhat cloudy, electron-dense materials that did not noticeably vary in electron density.

The Golgi apparatus was rounded, consisting of small stacks of a few laminated components and vesicles (Fig. 6). Vacuoles of the complex were seldom observed, and few dense granules were found in its vicinity. The mitochondria were smaller and fewer than in normal fibroblasts (Fig. 6, 7). The number of cristae also was decreased. Throughout the rest of the ground cytoplasm, there were numerous intracellular

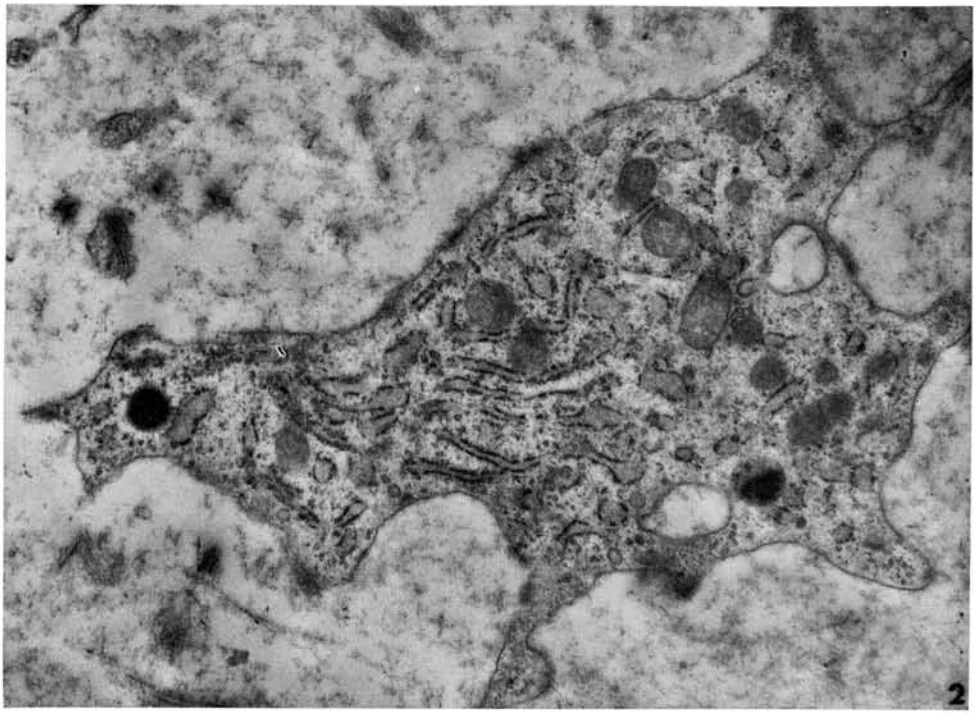

FIG. 2.—A portion of the peripheral cytoplasm of a fibroblast from the middle portion of guinea pig incisor pulp. Within the irregularly contoured cytoplasm are vacuoles, well-developed, rough-surfaced endoplasmic reticulum; dense bodies, and mitochondria. Materials in the cisternae of the flat, rough-surfaced endoplasmic reticulum are much more electron dense than the rest of the cytoplasm and the extracellular space (mag. approx. ×15,600).

FIG. 3.—A portion of the intercellular space from normal rat incisor pulp. The fine extracellular fibrils 100 to 120 A in diameter, and collagen fibrils are visualized (mag. approx. ×86,000).

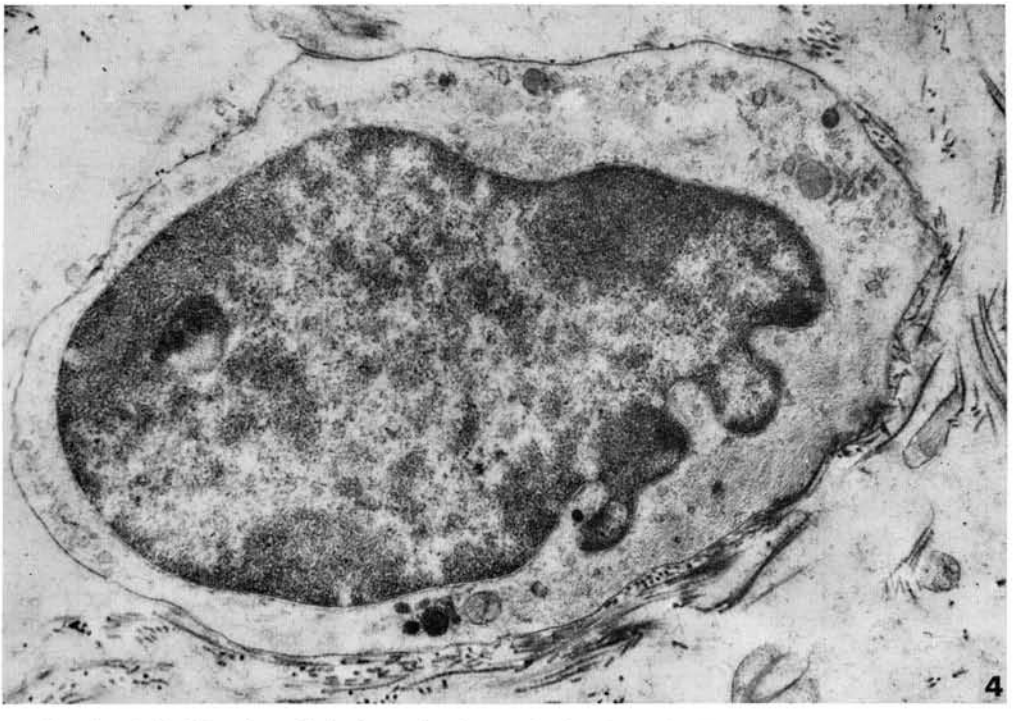

FIG. 4.—A fibroblast from the incisor pulp of a scorbutic guinea pig. Notice the rare occurrence of the rough-surfaced endoplasmic reticulum and small mitochondria, which are much fewer in number and poorly developed. Numerous intracellular fine fibrils appear to fill up much of the cytoplasm (mag. approx. ×10,000).

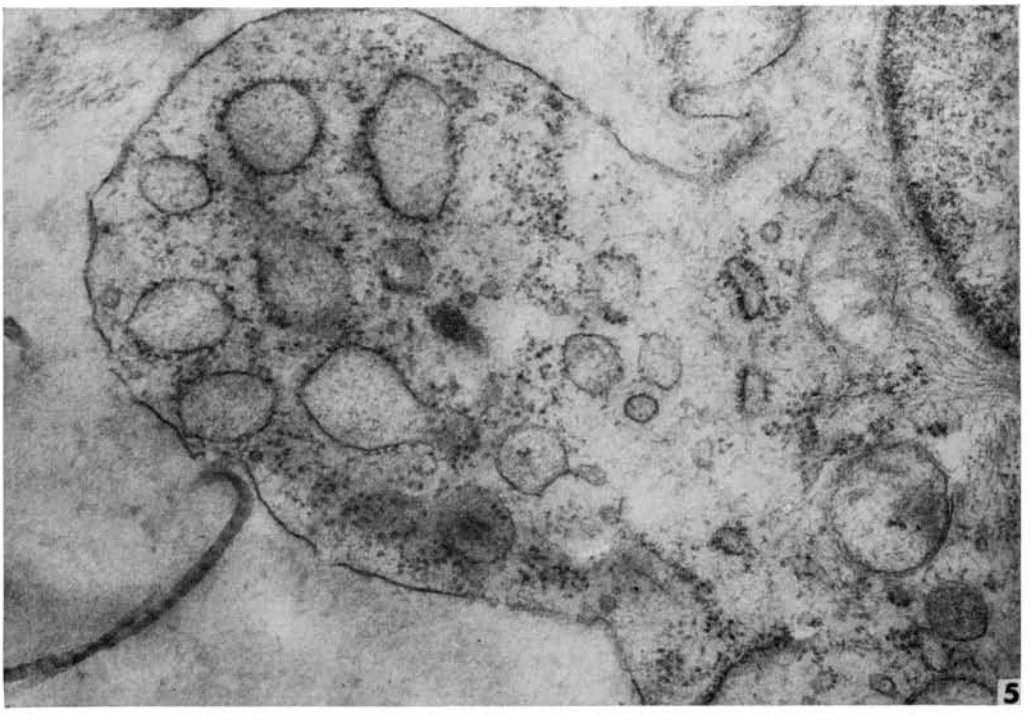

FIG. 5.—A portion of the fibroblast from the incisor pulp of a scorbutic guinea pig. The rough-surfaced endoplasmic reticulum has round profiles, and free ribosomes in the cytoplasm appear to have lost polyribosome arrangement (mag. approx. ×20,000).

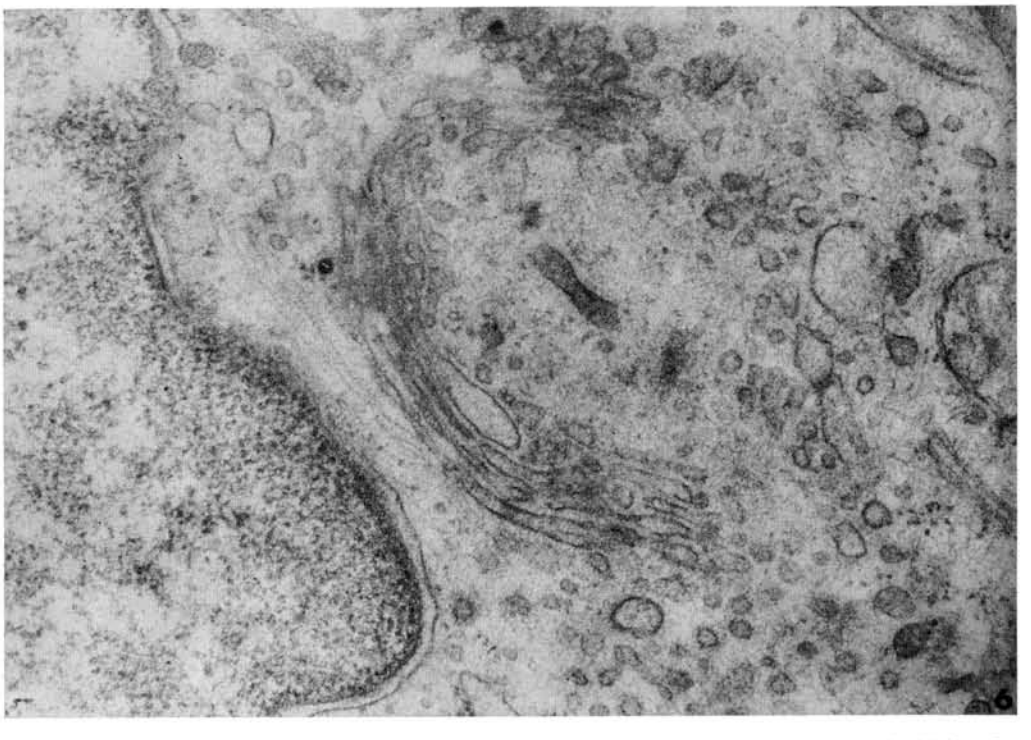

FIG. 6.—A Golgi apparatus in the fibroblast from the incisor pulp of a scorbutic guinea pig. Notice the rounding up of the stacked lamellae. Relatively few vesicles are present (mag. approx. ×36,600).

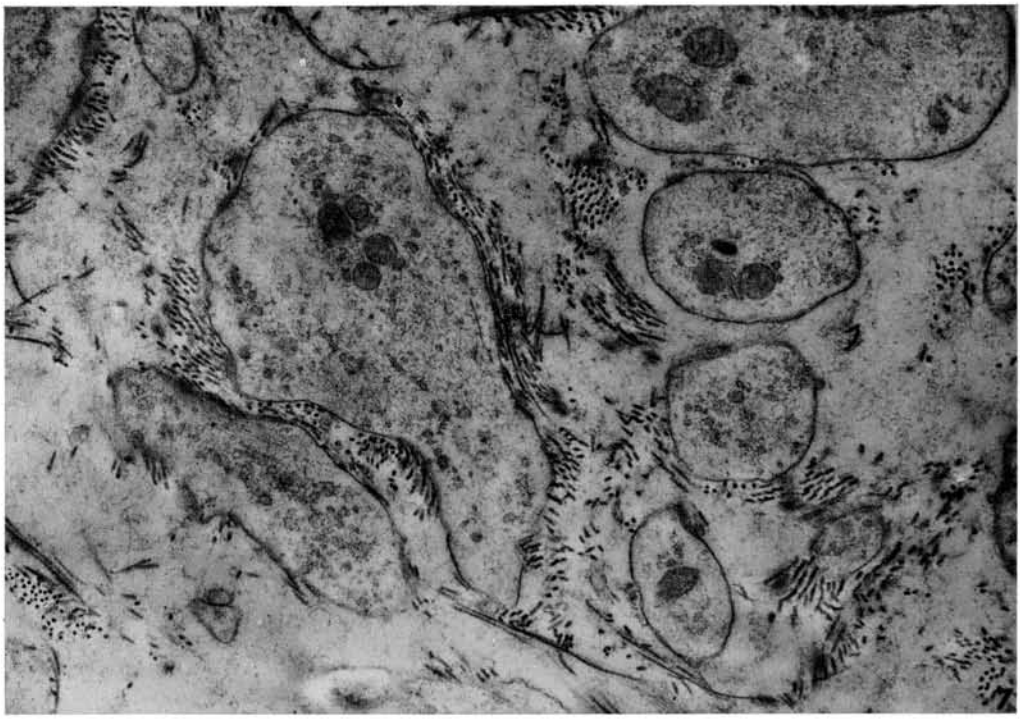

FIG. 7.—A portion of the incisor pulp from a scorbutic guinea pig. The peripheral processes of the fibroblasts that are shown in the field contain only a small number of tubular profiles of the rough-surfaced endoplasmic reticulum and a few small rounded mitochondria. The cytoplasm has abundant fine fibrils in it.

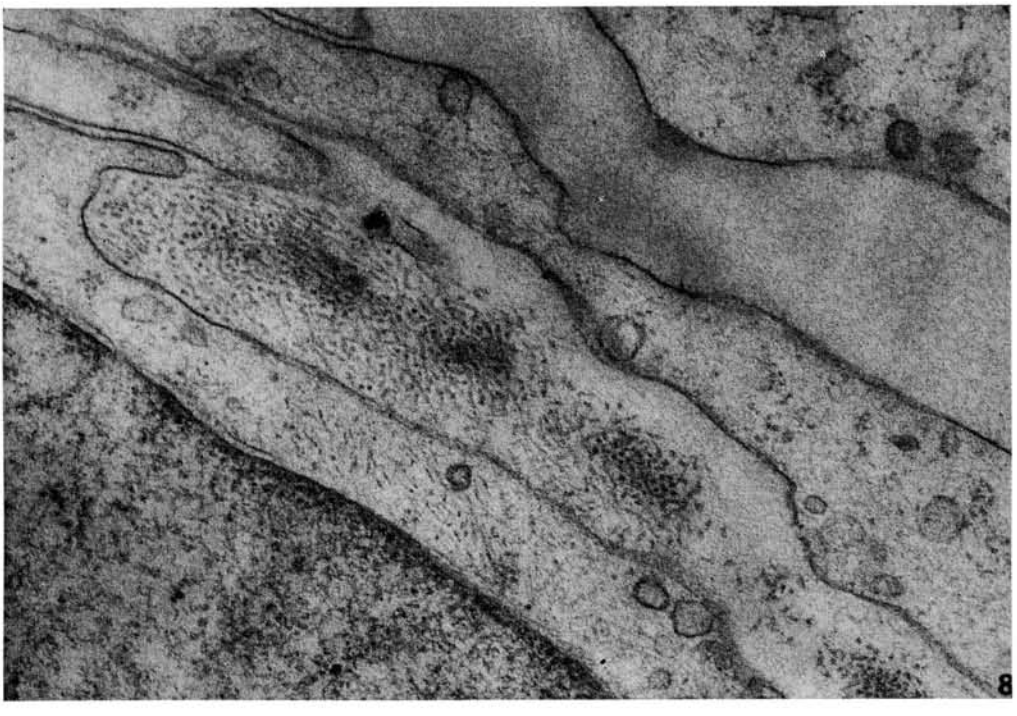

FIG. 8.—The appearance of fine extracellular fibrils (100 to 120 Å) in the incisor pulp of a scorbutic guinea pig. The fibrils are covering the surface of the cell by forming small bundles (mag. approx. ×52,000).

fibrils that were similar to those seen in normal fibroblasts but that were relatively greater in number (Fig. 4, 7).

MODIFICATIONS OF INTERCELLULAR SUBSTANCES.—A rather striking change was observed in the intercellular region, especially near the cell surface. Although the two types of extracellular fibrils appeared similar to those of the controls, there was a definite increase in the numbers of small fibrils along the surfaces of the fibroblasts (Fig. 8). The degree to which such accumulations were formed varied greatly (Fig. 9). When cells were transversely sectioned, the accumulation presented a profile of an aggregate fibril bundle rather than that of a sheet. This might account for the great variation in thickness described earlier. The bundles might be located somewhat away from the cell as well as being closely related. The number of mature collagen fibrils appeared to be small, yet they were observed throughout the pulp. The collagen fibrils often were found in close association with the fine fibrils.

Discussion

Ultrastructural changes observed in the dental pulp in ascorbic acid deficiency reveal the fibroblast to be one of the primary sites affected. The intracellular organelles were decreased both in size and in number, indicating decreased cellular function. The mitochondria, for example, not only were found to be smaller and fewer, their internal structure was affected. This implies an alteration (a possible decrease) in oxidative phosphorylation, which is directly related to the energy consumption of the cell. The dilatation of the endoplasmic reticulum and the decreased number of ribosomes on reticular surfaces is in agreement with the findings of Peach[15] and Ross and Benditt.[16, 17, 19] The recent observations of Ross and Benditt[19] of the disappearance of spiral or rosette forms of the ribosomes in the fibroblasts of healing wounds of scorbutic guinea pigs were not as apparant in the pulpal fibroblasts. This may be due to a difference in the metabolic state between

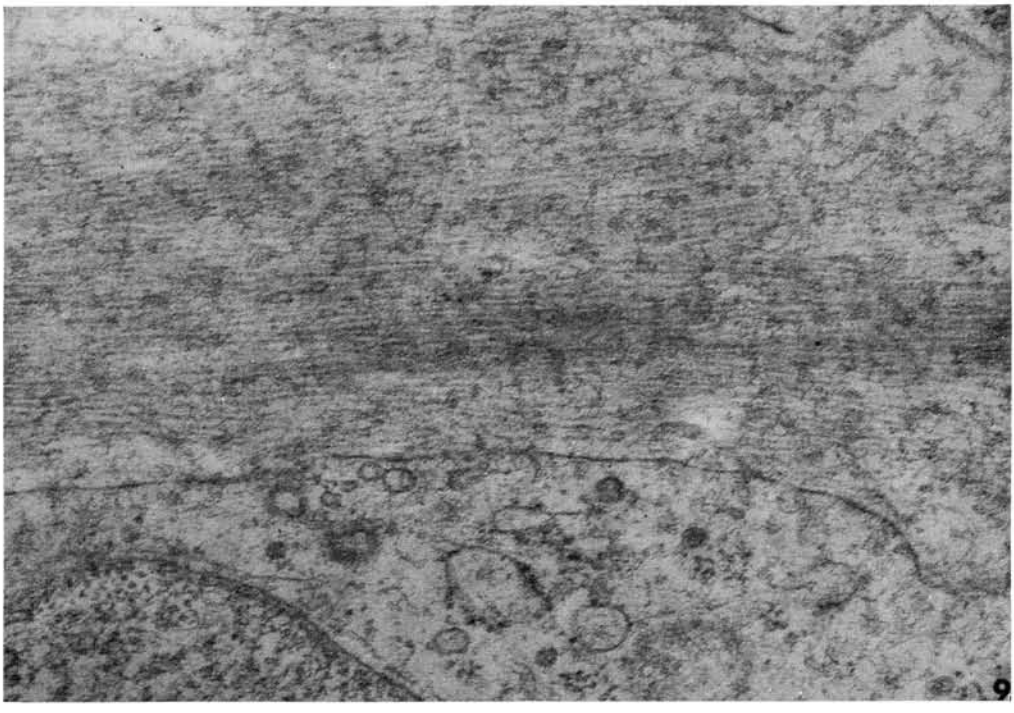

FIG. 9.—The appearance of fine extracellular fibrils in the incisor pulp of a scorbutic guinea pig. The covering of the cell surface by fine extracellular fibrils may be as thick as 1 μ or more (mag. approx. $\times$48,000).

fibroblasts of the wound and the less rapidly growing pulpal cells. The appearance of intracellular fibrils in the scorbutic pulpal fibroblasts is of interest. Although these fibrils were observed in the normal cells, the fibrils seemingly were unaffected in the scorbutic cell and, therefore, appeared more prominent in the latter. These fibrils might be regarded as part of the cytoskeleton of the cell, possibly being less active and therefore less affected by scorbutic changes in the cell. More definite answers will have to come from correlative biochemical studies.

The significance of small (100 to 120 Å in diameter) extracellular fibrils accumulated on the surface of the cell cannot be stated definitely at this time. It is possible that this represents an accumulation of abnormal tropocollagen produced by cells incapable of polymerizing into collagen fibrils.

Conclusions

The differentiating fibroblasts of the guniea pig incisor pulp tissue were stellate with long cytoplasmic processes. These cells were characterized by well-developed, rough-surfaced endoplasmic reticulum, Golgi complex, and mitochondria.

The ground cytoplasm of these cells was dense and contained varying amounts of intracellular fibrils measuring approximately 60 Å in diameter. Along the inner surface of the plasma membrane, there were a number of vesicles and vacuoles.

Extracellular fine fibrils, 100 to 120 Å in diameter, were found usually in groups surrounded by aggregations of finely granular ground substance. Collagen fibrils, 400 to 700 Å in diameter with characteristic cross banding, were also found near the cells.

In the scorbutic guinea pigs, the fibroblasts appeared smaller and the intracellular organelles were diminished in number. Many cells were devoid of endoplasmic reticulum which, when present, appeared dilated with a decreased number of ribosomes on the surface.

A significant increase in the number of intracellular and intercellular fine fibers was found. Intercellular fibrils were situated near the plasma membrane, and some appeared in isolated bundles.

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