WOUND HEALING AND COLLAGEN FORMATION

II. Fine Structure in Experimental Scurvy

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ABSTRACT

The sequence encountered in healing skin wounds in scorbutic guinea pigs has been examined by methods of light and electron microscopy. Linear incisions in the skin of female guinea pigs fed a scorbutigenic diet were allowed to heal. The wounds were removed for examination at 1, 3, 5, 9, and 14 days after wounding. The fibroblasts of the scorbutic wounds differ from those of the controls in three major aspects. First, little collagen is present within the intercellular spaces, although small groups of individual collagen fibrils can be found adjacent to some of the fibroblasts; in addition, large amounts of somewhat fibrillar, poorly structured, dense matter are present throughout the extracellular regions. The second alteration consists of large aggregates of intracytoplasmic lipid deposits present within the majority of the fibroblasts. Third, the endoplasmic reticulum of the fibroblasts is altered in form from that of the controls. The profiles of the cisternae are round, non-continuous within the plane of section, and are less extensively developed than in the controls. An increased macrophagic activity of the histiocytes is also described. These changes are discussed in light of the available biochemical data associated with this abnormality of protein synthesis.

INTRODUCTION

Defective wound healing in individuals suffering from scurvy was recorded centuries ago. By the eighteenth century, it was noted that this disease could be traced to individuals whose diet was lacking in fresh fruit and vegetables. Anson (1) in 1748 and Lind in 1772 (2) both commented on the propensity for old, healed scars to break down and rupture in persons afflicted with this disease. Even at this early time, it was apparent that the same dietary constituent was necessary for both proper wound healing and maintenance of scar tissue. With the discovery by Holst and Frölich (3, 4) in 1907 that the guinea pig was subject to scurvy, it became possible to study experimentally the deficiency and to determine not only the factor whose absence caused the disease but also the tissue components affected in scurvy. Much of the early work leading to the establishment of ascorbic acid, or Vitamin C, as the factor necessary for prevention of scurvy was carried out by Harden and Zilva (5–8). The classical experiments of Höjer (9) and Wolbach (10, 11) clearly demonstrated a defect in the formation of reticulin and collagen in ascorbic acid–deficient guinea pigs. Wolbach and Bessey (12) went on to state that the protein substructure of the intercellular substances of the supporting tissues, namely, collagen, is either not produced or is produced in defective form in scurvy. The necessity of ascrobic acid for the maintenance of wound collagen has been recently confirmed by Abt and co-workers (13, 14).

The present study was designed to investigate, with the electron microscope, healing wounds of scorbutic animals and compare them with the wounds of normal animals. This is, therefore, an

extension of the classical studies of Wolbach (10, 11) on scurvy utilizing the increased resolution of the electron microscope to provide additional data for analysis of this connective tissue alteration. In our previous report (15) the components of the healing wounds of non-scorbutic animals were examined and characterized. The healing wound in the scorbutic environment affords a reproducible system in which modification of collagen synthesis may be studied. From this system, basic information may also be gained concerning collagen synthesis as a pathological variant of protein synthesis.

MATERIALS AND METHODS

Tissue Preparation for Light Microscopy

Wounds were removed at 1, 3, 5, 9, and 14 days from 300-gram female guinea pigs and were embedded in paraffin in the same way as described in the previous report (15), the only difference being that the diet (Nutritional Biochemicals) was identical in form and consistency but totally lacking in ascorbic acid.

In addition, one-half of the wounds were fixed in formalin, embedded in polyethylene glycol (Carbowax), and stained with Oil Red O and Harris hematoxylin modified from a method by Zugibe *et al.* (47, 48) for the identification of lipids.

Tissue Preparation for Electron Microscopy

The tissues were prepared as previously stated for examination with the electron microscope. They were fixed in osmium tetroxide, fixed again in formalin, and embedded in epoxy resin (Epon 812) (50). Some of the tissue sections were stained with phosphomolybdic acid in addition to uranyl acetate.

Preparation of Animals; Criteria for Determination of the Scorbutic State of the Experimental Animals

To be certain that all the animals in the experimental group were truly scorbutic, the epiphyses of the long bones, the mandibles, and teeth were routinely examined. The classical "Gerüstmark" in the epiphyses and classical changes in the odontoblastic layer of the pulp of the teeth were taken as adequate indices of the scorbutic state in these animals. The daily food intake and the weights of the experimental animals were measured and compared with those of the controls which were reported in the previous communication (15). Höjer, in his studies on scurvy (9), noted that 300-gram guinea pigs on a scorbutigenic diet began to slowly lose weight between the 10th and 14th day, and that they proceeded on a downhill course and died some 30 to 35 days from the time the diet was begun. Our experimental animals behaved in a comparable fashion. The daily measurements indicated that food intake in the scorbutic animals did not appreciably decrease until approximately the 25th day on the diet. This being so, our animals were fed the deficient diet for a total of 24 days, which included a 10-day period on the diet before wounding and removal of the wounds at intervals of 1, 3, 5, 9, and 14 days after wounding.

LIGHT MICROSCOPE OBSERVATIONS

The sequence of events observed in the scorbutic wounds was determined by counting cells with an ocular grid (Fig. 1). Grading of zero to three was based on numbers of cells per high power field, a grade of one being equivalent to 2 to 4 cells, grade two representing 4 to 10 cells, and grade three representing more than 10 cells.

Within 12 hours the wounds contain an exudate consisting of fibrin, polymorphonuclear leukocytes, erythrocytes, and some macrophages. The quantity of these cells increases for the first 24 hours, the number of macrophages lagging a little behind that of the polymorphonuclear leukocytes in time. Mononuclear cells described by many observers as "immature fibroblasts" begin to appear after 24 hours and continue to increase in number until the 6th postoperative day. The number and appearance of the fibroblasts remain unchanged from the 6th day through the 14th day of observation. The polymorphonuclear leukocytes rapidly decrease in number after the first 24 hours. The number of macrophages reaches a maximum by 72 hours and decreases thereafter; however, many more macrophages remain within the scorbutic wounds than in the controls at the later time periods. Many of these macrophages display erythrophagocytosis and contain hemosiderin within their cytoplasm. Little if any collagen or reticulin can be detected by the classical light microscopic staining techniques for these substances, at any time during the experimental period. Capillaries also make their appearance

later within the scorbutic wounds than in the controls.

The panorama of changes which occur in scorbutic wounds is largely demonstrated in Fig. 2. This represents an adjacent thick section of the material embedded for electron microscopy, sectioned at approximately 1 to 2 microns and stained with Azure II-methylene blue (49). These micrographs display representative areas from 7-day-old wounds from both the control and the scorbutic animals. The relative decrease of moderately dense material in the extracellular spaces of the scorbutic wound represents the deficit in collagen formation. The dark bodies in the cyto-

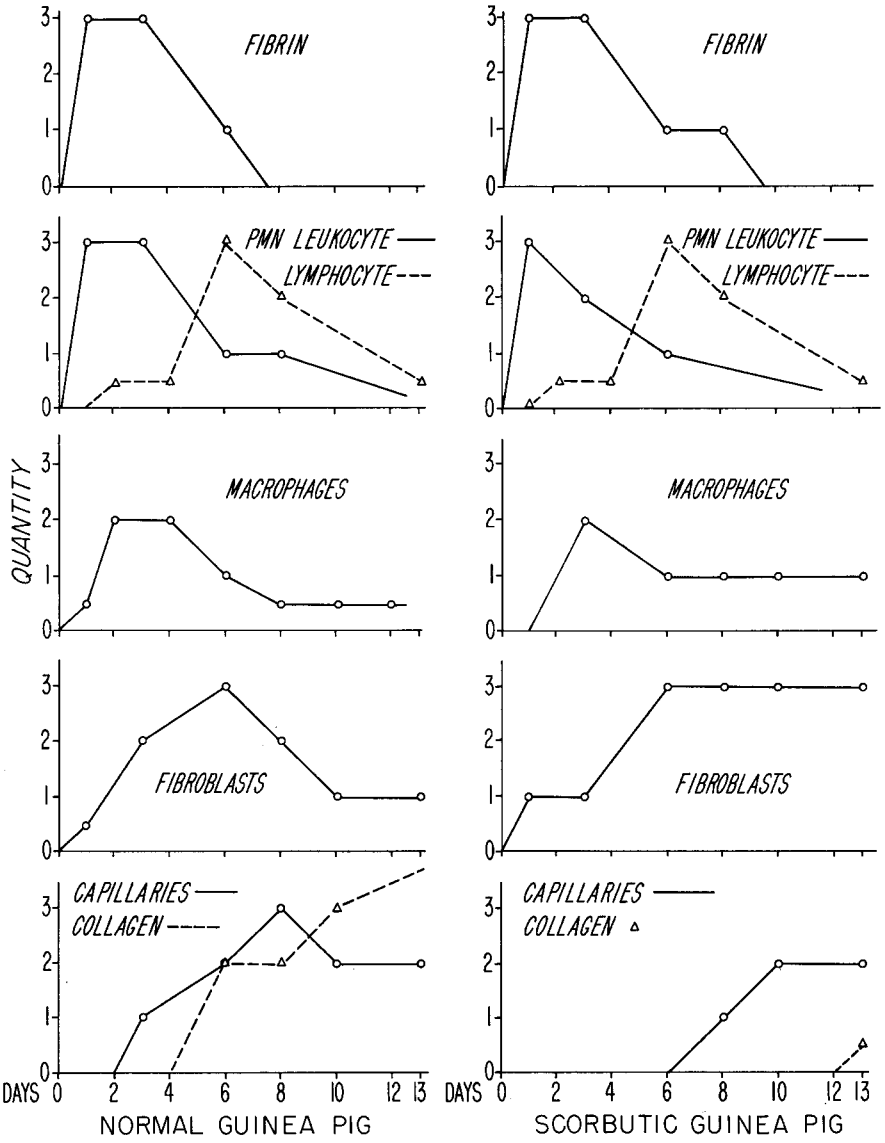


FIGURE 1

The graphs show the comparison between the control and the scorbutic animals. The major differences are seen in the fibroblasts, macrophages, capillaries, and collagen. The estimates listed as "quantity" are derived from the numbers of cells or elements per high power field, as seen in five sections of each wound, 20 fields per section.

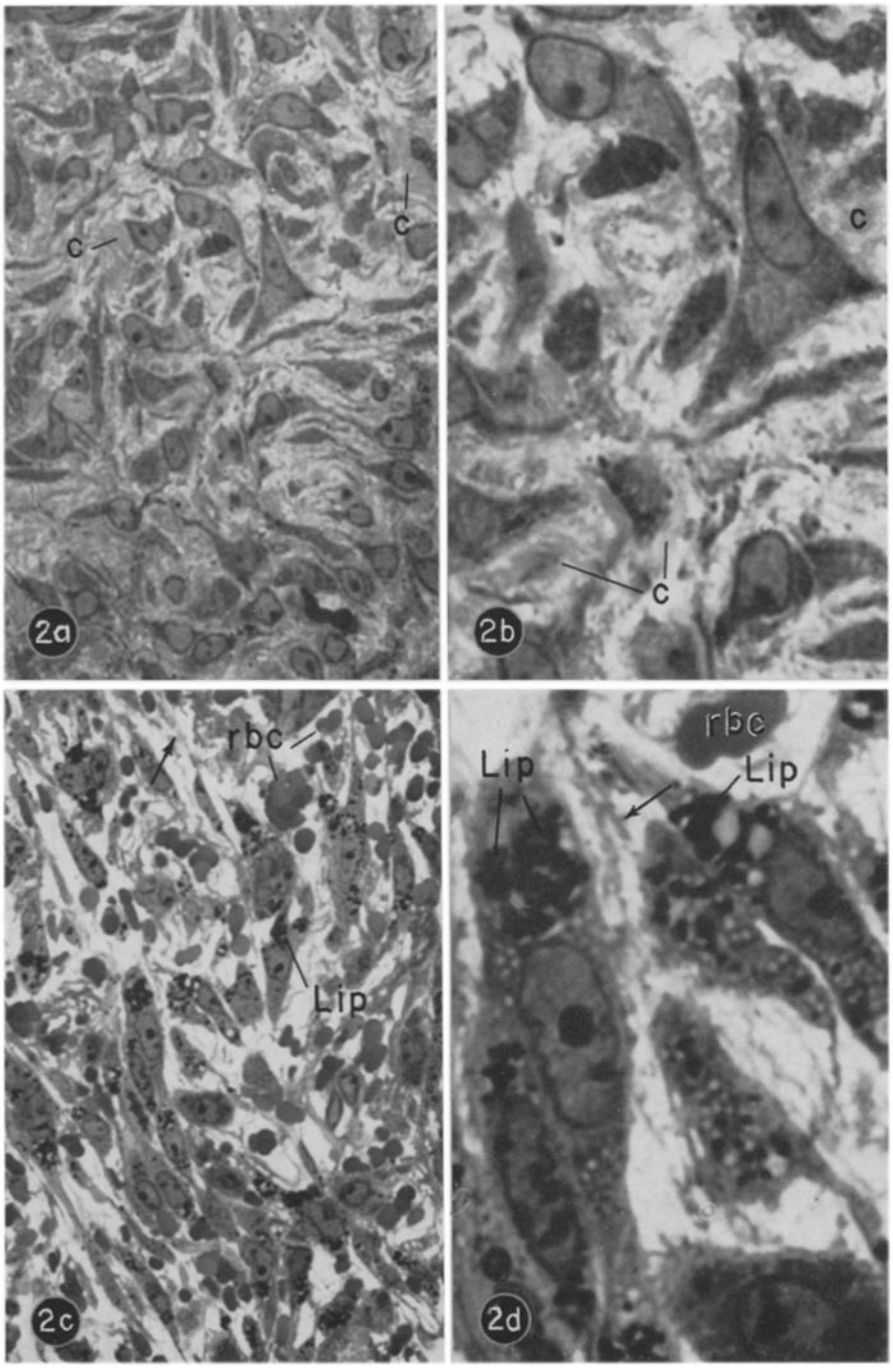


FIGURE 2

Figs. *a* and *b* are low- and high-power photomicrographs of a 7-day control wound, embedded in Epon and stained with Azure II and methylene blue. Figs. *c* and *d* are the same magnifications of a similar area from a 7-day scorbutic wound. The contrast in cell and wound morphology is striking. The lipid droplets (*lip*) can be seen in the majority of the scorbutic fibroblasts, and the lack of collagen (*c*) deposition is evident in the intercellular spaces of the scorbutic wounds. Extravasated red blood cells (*rbc*) and strands of extracellular matter (*arrows*) are present throughout the wounds. *a* and *c*, × 460; *b* and *d*, × 1800.

plasm of the scorbutic fibroblasts are lipid deposits which will be discussed later. Numerous extravasated erythrocytes can be seen to be dispersed throughout the scorbutic wounds.

ELECTRON MICROSCOPE OBSERVATIONS

The sequence of events as observed in the electron microscope is as follows. At the 24-hour period three distinct cell types are identified within the wounds. The first of these is the polymorphonuclear neutrophilic leukocyte with its characteristic granules and moderately dense cytoplasmic matrix. The granules of these cells are membrane-bounded and vary in density from a mottled light to an extremely dense granule in which no substructure can be seen. The second cell type is the macrophagic histiocyte. These cells contain irregularly shaped nuclei with a fairly large, well developed Golgi complex. The rough surfaced endoplasmic reticulum of these cells varies in extent from cell to cell, but is clearly less extensive than that of the fibroblasts. Throughout the cytoplasm of these macrophages are found vacuoles, varying in size and containing dense bodies of irregular shape, and numerous myelin figures (see *v* in Fig. 3). Another component of the cytoplasm of these cells is slightly dense, homogeneous, irregularly shaped bodies which range from 0.5 to 1.5 μ in diameter (see *b* in Fig. 3).

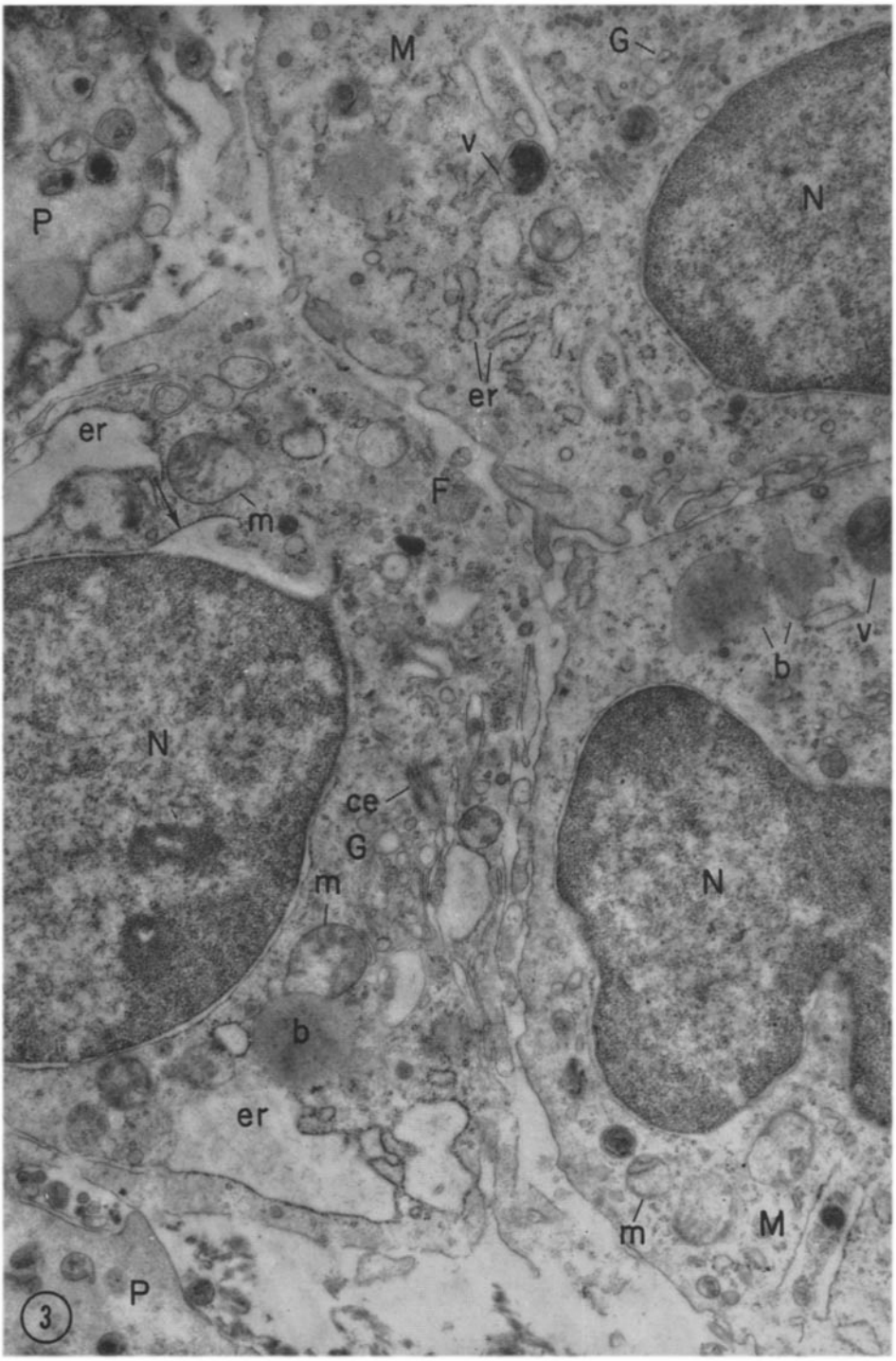
The third cell type, the fibroblast, displays a fairly extensive endoplasmic reticulum the cisternae of which are quite dilated and irregular in many regions (Figs. 3 and 4). The mitochondria of the fibroblasts are large and many of them have cristae which appear shortened. Diffusely scattered throughout the cytoplasmic matrix of these cells are groups of particles, presumably ribosomes. Occasionally, an irregular, amorphous, slightly dense body, not clearly membrane-bounded, can be found within the cytoplasm of these cells. Filaments approximately 50 A in diameter are seen sporadically within these fibroblasts. Numerous intercellular strands of fibrin, identified by their characteristic banding, are present coursing throughout the wound. Also present at this early period are numerous extracellular granules corresponding in morphology to the granules of the polymorphonuclear neutrophilic leukocytes. These granules are found dispersed throughout the extracellular spaces as well as in vacuoles within the cytoplasm of macrophages. This was noted previously in healing wounds of normal animals (15). It is difficult to see any differences in the electron micrographs between control and scorbutic wounds at 24 hours.

3 Days

By the 3rd day the polymorphonuclear leukocytes have largely disappeared and the scorbutic wounds consist of essentially three cell populations: the macrophages, the fibroblasts, and numerous extravasated erythrocytes. Throughout the period of study the morphology of the macrophages remains unchanged. Numerous vacuoles containing myelin figures (Fig. 5), ferritin, and portions of engulfed erythrocytes are present within these cells. The endoplasmic reticulum remains poorly developed in contrast to that of the fibroblasts, and the borders of these cells are extremely irregular, with numerous fine processes, many of which appear like microvilli. The mitochondria of these macrophages are smaller than those of the fibroblasts, and the cristae are quite regular. The Golgi complex continues to be located in a juxtanuclear position and is well developed, consisting of numbers of flattened parallel sacs and small vesicles.

The fibroblasts have a variable appearance at this time. Characteristically, the endoplasmic reticulum is well developed in these cells and appears either quite dilated or flattened, varying from cell to cell. The cisternae which are dilated contain a relatively dense, flocculent material which seems to be made up of short filaments in some regions (Figs. 6 and 7). The mitochondria are large, irregular, and have cristae which appear shortened and a matrix which in some regions is very pale. Their appearance in general is similar to that of the mitochondria of the fibroblasts in the control wounds. It can be seen that the vesicles and cisternae of the Golgi complex in the fibroblasts are dilated.

The fibroblasts of the scorbutic wound have an additional component not present within the previously described control wounds (15). It consists of numerous, irregular, very dense bodies which have no obvious limiting membrane (Fig. 6). These bodies appear brown when adjacent thick sections are viewed in the light microscope, indicating osmiophilia. They range in size from 0.5 μ to 1.5 μ and are randomly located throughout the cytoplasm of the fibroblasts.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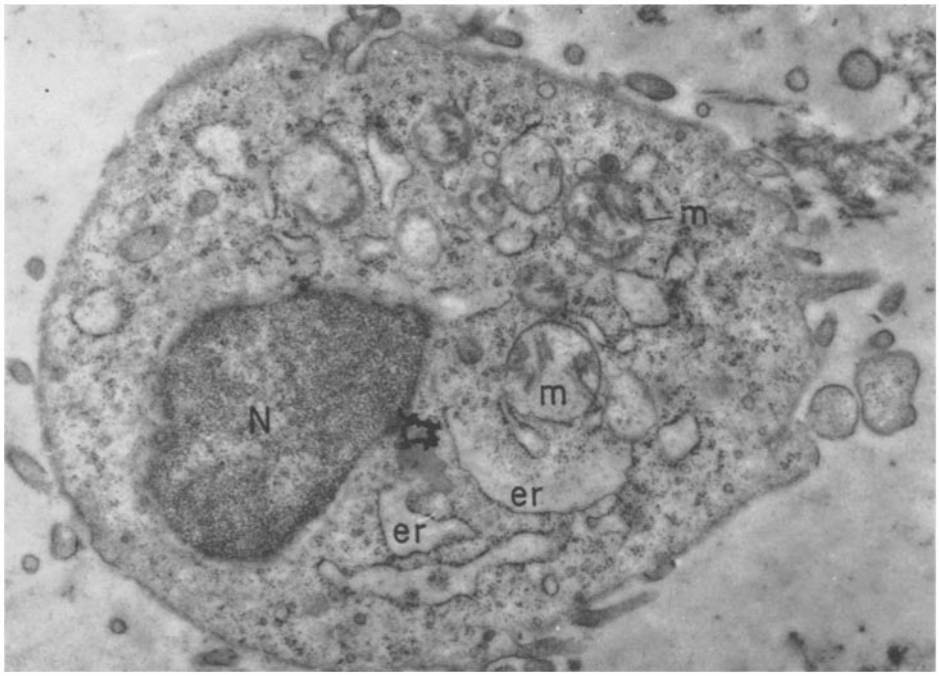


FIGURE 4

Micrograph of a 24-hour wound. This cell is typical of the early fibroblasts which proliferate in both the control and the scorbutic wounds. Typically, the mitochondria (*m*) are enlarged, the cisternae of the endoplasmic reticulum (*er*) are dilated, and there are numerous groups of ribosomes scattered throughout the cytoplasm. The nucleus (*N*) and numerous small intracytoplasmic vesicles are also apparent. The black irregular fragment seen near the nucleus is artifact and represents debris overlying the tissue section. × 19,000.

More than 50 per cent of the fibroblasts examined at the 3-day interval contain these bodies.

No identifiable collagen is evident in the wounds at this time, although large strands of fibrin are still found throughout the wound area.

5, 9, and 14 Days

The appearance of the scorbutic wounds from the 5th through the 14th day is similar. The cell populations continue to consist of erythrocytes,

FIGURE 3

A micrograph of a portion of a 24-hour wound showing the two major cell types characteristic of these wounds. The fibroblast (*F*) with its dilated cisternae of the endoplasmic reticulum (*er*) is contrasted with the macrophagic histiocyte (*M*) with its less well developed endoplasmic reticulum (*er*). The mitochondria (*m*) of the fibroblast are enlarged and the cristae are irregular. The Golgi apparatus (*G*) and a centriole (*c*) of the fibroblast are present in this illustration. The vacuoles (*v*) of the histiocyte contain myelin figures and ingested matter. Somewhat dense bodies (*b*) are seen in both the histiocyte and the fibroblast. These resemble lipid in their appearance. Portions of two polymorphonuclear neutrophilic leukocytes (*P*) are present in the left corners of the micrograph. The nuclei (*N*) of these cells appear similar in this section, and continuity between the endoplasmic reticulum and the nuclear envelope can be seen (arrow). × 18,00

macrophages, and fibroblasts. Throughout the period studied, the macrophages retain the same features that were evident by the 3rd day.

The fibroblasts display variation. From the 5th day on, the cisternal profiles of the endoplasmic reticulum appear round or irregular. They are not so extensively developed, nor do they appear so obviously interconnected as they do at earlier times in scorbutic wounds or at any time in the control wounds. Fine filaments, approximately 50 A to 80 A wide, are present throughout the cytoplasm of many of these fibroblasts, as well as the marginal filamentous condensations seen so commonly within the fibroblasts of the control wounds (Figs. 8 and 9).

The dense osmiophilic bodies continue to be present within the cytoplasm of the large majority of the fibroblasts (Fig. 10) at these times and they vary in shape and size. Many of them are irregular and have a beaded border along one edge, while others appear to have smooth contours. When these sections are stained with phosphomolybdic acid, the irregular bodies display less dense filament-like lines which course in various directions throughout their structure (Fig. 10).

An additional finding of interest in the scorbutic wounds at these times is the appearance of individual fibrils of collagen near the surface of some of the fibroblasts (Fig. 10). Although no bundles of collagen fibrils large enough to be visualized as fibers in the light microscope are present, there are some individual fibrils with a 700 A periodic banding which are adjacent to some scorbutic fibroblasts. Extracellular masses of a moderately dense material having no identifiable structure are also present throughout the wound.

CHARACTERIZATION OF THE OSMIOPHILIC BODIES

Electron micrographs taken of sections of wounds which were exposed to propylene oxide, a lipid solvent, for 24 hours display empty spaces corresponding to the sites of the dark osmiophilic bodies.

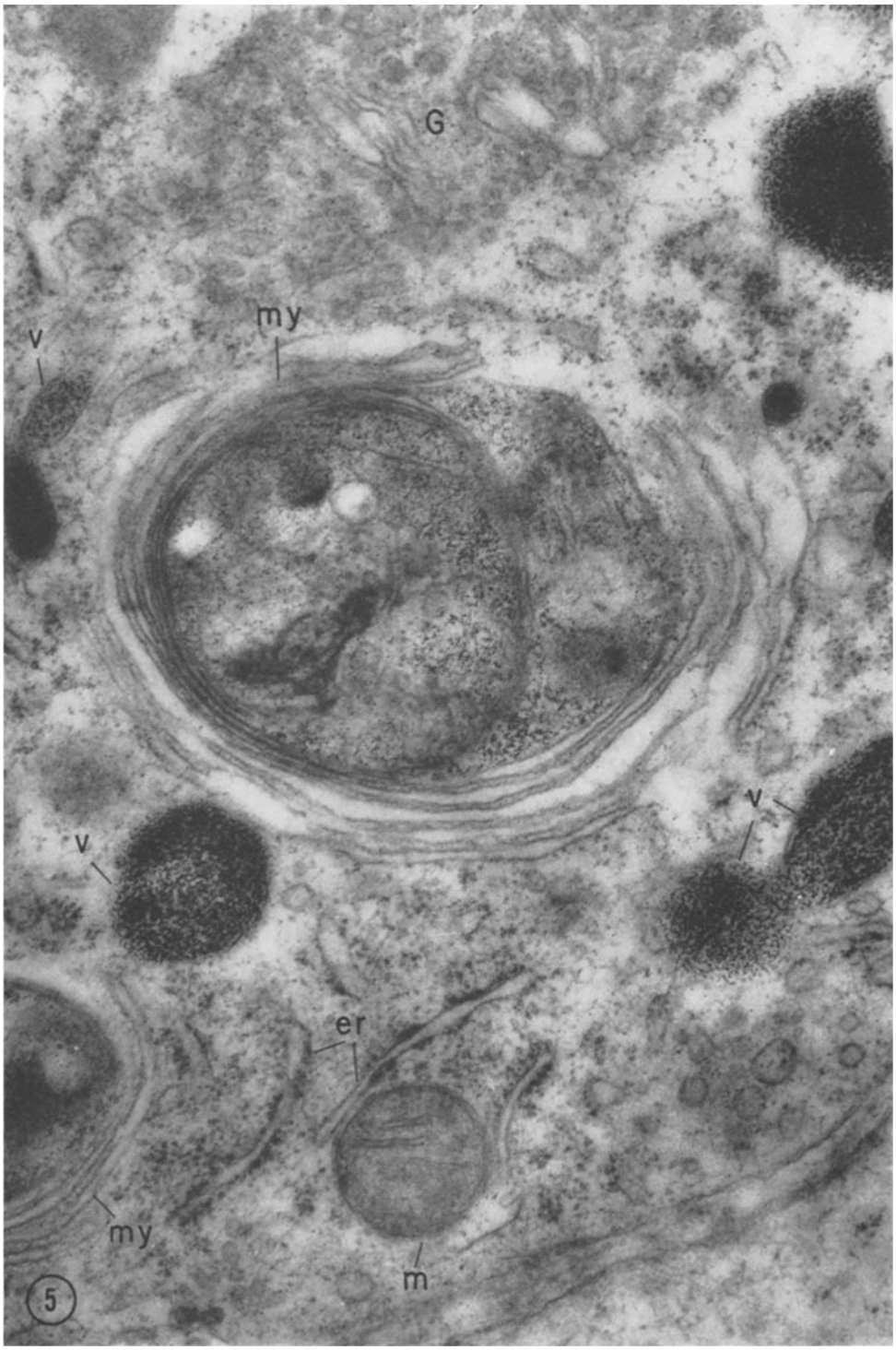
When these wounds are embedded in polyethylene glycol (Carbowax), large numbers of the fibroblasts contain numerous Oil Red O positive deposits within their cytoplasm at all the times examined after 24 hours. In contrast, the control wounds at these times lack these deposits. Adjacent thick sections of 7-day-old scorbutic and control wounds (Fig. 2) stained with Azure II and methylene blue (49) demonstrate the extensive lipid deposits in the scorbutic cells as prominent green bodies. Examination of the electron micrographs taken of adjacent sections of these wounds show that, after 24 hours, the dense, osmiophilic bodies clearly correspond to the lipid deposits seen in the thick sections. It is interesting that the thin sections stained with phosphomolybdic acid, instead of uranyl acetate, demonstrate the less dense lines in the structure of the lipid droplets (Fig. 10). It is not clear whether these lines represent sites which do not have affinity for the stain or regions which are in some fashion removed upon staining with this heavy metal.

DISCUSSION

The series of events seen in the light microscope in scorbutic wounds was elaborated in the classical studies of Wolbach and Howe (10, 11) and Wolbach and Bessey (12). They stated succinctly the consequences of ascorbic acid deficiency, noting "a failure of formation, and maintenance of intercellular materials" during this deficiency state. Wolbach made mention also of the "vacuoles that form in fibroblasts in the scorbutic guinea pig." He felt that these vacuoles might contain the source of the extracellular liquid seen in scurvy, and postulated that this liquid might represent a precursor of collagen which was stored within the cells. The present study confirms the very careful observations made by these workers, and extends these observations to the changes

FIGURE 5

A micrograph of a 3-day wound showing a portion of a macrophagic histiocyte. Several large myelin figures (*my*) are seen as well as vacuoles (*v*) containing ferritin. The cisternae of the endoplasmic reticulum (*er*) are narrow and the ribosomes lining their membranes are missing in regions. A mitochondrion (*m*) and a portion of the Golgi apparatus (*G*) can be seen. Numerous small vesicles are present throughout the cytoplasm. × 51,000.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which can now be visualized with the increased resolution of the electron microscope.

Hunt (19), Bourne (20), Murray and Kodicek (21), and, more recently, Fromm and Nordlie (22), and Johnson and McMinn (23) have examined the problem of wound healing in scurvy. The necessity of ascorbic acid not only for the formation of collagen but also for its maintenance in the healed wound has been observed by Bourne (20), Pirani and Levenson (24), and by Abt, von Schuching, and Roe (13, 14). In his study of wound healing, Hunt (19) indicated that there is a delay in conversion of argyrophilic fibers to fibers which have the staining properties of collagen. The same results have been shown in other structures which consist predominantly of connective tissue, namely, bone, teeth, and cartilage (17, 21, 25–31).

Ascorbic acid appears to have a direct local action in collagen formation. Gould (32) demonstrated this in polyvinyl sponges implanted in the skin of scorbutic guinea pigs. He found a rapid synthesis of collagen in the vitamin C-treated sponge whereas none was formed in an untreated sponge implanted within the same animal.

Chemical analysis of collagen formation in healing wounds has confirmed the necessity of ascorbic acid in the formation and maintenance of collagen. However, the specific role played by the vitamin in these phenomena is still not understood. The amino acid hydroxyproline is characteristically found in considerable quantity in collagen and has been used as an index of collagen formation (16, 33). Some investigators (34–40, 46) have attempted to correlate the importance of hydroxyproline as a constituent of collagen and the conversion of proline to hydroxyproline with the action of ascorbic acid in collagen formation. It is considered that the morphologic alterations, both intra- and extracellular, might provide additional information in relation to the available biochemical data.

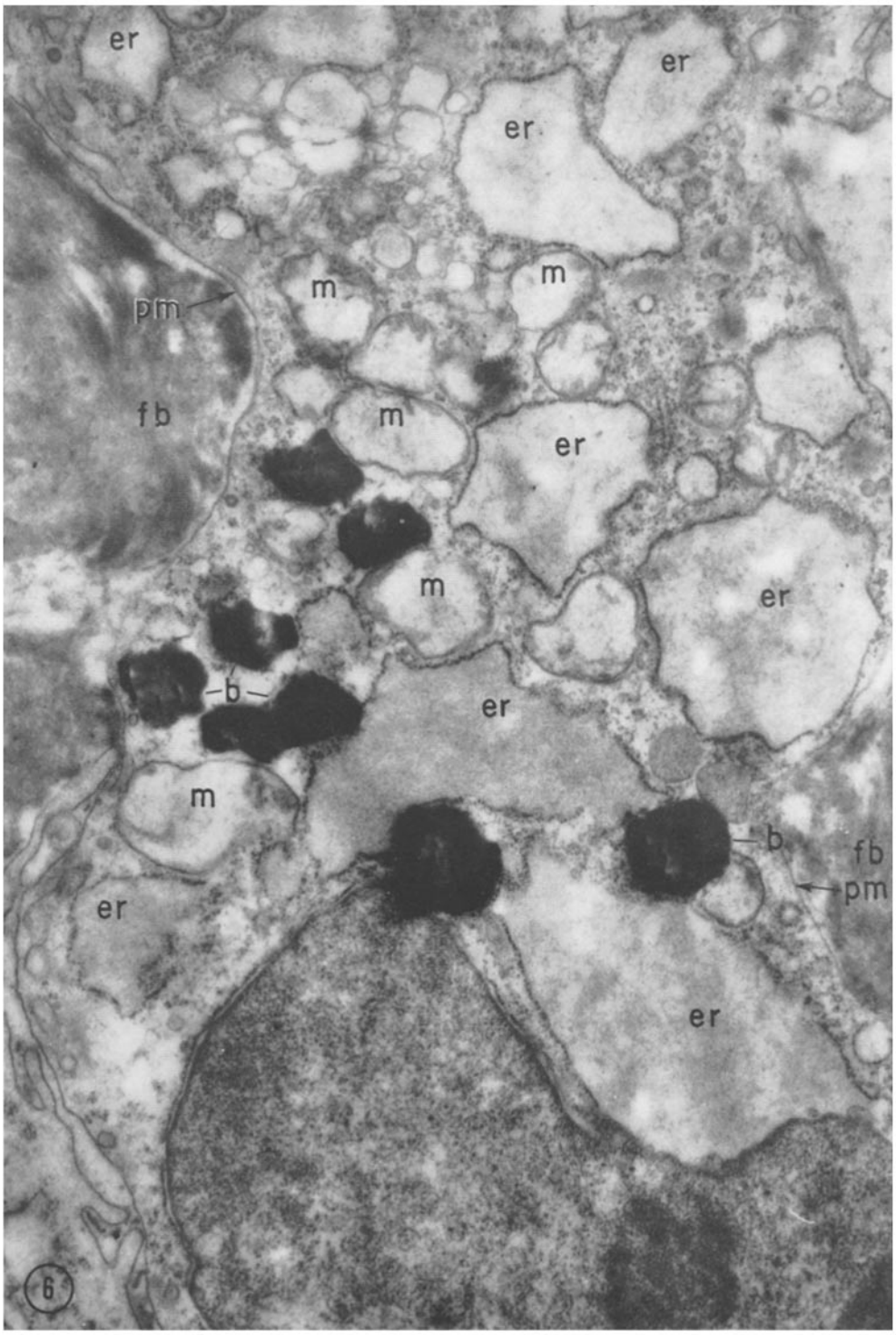
The present study was undertaken to define further the morphologic changes which occur in scorbutic fibroblasts and perhaps help to establish a relationship between the deficient collagen formation and these changes. The scorbutic wounds clearly differ from the controls in three major aspects. First, there is a marked decrease, although not a total absence, of collagen fibrils, with a large amount of somewhat amorphous, dense material within the intercellular space. Second, the great majority of the scorbutic fibroblasts contain irregularly shaped deposits of lipid which are not obviously membrane-bounded. Third, the endoplasmic reticulum of the scorbutic fibroblasts appears as rounded, often dilated, but rarely interconnected profiles, and this system is not so extensively developed as it is in the fibroblasts of the control wounds (Fig. 9). An ancillary finding is that the macrophages of the scorbutic wound are very actively involved in phagocytosis of the numerous extravasated erythrocytes and contain not only ingested erythrocytes but also numerous vacuoles filled with ferritin (Fig. 5).

In relation to the above, the presence of very few individual mature collagen fibrils adjacent to the border of some of the fibroblasts in the scorbutic wounds (Fig. 10) may be due to the fact that collagen is forming at a very slow rate, as postulated by Gould (41). Another possible explanation for the presence of very few fibrils is the one presented by Gross (42) in studying the neutral salt–soluble extracts of skin collagen. He could find no collagen in the extract of the skin from scorbutic guinea pigs. From this he concluded that, in intact skin, ascorbic acid deficiency may either interfere with collagen synthesis or cause its destruction and removal as rapidly as it is formed.

The lipid deposits present in the scorbutic fibroblasts are numerous and irregular in shape and disposition within the cells. At times these dense bodies have a beaded border along one edge, and

FIGURE 6

A micrograph of a region of a 3-day wound showing a portion of a fibroblast demarcated by its plasma membrane (*pm*). The cisternae of the endoplasmic reticulum (*er*) are dilated and contain a thread-like or flocculent-appearing material. The mitochondria (*m*) are enlarged, pale, and contain irregular cristae. Very dense, irregular bodies (*b*), not obviously membrane-bounded, are present in a portion of the cytoplasm. A dense, fibrillar material (*fb*) is seen in the extracellular regions. This material displays no banding and is not identifiable as fibrin. × 18,000.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when stained with phosphomolybdic acid they appear to contain less dense lines within their matrix. Penny and Balfour (18) made incidental mention of lipid deposits seen in occasional fibroblasts in scorbutic granulation tissue. Several possibilities are worthy of consideration. The fact that protein synthesis is altered in scurvy is manifest by the change in appearance of the endoplasmic reticulum of these cells as well as by the marked decrease in collagen formation. Smuckler, Iseri, and Benditt (43) have presented evidence in carbon tetrachloride intoxication in rats that the fatty metamorphosis in the liver may be due to an impairment of protein synthesis and consequent lack of transport of triglyceride from the liver cells. The first change observed by these workers is in the endoplasmic reticulum of the liver cells. The cisternae appear dilated and rounded, and many detached RNP particles are seen within the cytoplasm of the liver cells. At a somewhat later time, lipid droplets accumulate within the liver cells. These changes were correlated with a decrease in the synthesis of both albumin and fibrinogen. With altered protein synthesis in scurvy, it is possible that the lipid accumulations seen here are due to the same mechanism. Another possibility is that pointed out by Banerjee and Ghosh (44) who noted that scorbutic guinea pigs display an increased total body cholesterologenesis, apparently due to hypoinsulinism, for upon administration of insulin this alteration is corrected. We have observed that insulin administration to scorbutic guinea pigs does not affect the appearance of the lipid droplets (unpublished data). A third possibility is that of an association of these lipid deposits with the inanition seen in the later stages of scurvy. Palade (45) has demonstrated accumulation of lipid in the pancreatic acinar cells during starvation. This possibility, however, seems doubtful in the present study, because these lipid deposits are seen early before any evident inanition has taken place.

Mention should be made of the poorly structured, dense material found in the intercellular spaces. Its nature is, as yet, not clear. It may include not only mucopolysaccharides secreted by the fibroblasts but also a collagen precursor which is in some fashion either incomplete or unpolymerized. It is also possible that this represents some other substances synthesized by scorbutic fibroblasts alone or in combination with material derived from the plasma.

We conclude from these studies that the changes in the fine structure of scorbutic wounds are consistent with the concept that interference with protein synthesis is the major defect in the scorbutic fibroblast.

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Figure 7

Micrograph of a section of a 3-day wound showing a portion of a fibroblast. In this region of the fibroblast the Golgi apparatus (*G*) consists of dilated, parallel sacs and numerous vesicles. Portions of dilated cisternae of the endoplasmic reticulum (*er*) and one of the many enlarged mitochondria (*m*) characteristic of these cells are present. Two centrioles (*ce*) situated at right angles to each other can be seen as well. × 46,000.

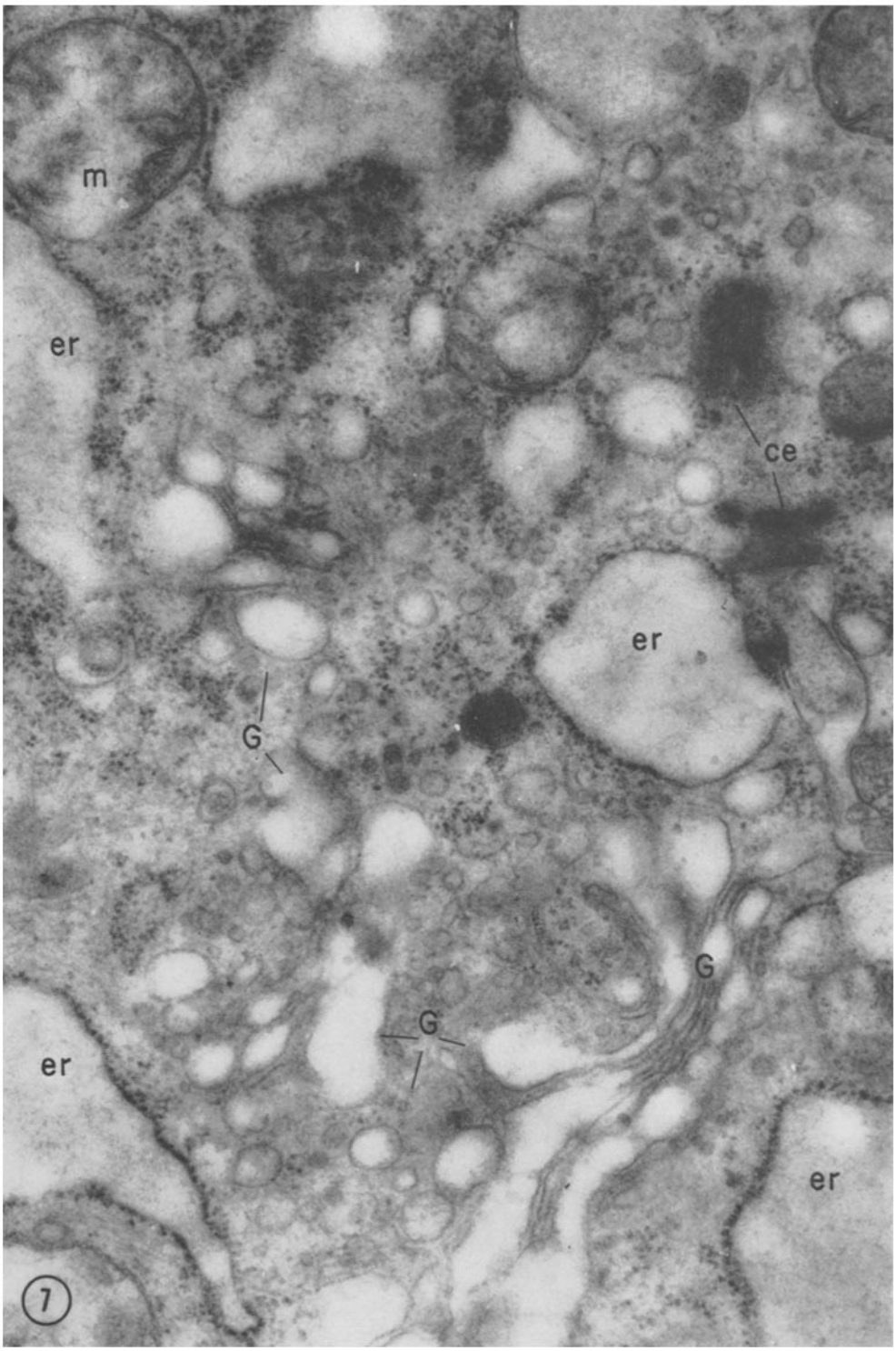
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FIGURE 8

Micrograph of a portion of a 14-day wound. The fibroblast (*F*) in this area contains the typical, irregular, dense bodies (*b*) seen throughout the cytoplasm of the majority of fibroblasts within the scorbutic wounds. The rounded, irregular cisternal profiles of the endoplasmic reticulum (*er*) are also evident. Marginal filamentous condensations (*f*) and numerous small vesicles (*v*) also can be seen. The extracellular fibrillar material (*fb*) displays no banding or other identifiable structure. × 47,000.

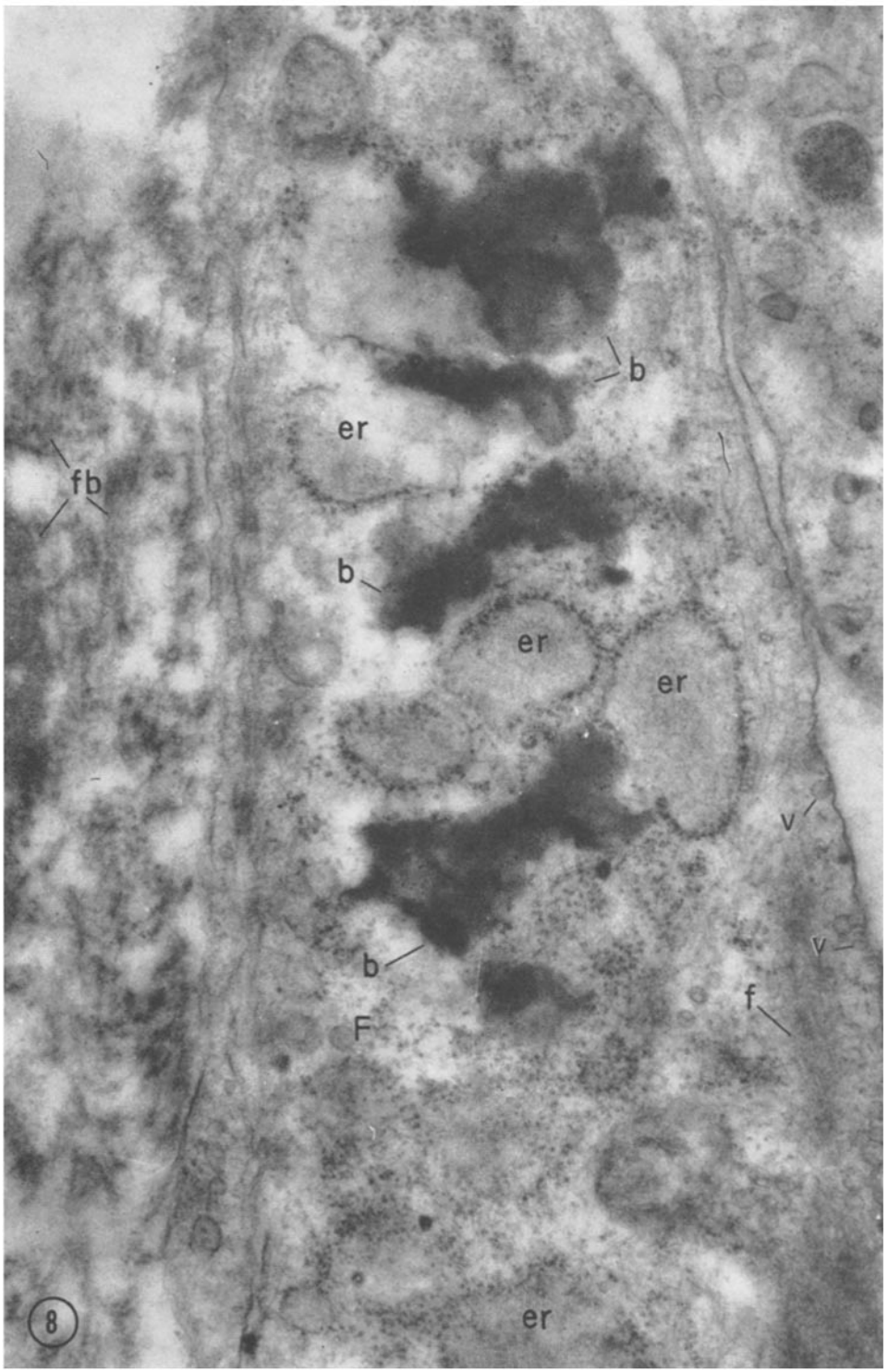
b
er
fb
b
er
er
v
v
b
f
F
8
er

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FIGURE 9

Micrograph of a 14-day wound showing portions of a fibroblast (*F*) and a macrophage (*M*). The fibroblast contains rounded cisternal profiles (*er*), irregular dense bodies (*b*), intracytoplasmic filaments (*f*), and enlarged mitochondria (*m*). The nucleus (*N*) of this cell can be seen at the right edge of the micrograph. The histiocyte contrasts with the fibroblast in its sparsity of endoplasmic reticulum and absence of dense bodies which have been identified as lipid. Numerous vacuoles (*v*), the nucleus (*N*), and portion of the Golgi apparatus (*G*) are present. × 27,000.

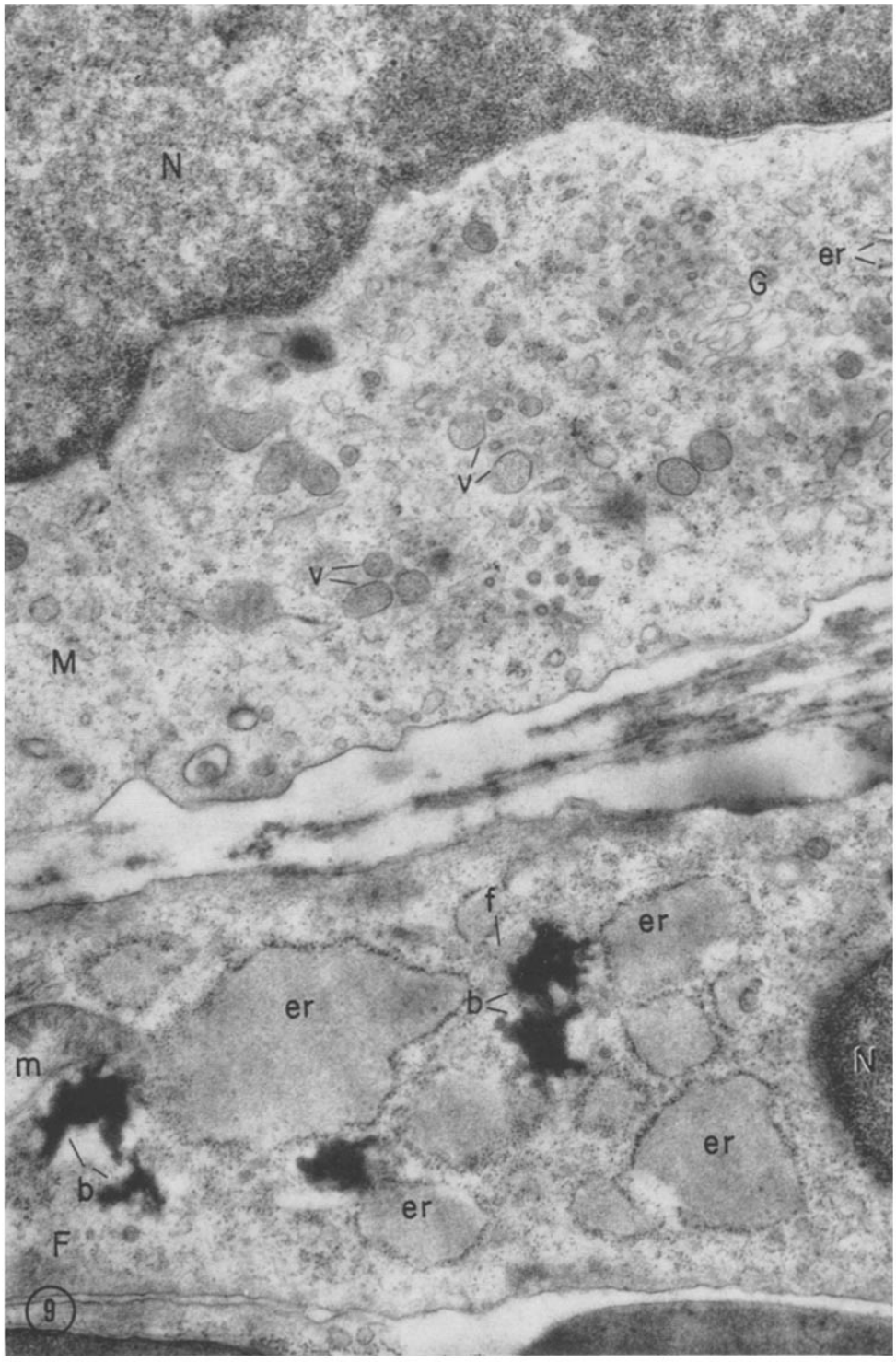
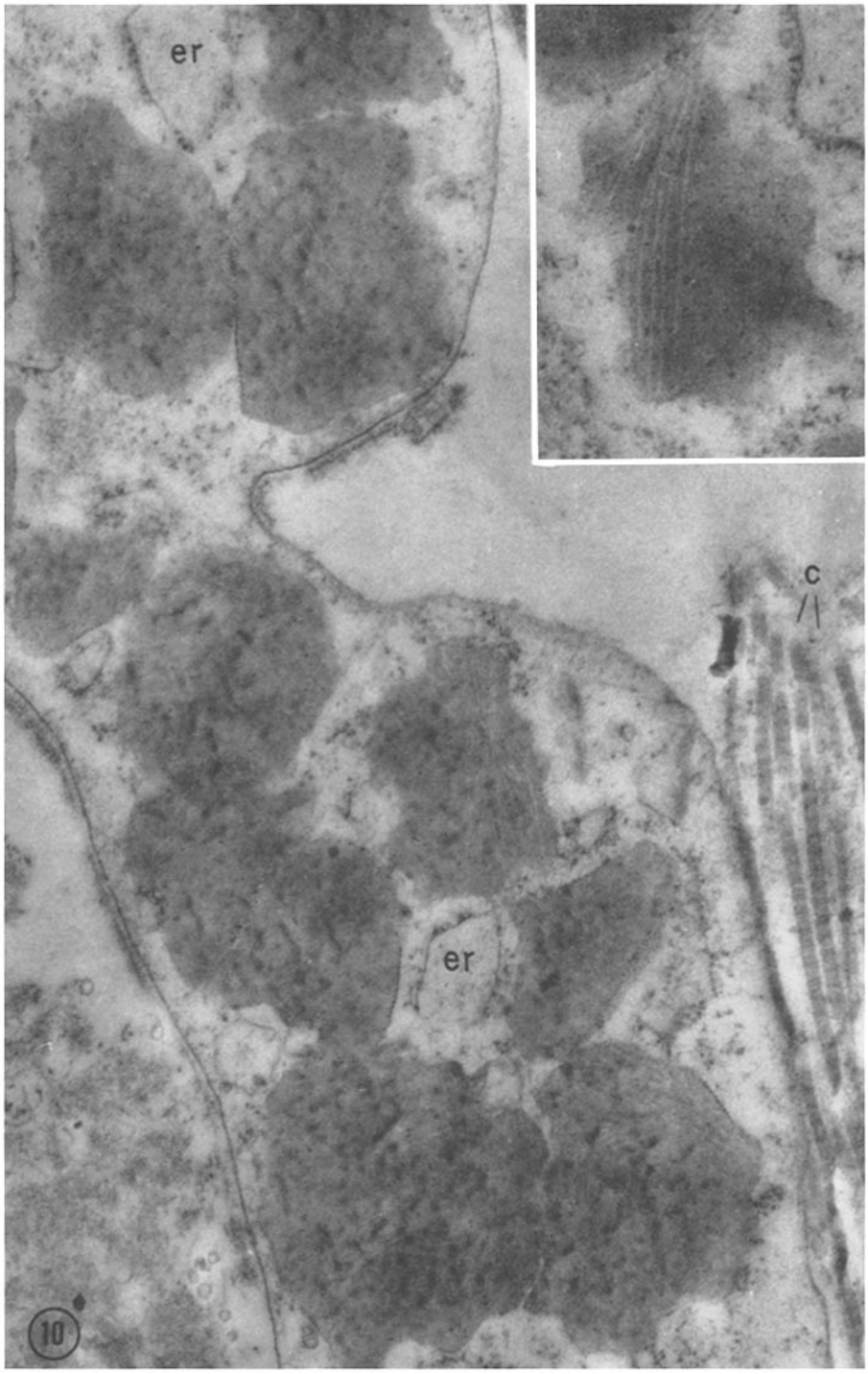

N
er
G
v
v
M
f
er
er
b
m
N
b
er
er
F
9

FIGURE 10

Micrograph of a 5-day wound stained with phosphomolybdic acid. The dense bodies appear to have a beaded border along one edge, and fine, dark, individual particles can be seen in their substance. The rounded profiles of the endoplasmic reticulum (*er*) are apparent as well as a few extracellular collagen fibrils (*c*) near the cell surface. × 40,000. In the insert, the less dense lines in these bodies are evident. Insert, × 56,000.


er
c
er
10