71-12,619

STINSON, Warren William, 1936-

STUDIES ON THE GROWTH, DEVELOPMENT, AND CELLULAR ACTIVITY OF EXPERIMENTALLY INDUCED CONNECTIVE TISSUE COMPLEXES IN THE HUMAN.

The University of Oklahoma, Ph.D., 1971

Anatomy

University Microfilms, A XEROX Company, Ann Arbor, Michigan

THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

STUDIES ON THE GROWTH, DEVELOPMENT, AND CELLULAR ACTIVITY OF EXPERIMENTALLY INDUCED CONNECTIVE TISSUE COMPLEXES IN THE HUMAN

A DISSERTATION

SUBMITTED TO THE GRADUATE FACULTY

in partial fulfillment of the requirements for the

degree of

DOCTOR OF PHILOSOPHY

BY

WARREN WILLIAM STINSON

Oklahoma City, Oklahoma

1970

STUDIES ON THE GROWTH, DEVELOPMENT, AND CELLULAR

ACTIVITY OF EXPERIMENTALLY INDUCED

CONNECTIVE TISSUE COMPLEXES

IN THE HUMAN

APPROVED BY

DISSERTATION COMMITTEE

ACKNOWLEDGMENTS

It is with sincere appreciation that I acknowledge all of those with whom I have been associated during my years of graduate study at the University of Oklahoma Medical Center. Their understanding and assistance has made this work possible.

Dr. Kenneth M. Richter, Department of Anatomical Sciences, has rendered in depth training, guidance, timely assistance, and set an enviable example as an investigator. Through Dr. Richter, the Helen Hay Whitney Foundation and the Lawrence and Jane Edwards Microscopic Anatomy Trust Fund provided the funds and the facilities necessary for this research. For all of these things, I am truly grateful.

Appreciation is extended to Drs. William J. L. Felts, Ernst Lachman, Garman Daron, Kenneth K. Faulkner, and Lawrence G. Gumbreck of the Department of Anatomical Sciences, and Dr. Jacqueline J. Coalson, Department of Pathology, for the interest they have exhibited, knowledge they have shared, and enlightenment they have provided.

The members of the reading committee for this dissertation, Drs. John E. Allison, John F. Lhotka, Robert E. Coalson, Department of Anatomical Sciences, A. L. Dee, Department of Pathology, M. J. Keyl, Department of Physiology, and Paul B. McCay, Department of Biochemistry, deserve special thanks for their time and learned advice.

Acknowledgement is given to Dr. John A. Schilling, Department

of Surgery, for providing the basic study material for this research.

Dr. Philip E. Smith, Associate Dean, Graduate College, is thanked for the personal interest, deep understanding, and willing helpfulness he extends to students.

A note of gratitude is given to Mr. Charles Palmer who has given of his time and expertise in technical assistance.

To my wife, Joni, who has patiently stood beside me during this study, rendered encouragement and understanding, I extend the gratitude of an appreciative husband.

TABLE OF CONTENTS

STUDIES OF THE GROWTH, DEVELOPMENT, AND CELLULAR ACTIVITY OF EXPERIMENTALLY INDUCED CONNECTIVE TISSUE COMPLEXES IN THE HUMAN

CHAPTER I

INTRODUCTION

It is generally accepted that the fibroblast is the cell primarily responsible for the production of ground substance and collagenous fibers in the regeneration and development of the connective tissues proper in the healing wound. Schwann in 1839 and Flemming in 1891 suggested that collagenous fibers were formed within the cell cytoplasm and that fibrillogenesis represented a direct transformation of the cytoplasm (Arey, 1936).

This theory was modified by the works of Hansen (1899), Mall (1902), Mallory (1903), and Studnicka (1903, 1907). These investigations were later upheld by Lewis (1917), and Mallory and Parker (1927). These early investigators suggested that the specific connective tissue cells (fibroblast, chondroblast, or osteoblast) were responsible for the formation of collagenic fibers or the precursory collagenic substance, a concept reiterated by Godman (1961). These investigators further reported that the connective tissue cells responsible for forming the collagenic

material were divided into an endoplasmic region and an exoplasmic region which was suggested to be the area of accumulation of precursory collagenic material. Subsequently overt collagenic fibrous material was formed in the exoplasm which is concurrently converted to an extracellular position where it became the intercellular components of connective tissue, the amorphous ground substance and the collagenic fibrous material.

These reported observations, while suggesting a specific secretory activity, gave rise to the theory that collagenic fibers were formed intracellularly.

In contrast such investigators as Henle in 1841, Kölliker in 1850-1854, Ranvier in 1875, von Ebner in 1896, and Merkel in 1909 were of the opinion that collagen was formed by a sort of crystallization of a semifluid, amorphous, secreted substance in their intercellular space (Arey, 1936). This theory was supported in part by Baitsell (1916, 1917), Alfejew (1926), Maximow (1928), and Wolbach (1933). These investigators observed fibrils forming in the amorphous intercellular material far distant from the cells. They further suggested that the amorphous intercellular material was secreted by the connective tissue cells.

Both of these theories, while being in direct contention, alluded to the broad concept that specific cell contact is required in the process of fibrillogenesis. Investigative observations referable to this concept were reported by Sterns (1940a, 1940b) and reemphasized by Porter (1964) and, Richter and Schilling (1969).

The ultrastructural changes in the cell during fibrillogenesis, the method of synthesis, the morphology of the collagen molecules and its polymerization into the overt collagenic fibril are matters of

current interest and will be discussed in the discussion section of this investigation.

The literature contains many ultrastructural studies on experimental animals concerning inflammation and connective tissue formation. Most of the investigations were executed with laboratory animals such as the rat (Gross, 1950; Wasserman, 1954; Peach, et al., 1961; Fernando and Movat, 1963); the guinea pig (Schilling, et al., 1953b; Chapman, 1961; Ross and Benditt, 1961, 1962a, 1962b), the mouse (Abercrombie, et al., 1957; Karrer, 1958; Silberberg, et al., 1961), and dogs (Schilling, et al., 1959, 1961; Richter, et al., 1964a, 1964b, 1964c, 1966; Richter and Schilling, 1969).

The granulation tissue for study was usually induced by direct incision (Ross and Benditt, 1961, 1962a, 1962b, 1968; Schilling, et al., 1953; Hay and Revel, 1963), by tissue culture methods (Porter and Vanamee, 1949; Jackson, 1953; Abercrombie, et al., 1957), and induction by formation of the carrageenin granuloma (Jackson, 1957; Lowther, et al., 1961; Barton, 1962).

One method of experimental investigation involves the implantation into animals of specially structured mechanical devices. These devices and their implantation provide a sterile dead space which serves as: 1) an inducer of the connective tissue regeneration phase of the healing wound and 2) a substrate for the developing connective tissue complex. Some of the devices used include polyvinyl sponges (Dumphy and Udupa, 1955; Noble and Boucek, 1955; Bollet, et al., 1958; Schilling, et al., 1959), perforated celluloid spheres and methacrylate cylinders (Guyton, 1963), knitted dacron cylinders (Florey, et al., 1962; O'Neal,

1964), and stainless steel and tantalum wire mesh spheres or cylinders (Schilling, et al., 1953a, 1953b; Shetlar, 1959; White, et al., 1959; White, et al., 1961; Richter, et al., 1964c).

The use of the mechanical devices has the advantage of producing standardized highly ordered fibrocollagenous tissue complexes without the interference of epithelization. Further, the separation and characterization of certain cellular and fluid components of the regenerating connective tissue for biochemical, physiological, and morphological studies has been made possible by the use of mechanical devices.

Previous studies by this laboratory have utilized stainless steel wire mesh cylinders implanted in experimental dogs for the purposes of studying blood and lymph vascular components (Schilling, et al., 1959; Schilling, et al., 1961), blood and wound fluid (Richter, et al., 1964c; White, et al., 1959, 1961), extravascular fibrocollagenous tissue components (Schilling, et al., 1959; Schilling, et al., 1961; Richter, et al., 1964a, 1964b; Richter and Schilling, 1969), and the morphologic structure of the tropocollagen molecule (Richter, et al., 1966; Richter and Schilling, 1969; Richter and Schilling, 1970).

In regard to ultrastructural studies of wound repair in man, little information has been available. Hartwell's observations (1955) on mechanisms of healing in man has suggested certain differences. Ultrastructural studies on human wound repair by Ross and Odland (1968), and Odland and Ross (1968) were felt to compare favorably with previous studies on guinea pigs and rats. It should be noted that their model wound was a direct incision with skin plugs removed from the wound.

The purpose of this study is to a) morphologically characterize

fibrocollagenous tissue complexes structured in vivo in human male volunteers with reference to changes during the time course of fibroplasia, b) investigate the fine structural aspects of the extravascular tissue complexes which bear on the problem of collagenic fibrillogenesis, and c) analyze and compare the findings with previously reported morphological data related to the experimental fibrocollagenous tissue complexes structured in vivo in the dog and pertinent findings by other investigators.

CHAPTER II

MATERIALS AND METHODS

Fibrocollagenous tissue complexes were induced in a group of 22 human male volunteers who had received prior physical examinations to determine their state of health and suitability for implantations of specially structured stainless steel wire mesh cylinders. Induction of the fibrocollagenous tissue complexes followed procedures previously described by Schilling, et al., (1959), and Richter and Schilling (1969). The steel mesh cylinders (7.2-7.6 cm long by 1 cm in diameter with closed ends) were fabricated from mesh no. 40, surgical foundation wire mesh which has openings 0.18 mm and 195 openings per square centimeter.

Using local anesthesia and aseptic conditions, two cylinders were implanted in the superficial fascia of the upper right abdominal quadrant of each volunteer. The inducing cylinders were reclained using local anesthesia at intervals of 2, 3, 4, 8, 12, and 16 weeks. The physical examinations and all surgical techniques were performed by Dr. John A. Schilling, Department of Surgery, University of Oklahoma Medical Center.

Upon reclamation, the cylinders were opened longitudinally with wire cutting scissors and the induced tissue complexes were immediately immersed in the appropriate fixatives for light or electron microscopic studies. The tissue was dissected away from the steel mesh during the

first few minutes of fixation.

The collagenous tissue complexes for light microscopy were fixed in 10 per cent neutral buffered formalin solution (pH 7.0). Subsequent washing, dehydration, clearing, paraffin embedment, sectioning, and staining was performed by Dr. Walter Joel's laboratory, Oklahoma Medical Research Foundation. Additional sections were cut and stained by the investigator. The stains employed were Harris's hematoxylin-eosin (Armed Forces Institute of Pathology, 1960), Heidenhain's "Azan" (Lillie, 1954), periodic acid-Schiff (Armed Forces Institute of Pathology, 1960), elastica (Kornhauser, 1952), Gomori's reticular stain (Armed Forces Institute of Pathology, 1960), and Van Gieson's picrofuchsin (Armed Forces Institute of Pathology, 1960). The sections were examined with an AO Spencer research microscope and a Wild M20 research microscope.

A 12-week-old tissue complex was cleared of paraffin with xylene, hydrated through descending alcohol concentrations to water, placed in a 1 per cent aqueous osmium tetroxide solution for three weeks and sectioned free-hand in a transverse plane. The gross structure of the connective tissue complex was studied with an AO Spencer dissecting microscope.

The tissue complexes for electron microscopic study were immediately immersed in cold fixative (5° C), dissected from the steel mesh and minced into 1 mm square pieces. The fixative and fixation time employed was either a 1 per cent osmium tetroxide solution buffered with Sorenson's phosphate buffer (pH 7.4) for 2 hours or a 6.25 per cent gluteraldehyde buffered to pH 7.4 with s-collidine (Bennett and Luft, 1959) for 2 hours and post-fixed with 1 per cent aqueous osmium tetroxide for 1 hour. The tissue was washed in the respective cold buffer for 30

minutes, dehydrated in ascending ethyl alcohol concentrations (35, 50, 80 per cent for 15 minutes each, 95 per cent for 40 minutes and 100 per cent for 1 hour with 3 changes), and infiltrated. Infiltration was accomplished with a 50/50 absolute ethyl alcohol and methacrylate mixture (25 per cent n-ethyl- and 75 per cent n-butyl-methacrylate) for 45 minutes with frequent changes. The mixture was changed to a 100 per cent methacrylate mixture containing 3 per cent benzoyl-peroxide catalyst for 1 hour at room temperature with 3 or more changes. Next, the tissue underwent a prolonged infiltration (12-18 hours) in the methacrylate-catalyst mixture at 5-10° C. Prior to embedment the tissue was returned to room temperature and changed to a fresh methacrylate-catalyst mixture. The infiltrated tissue was placed in Beem plastic capsules and polymerized with U-V light (Tissue to source, 2 cm, temperature 27-35° C) until the tissue embedment was sufficiently firm for cutting.

Precision trimming and thin sectioning (2-3 microns) were accomplished with a Porter-Blum MT-2 microtome. The thin sections were mounted on glass slides, the embedment removed with xylene, hydrated through descending ethyl alcohol concentrations to water, and stained with hematoxylin-eosin or hematoxylin-phloxine. The stained sections were dehydrated through ethyl alcohol solutions, cleared with xylene, and mounted with permount for study with a light microscope.

Ultrathin sections were cut with a Porter-Blum MT-1 microtome. The ultrathin sections were cut onto a water flotation bath and flattened with toluene or xylene vapors. The sections were mounted directly from the flotation bath onto formvar coated specimen screens.

The various stains and stain combinations investigated were

phosphotungstic acid, phosphomolybdic acid, uranyl acetate, and various lead stains. Staining with phosphomolybdic acid was found to be unsatisfactory. The optimum staining with phosphotungstic acid was achieved with a 1 per cent aqueous solution for 1 hour followed by a 30 minute wash in distilled water with 3 changes. The optimum uranyl acetate staining was achieved with a 1 per cent aqueous solution for 5 minutes in a 60° C oven, followed by a 30 minute wash in distilled water with several changes. The lead stains were used in conjunction with uranyl acetate to improve general contrast as suggested by Kay (1965). The lead stains tested were lead hydroxide (Watson, 1958; Dalton and Ziegel, 1960; Karnovsky, 1961), lead tartrate (Millonig, 1961) and lead citrate (Reynolds, 1963; Venable and Coggeshall, 1965). The Venable and Coggeshall lead citrate stain proved very satisfactory. The stain was prepared by dissolving 20 mg of commercial lead citrate (K & K Laboratories) in 10 mls of 0.1 N NaOH. The sections were stained for 45 seconds, and washed for 3 seconds in 0.02 N NaOH followed by a 1 minute wash in distilled water. The ultrathin sections were examined with an RCA EMU-4A electron microscope.

CHAPTER III

OBSERVATIONS

General and Light Microscopic Observations

When the wire mesh cylinders were retrieved and the connective tissue complexes dissected from the mesh, it reflected the long cylindrical shape and size of the induction device. As seen in cross section (Figure 1), an osmicated 12-week-old induced connective tissue complex is cylindrical with a smooth lumen. Further, the osmicated tissue complex exhibits concentric osmiophilic and osmiophobic zones which impart a lamellated appearance to the complex. Additional investigation found the osmiophilic zones to be primarily cellular and the osiophobic zones to be composed predominantly of fibrous material. The tissue complexes after 12 to 16 weeks of growth have a measurable wall thickness of 0.8 mm to 1.5 mm.

The 2-week-old tissue complexes are primarily cellular with a minor amount of fibrous material. In cross sectional profile, the cells around the lumen are oriented with the long axis of the cell parallel with the lumen. The cells more peripherially located exhibit no apparent orientation. The fibrillar material has a random interwoven appearance and exists mainly around the periphery of the cells. The 3-week-old tissue complexes are predominantly cellular; however, in the mid-region of the tissue (between the periphery and the lumen) a zone of

fibrous material has developed. This zone while being primarily fibrous does contain cells which are widely spaced due to the amount of fibrillar material between them. The fibrous zone follows the general shape of the tissue complex, i.e.; a cylindrical zone of fibers coursing parallel to the lumen. By the 4th week of growth (Figure 2) more than one fibrous zone has developed and the fibrous zones are more discretely organized. Between the fibrous zones, cellular zones exist in which the cells are closely packed with a minor amount of fibrillar material deposited between them. The luminal and peripheral aspects of the tissue complexes are descriptively the same in all ages as described for the 2 and 3 week-old complexes. The 8, 12, and 16-week-old tissue complexes are arranged essentially like the 4-week-old tissue complex with the exception of having more fibrous zones developed (compare Figure 1 and Figure 3 to Figure 2). Thus, the older tissue complexes can be divided into 3 regions: a peripheral region which is primarily cellular, a mid-region which consists of alternating cellular and fibrous zones, and a luminal region which is predominantly cellular.

As more fibrous zones are developed with age, the peripheral zone of the tissue complexes diminishes in width. Further, the wider fibrous zones are located more peripherally with a diminution of the measurable width of the fibrous zones which are succeedingly closer to the lumen (Figure 1).

The primary cell type identified in the connective tissue complexes is the fibroblast. Based on cytoplasmic basophilia some of the fibroblasts of the 2-week-old tissue complexes (with the exception of the luminal region) and the fibroblasts in the peripheral region of complexes

of all ages are comparatively less active than the fibroblasts of the cellular zones in the mid-region. The characteristics exhibited include an ellipsoidal nucleus containing one or more nucleoli, an abundant cytoplasm with little or no basophilia and multiple cytoplasmic processes. There is an increased basophilia in fibroblasts of the peripheral zone which lie adjacent to a fibrous zone.

The fibroblasts of the luminal areas exhibit characteristics of a more active nature. They appear as long attenuated bipolar cells with scanty cytoplasm but of a more basophilic nature. The long spindle-shaped nucleus conforms to the shape of the cell and usually contains multiple nucleoli. These luminal cells, when viewed in a plane parallel to the luminal surface appear as large irregularly shaped cells. Their overall configurations would descriptively resemble that of squamous epithelial cells.

In the region of alternating fibrous and cellular zones, of the older tissue complexes (4, 8, 12, and 16-week-old) the fibroblasts present several different configurations. Some of the fibroblasts of the cellular zone resemble the less active cells of the peripheral regions, however most of the cells exhibit a marked increase in basophilia and are considered more active (Figure 4).

The fibroblasts of the fibrous zones may be long attenuated polar cells, or cells in various stages of cell death (Figure 5, Figure 6). The nuclei range from long spindle-shaped nuclei with multiple nucleoli to pycnotic nuclei. Cytoplasmic basophilia is variable from cell to cell; however, it is generally decreased in comparison to cells of the cellular zone. Fragmented remnants of cells are also present in

the fibrous zone. Mitotic figures were not observed in any region of the tissue complex.

Other cell types observed in the connective tissue complexes include eosinophiles, lymphocytes, macrophages, and foreign body giant-cells. The eosinophiles and lymphocytes were generally located near the periphery of the tissue complex.

A particular type of macrophage was found in the peripheral regions of some of the older tissue complexes (8, 12, and 16-week-old). These cells contained a foamy or reticulated, clear staining cytoplasm. The nucleus is oval and contains a single, slightly eccentric nucleolus. These cells are identified as "foamy" histiocytes (Figure 7).

Foreign body giant-cells (Figure 8) were observed in a number of the tissue complexes (3, 8, 12, and 16-week-old). Their occurrence is observed only in the extreme peripheral areas of the tissue complex.

In determining the fibrous component of the tissue complexes, the elastic tissue stain was negative in all ages of development. The reticular stain was positive (Figure 9) and small argyrophilic fibers were observed around the peripheral margins of the fibroblasts. Based on other stains, (Van Gieson's picrofuchsin, Heidenhain's "Azan", and hematoxylin-eosin) the fibrillar material was found to be collagenic.

The periodic acid-Schiff stain applied to the human connective tissue complexes was negative although a false positive was obtained with prolonged oxidation and staining.

In the 3 through 16-week-old tissue complexes cell-lined tissue spaces were observed (Figure 2, Figure 3). The luminal areas presented as clean clear spaces unoccupied by cells or cell products in the light

microscopic preparations. These tissue spaces occur primarily in the predominantly cellular areas, particularly in the peripheral regions of the complexes although occasionally a tissue space is observed in a fibrous zone.

Electron Microscopic Observations

The predominantly cellular areas of the tissue complexes are found to be comprised mainly of healthy fibroblasts (Figure 10) with a minor amount of fibrillar material between the cells. The predominantly fibrous zones are comprised mainly of collagenic fibrous material with a few widely spaced cells and cell remnants in various stages of cytodestruction (Figure 11).

Cell-lined tissue fluid spaces are present in the cellular areas (Figure 12, Figure 13). The cells lining the tissue space or channel are identified as fibroblasts arranged in a cord-like fashion. Structurally, the fibroblasts have a normal, healthy appearance with a wide spectrum of organelle variation (Figure 13).

In the cellular areas, the fibroblasts display a wide spectrum of structural features which may be observed in the peripheral region of the tissue complex and in the alternating cellular zones. The mitochondrial complement (Figure 13, Figure 15) may present as: 1) a vesicular structure with short tubular cristae and fine filamentous structures in an electron-lucent matrix, 2) the matrix may appear dense with long tubular cristae, 3) the cristae may appear granular in a very dense matrix, 4) the mitochondrion may contain a dense granular matrix without demonstrable cristae.

The endoplasmic reticulum may appear as granule studded

membranes encompassing large dilated cisternae (Figure 10) or smaller well developed tubular cisternae which occupy a major portion of the cytoplasm (Figure 14). The ribosomes may be attached to form the rough endoplasmic reticulum or exist free in the cytoplasm.

The Golgi complex may exist as stacked, flattened, smooth membranes (Figure 13) or as small membraneous vesicles (Figure 15). The Golgi complex does not occur as a prominent feature in the fibroblasts studied.

The cytoplasmic matrix or hyaloplasm is of variable density. It may occur as a homogenous matrix which is relatively electron-lucent or the hyaloplasm may be an electron-dense condensation in the cortical regions of the cell next to the plasma membrane (Figure 14).

The cytoplasm contains a fine filamentous or fibrous network (Figure 22, Figure 23). These filamentous fibers measure from 50 Å to 90 Å in diameter. They are found distributed throughout the cytoplasm, forming a cytoskeleton. Condensations of these cytoskeletal fibers were observed in the cortical regions of the cell (Figure 15). In areas of the membraneous structures of the cell such as the nuclear membrane, the plasma membrane or the endoplasmic reticulum, the fibers appear to blend with or form a part of the membranes.

The nuclei of the fibroblasts are also variable in structure. The nuclear membranes generally appear as a normal double membrane (inner and outer) delimiting the nucleoplasm (Figure 15). There is a wide variation in the concentration and density of the chromatin material around the nuclear membrane and in the nucleoplasm (Figure 14, Figure 15).

Within the nucleus, spherical structures of varying size, numbers, and ultrastructural detail were observed (Figure 14, and Figures 16 through 21). These structures were identified as nuclear bodies. As many as four have been identified in any one nucleus. Their frequency of distribution indicated they are a normal complement of the fibroblast nucleus. The size range of the nuclear bodies measured was from 393 Å to 6,075 Å. The nuclear bodies are comprised of small filamentous structures which are arranged in a spherical or circular pattern. The filaments measured from 32.0 Å to 75.1 Å in diameter. Granules of various sizes were found in the center of some of the nuclear bodies (Figure 18, Figure 19). The size range of the granules measured was from 78 Å to 475 Å. The center of some of the nuclear bodies contained one large dense structure which is made of a condensation of smaller granules (Figure 20). One nuclear body observed was in a nucleus of the condensed chromatin type. There was a decreased affinity for stain; however, the filaments and granules were still recognizable. Nuclear bodies generally were not observed in the nucleus of cells undergoing cytodestruction.

The formation of collagenic fibrils was observed in relation to a spectrum of changes occurring at or near the surface of fibroblasts of the cellular zones. The changes involve the plasma membrane, the hyaloplasm, and the fibers of the cytoskeleton. The various changes may occur simultaneously within localized areas of the individual cell. For purposes of description, the cyclic changes are divided into four phases. The designations of these phases (a_1 through a_4) have been previously described by Richter and Schilling (1969) who studied fibrillogenesis in induced connective tissue complexes in dogs.

In the first phase (a_1) the plasma membrane appears as a normal intact membrane. The cortical hyaloplasm is of minimal thickness and the fibrils of the cytoskeleton are randomly dispersed (Figure 14, Figure 15).

The second phase (a_2) is denoted by the accumulation of an electron-dense cortical hyaloplasm and its subsequent hypertrophy. Within the cortical hyaloplasm, the fibers of the cytoskeleton may be condensed and course parallel to the plasma membrane which is still intact (Figure 14, Figure 15).

The third phase (a_3) is characterized by the disruption of the plasma membrane and the formation of overt collagenic fibrils within the electron-dense cortical hyaloplasm (Figure 14, Figure 15). The electron-dense hyaloplasm blends into the intercellular matrix locally and the collagenic fibrils forming within it. The fibers of the cytoskeleton, while discernible as separate entities from the collagenic fibrils, are in close apposition to and are ordered parallel with the long axis of the collagenic fibrils (Figure 22).

Subsequently, in the fourth phase (a_4), the plasma membrane is reformed behind the area of cortical condensation leaving the dense hyaloplasm, cytoskeletal fibers, and the overt collagenic fibrils in an intercellular position (Figure 14). The reformation of the plasma membrane occurs at the junction of the endoplasmic and cortical zone.

Contributions of collagenic fibrils to the intercellular areas in the previous manner is an apocrine-like secretory process. The condensation of the hyaloplasm and cytoskeletal fibers establishes a region of organizing collagenic fibrils. With the disruption of the plasma membrane, the region of organizing collagenic fibrils blends with the

homologous regions of adjacent cells in such a manner that a common region is established in which the individual contributions of each cell is not discernible (Figure 15). The deposition of collagenic fibrils and the concomitant loss of cytoplasm separates the distance between the adjacent cells.

Collectively, the newly formed collagenic fibrils in the region of organizing collagenic fibrils around the periphery of the cell corresponds to the argyrophilic fibers observed at the light microscopic level (compare Figure 14 to Figure 9). Further, the blending of collagenic fibrils in a common region establishes the collagen fiber discernible at the light microscopic level.

The fibroblasts and cell processes located in fibrous zones follow a different recognizable secretory cycle. The fibroblasts undergo an orderly pattern of regression or cytodestruction which is interpreted to be a holocrine-like secretory process. The descriptive phase designations (h_1 through h_3) have been previously described by Richter and Schilling (1969).

In the first phase (h_1) of holocrine-like deterioration (Figure 24), the nucleus is reduced in size. The outer nuclear membrane is only partially discernible and chromatin material is condensed around the periphery of the nucleus. The mitochondria are usually reduced in size and number. While a wide spectrum of variation has been noted, the mitochondria generally have long tubular cristae which may or may not be granular in a matrix of varying density or the entire mitochondrion is a dense granular structure with no recognizable cristae. The endoplasmic reticulum occurs as ill-defined membranes which are reduced in amount.

They are generally granule-studded and may occur as vesicles or fragments. The ribosomes are either attached to the endoplasmic reticulum or freely dispersed in the cytoplasm. The Golgi complex is infrequently seen; however, when observed it occurs as small vesicles.

The plasma membrane is well defined and an electron-dense cortical hyaloplasmic zone lies adjacent to the plasma membrane. The fibrils of the cytoskeleton may be masked due to the density of the hyaloplasm. This phase of holocrine-like deterioration descriptively resembles the cell surface changes in the a_2 phase of apocrine-like deposition. The greatly hypertrophied hyaloplasm and diminutive endoplasm provide the major exceptions.

With the disintegration of the plasma membrane, the second phase (h_2) commences (Figure 24, Figure 25). Collagenic fibrils form in the cortical condensation in the same manner as described in the apocrine-like secretory process, except the plasma membrane is not re-established as in the a_4 phase of apocrine-like secretion.

The cells of the second holocrine-like deterioration phase (h_2) have virtually no plasma membrane. The cell surface is now represented by an electron-dense hyaloplasm containing organizing collagenic fibrils and the associated cytoskeletal fibrils (Figure 23, Figure 25). The electron-dense hyaloplasm is greatly hypertrophied and may extend from the arbitrary cell surface to juxtanuclear positions.

The fibrils of the cytoskeleton are present but as noted in the h_1 phase, may not be discernible due to the density of the electron-dense cortical hyaloplasm. Remnants of the endoplasmic reticulum appear as scattered fragments. The ribosomes may be attached to these fragments

forming a rough endoplasmic reticulum, or more commonly are found free in the cytoplasm. The mitochondria are greatly reduced in number, and contain internally long granular cristae in a dense matrix or a totally granular matrix with no discernible cristae. The outer nuclear membrane may occasionally be present. The nucleus is either of the condensed chromatin type (Figure 25) or slightly pycnotic.

Due to the continued elaboration of collagenic material and regression of the cytoplasmic matrix, the fibroblasts occupying positions at the junction between adjacent cellular and fibrous zones are further removed from adjacent cells. These cells are now deeper in a fibrous zone which they have created around themselves.

With continued holocrine-like deposition of collagen, the cells enter the terminal phase (h_3) of regression and cytodestruction (Figure 11, Figure 26). In the final phase (h_3) of cytodestruction, the nucleus of the fibroblast is reduced in size, and the chromatin material is condensed throughout the nucleus (pycnosis). Progressive cytodestruction continues until the nuclear material fragments (karyorrhexis) leaving only nuclear debris. An occasional remnant of the outer nuclear membrane may be present; however, by this stage of wasting, the nuclear membrane has usually dissipated.

The endoplasmic reticulum and Golgi complex have usually deteriorated to the point that no recognizable fragments are observable. The only observable structures in the cytoplasm are the highly modified, dense, granular mitochondria and free ribosomes. These structures eventually disappear with continued wasting.

Except for an occasional remnant, the plasma membrane has

deteriorated. The perimeter of the cytoplasm of the h_2 phase is marked by a greatly hypertrophied cortical condensation consisting of the electron-dense cortical hyaloplasm, condensed fibrils of the cytoskeleton, and organizing collagenic fibrils. Within the condensed cortex in the h_3 phase, the electron-dense hyaloplasm comes to reside in a perinuclear position. The cytoplasm is now represented by insular-like cytoplasmic extensions among the collagenic fibrils and the small amount of perinuclear electron-dense hyaloplasm (Figure 26, Figure 27). The insular-like cytoplasmic extensions now occupy areas between the regions of organizing collagenic fibrils. The condensed hyaloplasm and cytoskeletal fibrils of the cells and cell extensions are gradational with the interfibrillar matrix. Collagenic fibrils are observed to form at both the surface of the intracellular hyaloplasm and in the interfibrillar matrix which was derived from the cortical hyaloplasm.

The fibroblasts undergoing holocrine-like deterioration contributed the major amount of fibrous material formed in the tissue complex. This is readily demonstrable by comparing Figures 14 and 15 to Figures 25 and 26. The cells in apocrine-like phases of collagenic fibril formation do so at localized areas of the cell surface. The amount of cytoplasm utilized in apocrine-like deposition is minor compared to that utilized in holocrine-like deterioration where fibroblasts contribute almost all of their cytoplasmic mass to the formation of collagenic fibrils and the interfibrillar matrix.

As described in the apocrine-like phases, the individual regions of organizing collagenic fibrils in holocrine-like deterioration blend to form a common region of organizing collagenic fibrils. With

differentiation of the fibrils, the common region is represented by the collagenic fiber discernible at the light microscopic level (compare Figure 27 to Figure 5).

Long cell processes (Figure 28, Figure 29) extending into the fibrous zone may show all changes of the holocrine-like deterioration phases h_1 through h_3. The cytoplasmic matrix consists totally of the electron-dense cortical condensation and contains organelles in a wide variety of changes generally associated with holocrine-like deterioration phases.

Within the hyaloplasm of cell processes and cells associated with the holocrine-like secretory phases, electron-dense structures occur (Figure 29). In terms of density and size, two types of structures can be distinguished. One type consists of large, very electron-dense, spherical bodies occurring in a random distribution. The second type is smaller, less electron-dense spherical bodies which usually appear in ordered rows. Neither of the two are associated with observable membranous structures. High resolution micrographs (Figures 30 through 32) indicate that the larger structures are precursors or aggregates of the smaller structures. The size range of the smaller structures is from 129.2 Å to 252.5 Å with a mean value of 163.0 Å. The large dense structures are quite variable in size and have measured as large as 402.4 Å.

Within the structure of the dense bodies, corpuscular units were observed. The corpuscular units were further comprised of a cable or rope-like structure in a coiled configuration to form the corpuscular units. The cable-like structure has a measurable diameter range of 24.2 Å to 28.1 Å with a mean value of 24.9 Å. The simple corpuscular

units possessing only one coil (2 cable diameters) around a central axis measure from 55.0 Å to 69.7 Å with a mean value of 64.3 Å. The more complex corpuscular units consisting of more than two cable coils measure from 78.6 Å to 129.2 Å with a mean value of 109.4 Å.

A section through the long axis of one corpuscular unit (Figure 32) indicates the structure to be pear-shaped. The narrow segment of the pear-shaped unit is 106 Å long. Its cross-sectional diameter corresponds to the small measurable corpuscular units. The bulbous portion of the pear is approximately 140 Å long and corresponds in cross-section to the larger corpuscular units. The total length of the pear-shaped unit is approximately 246 Å. Due to superimposition and various planes of cut, the pear-shaped units are rarely discernible in sections through the long axis.

The collagenic fibrils forming within the hyaloplasm (inter or intracellular) appear to be undifferentiated. The periodicity measurement is smaller than that of the differentiated fibrils. The average periodicity measurement of the undifferentiated collagenic fibrils is 490.1 Å (Figure 33, Figure 34). The conventional bands (a, b, c, d, and e) within the macroperiod are not readily discernible.

Corpuscular units comprised of an internal cable-like structure are present in the undifferentiated collagenic fibril (Figure 34). The morphologic description and mean value measurements are essentially the same as that described for the electron-dense structures of the cortical hyaloplasm of cells in holocrine-like deterioration.

The differentiated collagenic fibrils in the fibrous zones of the human connective tissue complex have a mean periodicity of 553.0 Å

(Figure 35) with the conventional a, b, c, d, and e bands within the structure of the macroperiod. The periodicity measurement is larger for the differentiated fibrils than that measured for the undifferentiated fibrils.

Corpuscular units are observed within the structure of the fibril. The units exhibit a wide range of measurable distribution, from 52.2 Å to 132.8 Å. The corpuscular units have an internal structure consisting of a cable or rope-like structure which is coiled around a central axis to form the corpuscular units. The cable-like structure exhibited a mean diameter of 23.1 Å. The simplest corpuscular unit is comprised of a cable in one simple coil around a simple axis such that the measurable corpuscular unit consists of 2 cable diameters plus the electron-lucent central axis. These units measured from 52.2 Å to 71.2 Å with a mean value of 61.6 Å. The more complex units contain more than two cable diameters (more than two coils). These units measure from 84.6 Å to 132.9 Å with a mean value of 107.0 Å.

On examining the interfibrillar matrix in areas of fully differentiated collagenic fibrils it was noted that the fibers of the cytoskeleton were generally reduced in number or not observed at all.

The collagenic fibrils of the human connective tissue complexes did not stain as readily as the collagenic fibers of connective tissue samples from dogs or rats. The most suitable stain was a 1 per cent aqueous phosphotungstic acid solution which at best gave very little contrast to the human collagenic fibrils.

CHAPTER IV

DISCUSSION

General Shape and Ordering

The human fibrocollagenous tissue complex upon reclamation reflected the size and shape of the sterile dead space imposed upon the superficial fascia by the induction device. The connective tissue complex presented a lamellated pattern similar to the fibrous or outer periostinum and the dura mater (Knese and Knoop, 1961b). Similar results have been reported using identical induction devices implanted in the panniculus carnosus of experimental dogs' backs (Schilling, et al., 1959; Schilling, et al., 1961; Richter, et al., 1964a; Richter, et al., 1964b; Richter and Schilling, 1969). Comparable patterns were produced by tissue complexes developing in other types of induction devices such as perforated methacrylate tubes and celluloid balls (Guyton, 1963), on the luminal surface of dacron cylinders used as aortic grafts (Flory, et al., 1962), or dacron cylinders suspended in the lumen of the aorta (O'Neal, et al., 1964).

The above data indicate that the shape of the fibrocollagenous tissue complex is regulated by the dimensions of the induction device. The induction device and its implantation would serve as: 1) an irritant initiating an inflammatory reaction, and 2) a substrate for the peripherally invading connective tissue cells.

The factors perpetrating the lamellated ordering of the alternating fibrous and cellular zones is at present an incompletely understood process. The lamellated pattern is not peculiar to hollow induction devices. Dr. J. Keyl and Dr. R. Bell (unpublished observations), Department of Physiology, University of Oklahoma Medical Center, created a lesion by inserting a balsawood splinter into the renal cortex of an experimental dog. The resulting fibrocollagenous tissue developed in lamellated cones parallel to the long axis of the balsawood splinter with the splinter serving as the central axis of the cones.

Other investigators have reported lamellations or have described the lamellated ordering using methods other than mechanical induction devices (Weiss and Ferris, 1956; Knese and Knoop, 1961b; Movat and Fernando, 1962; Hay and Revel, 1963).

The specific cellular and fibrous features characterizing the lamellations per se correlated directly to the structural and functional changes in the fibroblast during the synthesis of precursory collagenic material, its accumulation in the cortical regions of the cell and the organization and deposition in situ of overt collagenic fibrils.

Vascularity

The human connective tissue complexes are essentially avascular as are the previously reported dog connective tissue complexes. Other investigators employing different induction devices observed vascularization of connective tissue complexes. Guyton (1963) observed small recurrent vessels located peripherally in tissue induced in celluloid balls. The perforation size used was 1.0 mm with approximately 200 holes per ball. Florey, et al., (1962) using knitted dacron cylinders of

unknown hole size observed vascularization extending to a point near the lumen of the developing tissue complex. At present, it appears that the factors dealing with vascularization or the lack of it may not be referable to the number or size of perforations in the induction device (Richter and Schilling, 1969).

In view of the avascularity of the tissue complex, cell nutrient supplies are probably dependent on properties such as hydrostatic pressures, diffusion gradients, and osmotic pressures. Since the human tissue complex develops as a closed system, diffusion through the interstitial spaces would be a slow process. The cells more peripherally located would, by position, have the advantage in securing nutrient materials needed for specific activity. This would account for the formation of the larger fibrous zones located near the lumen.

Cell-lined Tissue Fluid Spaces

The cell-lined tissue fluid spaces occurring in the peripheral regions of the tissue complex have been observed in the dog connective tissue complex (Schilling, et al., 1959; Schilling, et al., 1961; Richter, et al., 1964a, 1964b; Richter and Schilling, 1969). At present the processes involved in the development or the function of these cord-like columns of cells are unknown. The structures seem to correlate to the small closed columns of endothelial cells and fibroblasts reported by Wolbach (1933) while studying fibrillogenesis in normal and scorbutic guinea pigs at the light microscopic level. The author reiterates that the cells with an endothelial appearance at the light microscopic level in this study are identified as fibroblasts at the ultrastructural level.

Fibrous Components

In establishing the fibrous component of the human connective tissue complex, collagenic fibers were the only demonstrable fibers present. Elastic fibers could not be demonstrated by either differential staining at the light microscopic level or by direct observation at the electron microscopic level.

An argyrophilic component was observed and interpreted to be immature collagenic fibrils (Figure 9, Figure 14). Argyrophilia has been reported to characterize both immature collagenic fibrils and reticular fibrils (Foot, 1925, 1927; Mallory and Parker, 1927; Maximow, 1928; Wolbach, 1933; Williams, 1957; Bairati, et al., 1964). The chemical nature has been reported to be the same for collagen and reticulin (Robb-Smith, 1945; Tomlin, 1953; Berrens and Vanadriel, 1962). Fibril argyrophilia has been reported to be associated with small immature collagenic fibrils which are acid or neutral salt soluable (Harkness, et al., 1954; Windrum, et al., 1955; Jackson, 1957). These small thin collagenic fibrils are prevalent in developing connective tissue (Gross, 1950) and Dumphy and Udupa (1955) have shown that with an increase of demonstrable differentiated collagen, there is a subsequent decrease in argyrophilic fibrils. Argyrophilic fibers have been observed and reported in the fibrocollagenous tissue complex induced in the dog (Schilling, et al., 1961, Richter and Schilling, 1969).

The reported observations concerning fibril argyrophilia and electron microscopic observations are consistent with the author's observations. The small argyrophilic fibers at the periphery of the cell cytoplasm in Figure 9 are referable to the undifferentiated collagenic

fiber on the periphery of the cell in Figure 14. As the distance from the cell periphery widens, the fibers differentiate and loose their argyrophilic character.

The negative periodic acid Schiff (PAS) stain reaction is incompletely understood. A positive PAS reaction was reported by Schilling, et al., (1959), Schilling, et al., (1961), and Richter and Schilling (1969). At present biochemical assays are being performed on the human connective tissue complex. While the data is not complete, the presence of acid and neutral mucopolysaccharides is indicated. A loss of mucopolysaccharides or the chemical masking of their reacting groups is suggested.

Cell Types Observed

Fibroblasts in the Cellular Areas

The predominant cell type of the human connective tissue complex is the fibroblast. In the cellular zones the fibroblasts are generally of a normal healthy active appearance (Figures 4, 10, 14). Their structural features conform to those generally described for this cell in various experimental animals (Hansen, 1899; Mall, 1901-1902; Mallory, 1903-1904; Karrer, 1958; Yardley, et al., 1960; Peach, et al., 1961; Barton, 1962; Chapmen, 1962; Porter, 1964; Biarati, et al., 1964), in the fibrocollagenous tissue complex induced in experimental dogs (Schilling, et al., 1959; Schilling, et al., 1961; Richter, et al., 1964a, 1964b; Richter and Schilling, 1969), and in the human subject (Ross and Odland, 1968).

The fibroblasts of the cellular areas are observed to exhibit

apocrine-like cyclic phases of fibrillogenesis. The apocrine-like phases of fibrillogenesis have been observed in the fibroblast by other investigators (Porter and Pappas, 1959; Yardley, et al., 1960; Godman and Porter, 1960; Chapman, 1961, 1962; Richter, et al., 1964a, 1964b; Richter and Schilling, 1969) or are directly observable in recorded electron micrographs of other investigators (Porter and Vanamee, 1949; Jackson and Smith, 1957; Bradbury and Meek, 1958; Knese and Knoop, 1961a; Peach, et al., 1961; Ross and Benditt, 1961, 1962; Hay, 1961; Revel and Hay, 1963b; Ross and Odland, 1968; and in many other micrographs in the literature). It is the author's observation that in almost every report in the literature utilizing ultrastructural studies on the development of fibrous connective tissue in the healing wound or cells concerned with fibrillogenesis, some phase of the apocrine-like secretory process is observed.

Fibroblasts in the Fibrous Zones

The fibroblasts located in the fibrous zones (Figures 11, 24, 25, 26) are characterisitc of cells undergoing cytodestruction or wasting, and as observed, contribute the major amount of fibrillar material to the fibrocollagenous tissue complex in a holocrine-like deterioration (Richter, 1969). This final holocrine-like deterioration of the fibroblast in fibrillogenesis has been suggested (Wasserman, 1954; Knese and Knoop, 1961b; Porter, 1964; Richter, et al., 1964a, 1964b; Richter and Schilling, 1969), and is observable in recorded electron micrographs (Jackson and Smith, 1957; Hay, 1958; Zelander, 1959; Silberberg, et al., 1961; Peach, et al., 1961; Barton, 1962; Movatt and Fernando, 1962).

Fibrillogenesis

As mentioned previously in the introduction, the early investigators (Hansen, 1899; Mall, 1901-1902; Mallory, 1903-1904; Studnicka, 1903, 1907; Lewis, 1917) using light microscopes, differential connective tissue stains, and simple biochemical extraction methods described fibrillogenesis to be related to specific cell changes. The visualized changes, primarily described by Hansen (1899), have been summarized and reiterated by Richter (1969). The gist of this is as follows: It was visualized that the source of collagenic fibrils or precursory fibrillar material is the specific connective tissue cell (fibroblast, osteoblast, or chondroblast). The specific collagen producing cell is internally ordered into an endoplasmic mass and an exoplasmic mass which is of variable thickness from area to area within the cell or may be absent within various areas of the same cell. Overt collagenic fibrils form in the cortical exoplasm from precursory collagenic material with the exoplasm being concurrently converted into the intercellular substance, including the ground substance, and the overt collagenic fibrils. This sequence is followed by the continual transformation of endoplasm. At this point, the cell may repeat the sequence or the cell may enter the holocrine-like deterioration phases. The endoplasm as well as the exoplasm begins to degenerate releasing chondromucoids, albuminoids, or converting directly into that which is destined to become the intercellular substance including the ground substance and overt collagenic fibrils. The degenerating cells are eventually reduced to cytoplasmic remnants between the fibrils and small nuclear masses which gradually waste away. The fibrils widely separated from the decorticated cells undergoing

cytodestruction continue to develop and differentiate without the specific conversion of endoplasm into exoplasm.

On examining the above sequence of events, it is noted that fibrillogenesis is a continuous developmental process leading from fibroblastic differentiation to cytodestruction. Further, the apocrine-like phases of fibrillogenesis blend into holocrine-like deterioration without a break in continuity. It is noted that Figures 10, 14, and 15 are descriptive of fibroblasts in apocrine-like fibrillogenesis and Figures 11, 24, 25, 26, and 27 are indicative of the final holocrine-like deterioration role of the fibroblast.

Other Cell Types Present

While the predominant cell of the human fibrocollagenous tissue complex is the fibroblast, other cell types such as lymphocytes, eosinophiles, foamy histiocytes, and foreign body giant cells were observed in the peripheral region of the tissue complexes. These cells were not observed in the identical fibrocollagenous tissue model induced in the dog (Schilling, et al., 1959; Schilling, et al., 1961).

The occurrence of these cells suggests a condition of chronic inflammation (Muir, 1964). In review and comparison of implantation procedures concerning the human and the dog, the induction device was implanted in the panniculus carnosus of the dog's back. In this position the animal was not likely to disturb the implanted device or the immediate area of skin covering the device. In the human volunteers, the induction devices were implanted in the superficial fascia of the upper right abdominal quadrant. In this position, the induction device is subject to disturbance due to 1) normal everyday movements which require

a bending or stretching of the trunk region, 2) the wearing of items of clothing such as a belt, or 3) the inadvertent scratching or rubbing of an area of irritation by the experimental subject. Further, these cells may not have been observed in the dog connective tissue complexes since primary emphasis was placed on the high degree of lamellated ordering in the mid-region rather than the peripheral region near the internal surface of the inducing wire.

Fibroblast Fine Structure

Nuclear Bodies

Small ordered structures within the nucleus termed nuclear bodies by Weber and Frommes (1963) have been reported in various animal, plant, and insect nuclei (Weber and Frommes, 1963; Nicander, 1964; Stevens, 1965; Lafontaine, 1965; Brooks and Siegel, 1967; Bouteille, et al., 1967; Krishan, et al., 1967; Popoff and Stewart, 1968). Nuclear bodies were reported in thymus gland fibroblasts by Henry and Petts (1969).

Based on histochemical studies and enzymatic digestion, nuclear bodies do not contain DNA or RNA but may be composed of proteins and histones (Nicander, 1964; Krishan, et al., 1967). The functional significance of the nuclear bodies is unknown; however, they are suggested to be associated with nuclear hyperactivity and protein synthesis (Popoff and Stewart, 1968; Bouteille, 1967; Henry and Petts, 1969).

The nuclear bodies (Figure 16 through Figure 21) in fibroblasts of the human connective tissue complexes correspond to the Type I, Type II, and Type III nuclear bodies described by Bouteille, et al., (1967),

the Type I and Type II nuclear bodies described by Popoff and Stewart (1968), and the simple type nuclear bodies described by Henry and Petts (1969).

The fibroblasts in apocrine-like phases of fibrillogenesis and the less active fibroblasts of the peripheral region have a high frequency of observed nuclear bodies such that the author is of the opinion that they are a normal nuclear complement of these cells. Cells in holocrine-like deterioration rarely have observable nuclear bodies. After reviewing the literature, this investigation represents the second reported observation of nuclear bodies occurring in fibroblasts.

Mitochondria

The mitochondrial changes observed were highly variable. These changes were noted by Richter and Schilling (1969) and a similar variation was observed in secretory cells other than fibroblasts (Dugan, 1970). The variation in the fine structure of the mitochondria is incompletely understood. It does not seem to relate to the cell secretory cycle uniformily but seems to relate to the individual activity of each mitochondrion independently.

Cytoplasmic Fibers

Fine fibers were observed in the general fibroblast cytoplasm, in the hypertrophied cell cortex and associated with collagenic fibrils in various stages of differentiation.

These fine cytoplasmic fibers comprising a cortical cytoskeleton (Figure 22 and Figure 23) are visualized by the author as being separate structural entities from the collagenic fibrils to which they are in

close contact. The origin, function, and fate of these fibers is unknown. As such, their presence is simply recorded. This approach is shared by other investigators (Wasserman, 1954; Jackson, 1957; Zelander, 1959; Yardley, 1961; Lowther, et al., 1961; Peach, et al., 1961; Chapman, 1961; Knese and Knoop, 1961a; Ross and Benditt, 1961, 1962a; Chapman, 1961, 1962; Fernando and Movat, 1963; Karasaki, 1964; Ross and Odland, 1968). The cytoskeletal fibers have been suggested by some investigators to be primary or protofibrils upon or around which tropocollagen molecules polymerize to form the overt collagenic fibril (Porter and Vanamee, 1949; Peters, 1956; Porter and Pappas, 1959; Godman and Porter, 1960; Silberberg, et al., 1961; Porter, 1964). While the close relationship of the cytoskeletal fibers suggests a possible function as an organizer or initiator of polymerization, the cytoskeletal fibers exist and maintain a close relationship to the differentiated fibril as well as the undifferentiated fibril. Other investigators relate the cytoskeletal fibers to a cytoskeleton or cytoplasmic reticulum (Wasserman, 1954; Peters, 1956; Hay, 1961; Revel and Hay, 1963a; Richter, et al., 1964a, 1964b; Richter and Schilling, 1969; Fernando, et al., 1964).

The cytoskeletal fibers in the cytoplasm (Figure 21) do bear an extremely close relationship to nuclear membranes, membranes of the endoplasmic reticulum, and the plasma membrane. Richter and Schilling (1969) have suggested that the fibrous cytoskeleton may be continuous with or comprise a part of the plasma and nuclear membranes. The fiber's orientation with the plasma membrane lends evidence to their existence as a cytoskeleton. Due to the cytoskeletal fiber's staining qualities they may be made of a structural protein. Further, their ability to exist

both intra- and intercellularly and their observed existence from condensation in the cortex to their continued association with collagenic fibrils through differentiation of the native fibril alludes to the stability of these fibers.

In considering the cytoskeletal fibers as a whole, they are composed of a relatively stable substance, probably protein. Their ordered condensation in the cortical hyaloplasm would maintain or give support to the structural stability of the membranous components of the cell, particularly the cell surface. Further, with the loss of the plasma membrane during the various phases of fibrillogenesis the condensed cortical cytoskeletal fibers and the electron-dense hyaloplasm would possibly serve as a semi-permeable membrane maintaining the cell's integrity. The close apposition to the organizing and fully differentiated collagenic fibrils would suggest a possible role as an organizer or initiator of polymerization of the tropocollagen units; however, there is no evidence that the cytoskeletal fibrils are incorporated into the collagenic fibril.

Endoplasmic Reticulum and Ribosomes

The active fibroblasts of the cellular zone are described as having an extensive rough endoplasmic reticulum (Figure 14). The basophilia observed at the light microscopic level and the well developed rough endoplasmic reticulum are suggested to be related, and indicative of protein synthesis (Porter, 1953; Porter, 1954; Jackson, 1957; Karasaki, 1964; Louisot, et al., 1967). Specifically, in the fibroblast the rough endoplasmic reticulum or the polysome formations are suggested to be related primarily to the synthesis of collagenic material (Karrer,

1960; Knese and Knoop, 1961a; Lowther, et al., 1961; Revel and Hay, 1963b; Goldberg and Green, 1964; Manner, et al., 1967; Papaconstantinou, 1967; Welsh and Meyer, 1967).

The structure of the endoplasmic reticulum is variable and within the limits of the same cell. The variation noted ranges from a well developed rough endoplasmic reticulum to free ribosomes with the endoplasmic reticulum occurring as small vesicles with or without attached ribosomes. As the density of the hyaloplasm changes, the character of the endoplasmic reticulum also changes. This is readily demonstrated by comparing the nature of the endoplasmic reticulum in the endoplasmic mass to the endoplasmic reticulum or its remnants thereof in an area of hypertrophied cortical hyaloplasm. Various other investigators have observed this variation in structure on the concurrent changes of the hyaloplasm and the endoplasmic reticulum (Yardley, 1961; Moore and Schenberg, 1960; Chapman, 1961; Hay, 1961; Peach, et al., 1961; Ross and Benditt, 1961, 1962a, 1962b; Knese and Knoop, 1961a; Goldberg and Green, 1964; Ross and Odland, 1968; Richter and Schilling, 1969).

Based on electron microscopic autoradiographic studies (Revel and Hay, 1963a; Ross, 1965) precursory collagenic material was synthesized by the endoplasmic reticulum and subsequently attached to the endoplasmic reticulum and Golgi apparatus. Several investigators have reported accumulations of precursory material in the Golgi membranes and endoplasmic reticulum with subsequent fusion of these membranes with the plasma membrane effecting a merocrine-like secretory function (Karrer, 1960; Hay, 1961; Revel and Hay, 1963a, 1963b; Goldberg and Green, 1964).

At no time has the author's work suggested a merocrine-like

secretory function and reviews of the literature by other investigators are consistent with this view (Porter, 1964; Richter and Schilling, 1969).

The literature and observations indicate the synthesis of precursory collagenic material within the endoplasmic area is associated with the ribosomes and membranes of the endoplasmic reticulum. This precursory material is subsequently accumulated peripherally in the hyaloplasm. The accumulation is either local or throughout the cell surface during the apocrine-like phases of fibrillogenesis or in the endoplasmic mass in the holocrine-like phases. This is followed by the conversion of these areas into the observed electron-dense cortical hyaloplasm (Hansen, 1899; Sterns, 1940a, 1940b; Hay, 1958; Jackson, 1957; Yardley, 1961; Chapman, 1961, 1962; Knese and Knoop, 1961b; Porter, 1964; Richter and Schilling, 1969).

The Hyaloplasm

The hyaloplasm is observed to be of variable density. During the apocrine-like phases of fibrillogenesis (Figure 14, Figure 15), the hyaloplasm appears as electron-lucent endoplasmic masses and localized hypertrophied masses of electron-dense cortical hyaloplasm. During the holocrine-like deterioration phases, the electron-dense cortical hyaloplasmic mass is hypertrophied to the point that only scant perinuclear endoplasmic masses are observed. The localized cytoplasmic hypertrophy in the apocrine-like phases and the massive general hypertrophy in the holocrine-like phases of fibrillogenesis are due in part to the accumulation of precursory collagenic material in the cytoplasm.

With the disappearance of the plasma membrane undifferentiated collagenic fibrils begin to organize directly from precursory collagenic

material, presumably the basic molecular collagenic unit, tropocollagen (Jackson, 1957; Ross, 1968) contained in the cortical hyaloplasm (Richter, et al., 1964a, 1964b; Richter and Schilling, 1969). As previously observed, the cell surface in these areas now consists of the electron-dense cortical hyaloplasm, the condensed fibers of the cytoskeleton, and organizing collagenic fibrils. The reestablishment of the plasma membrane in the apocrine-like phases or the further regression of the cytoplasm in the holocrine-like phases deposits the respective dense cortical hyaloplasmic masses in situ intercellularly.

The undifferentiated fibrils continue to grow and differentiate by incorporation of precursory collagenic material present in the now "intercellular hyaloplasm." The intercellular hyaloplasm would properly be termed the intercellular matrix or, not including the collagenic fibrils and cytoskeletal fibers, the ground substance. The actual amount of hyaloplasm retained as ground substance is unknown but it is suggested that the specific connective tissue cells (fibroblast, chondroblast, and osteoblast) do form the ground substance (Gersh and Catchpole, 1949).

Within the "intercellular hyaloplasm" far distant from cells, overt collagenic fibrils continue to form and differentiate indicating that precursory collagenic material is present in the "intercellular matrix".

These sequences or various portions of the sequences involving the apocrine-like or holocrine-like phases of fibrillogenesis have been observed or reported by many investigators and are referenced in the discussion of the apocrine-like and holocrine-like phases. The above sequences demonstrate both the intercellular and intracellular formation

of collagen, It is therefore suggested that the proponents of either theory would be essentially correct. Further, the ground substance or that portion of the ground substance formed from the hyaloplasm and the collagenic fibrils are deposited in situ. These observations are confirmed by Karrer, (1958), Porter, (1964), Richter, et al., (1964a, 1964b), and Richter and Schilling, (1969).

The broad concept that fibroblast contact is essential to fibrillogenesis is indicated by this investigation. The exact nature of fibroblast contact is not established or defined by previous investigators. It is suggested by the author that the synthesis of precursory collagenic material within the fibroblast, the accumulation of this material, its polymerization, and the deposition in situ of overt collagenic fibrils in either the apocrine-like or holocrine-like secretory processes should meet all the requirements of fibroblast contact.

Electron-dense Hyaloplasmic Structures

Within the hyaloplasm of some of the cells and cell processes demonstrating holocrine-like phases of fibrillogenesis, small spherical electron-dense structures were observed (Figure 29). These structures were typed arbitrarily into two categories based on size and electron density. The smaller less electron-dense structures are ordered in rows which is comparable to the configuration of ribosomes attached to the endoplasmic reticulum. The larger, highly electron-dense structures are randomly arranged in the cytoplasm. Neither of the structures is associated with discernible membranes.

It should be noted that these electron-dense structures were not observed in fibroblasts in the apocrine-like phases of fibrillogenesis

nor were they observed in all cells or cell processes undergoing holocrine-like deterioration.

Both the large and the small electron-dense structures are composed of pear-shaped units (Figures 30, 31, 32, 34). The pear-shaped unit's configuration is the reflection of an internal asymmetrically coiled cable or rope-like structure which has a mean diameter of 24.9 Å. The total measurement of the pear in the long axis is approximately 246 Å. The narrow segment measures 106 Å in the long axis with a mean diameter of 64.2 Å. The bulbous segment measures approximately 140 Å with a mean diameter of 109.3 Å.

In Figure 34, a large electron-dense structure, an organizing collagenic fibril and the hyaloplasm contains cross-sectional views of the pear-shaped units. Further, cross-sectional views of pear-shaped units are demonstrated in a differentiated collagenic fibril (Figure 35).

A structure of this approximate size and shape has been observed in native collagen fibrils in dog fibrocollagenous tissue and is suggested to be the basic molecular unit comprising the native collagenic fibril, the tropocollagen molecule (Richter, et al., 1966; Richter and Schilling, 1969, 1970). The reported mean dimensions are as follows: 1) a long axis length of 263 Å, 2) a cable diameter of 22.9 Å, 3) a long axis length of the narrow segment if 119 Å with the mean cross-sectional diameter of 57.2 Å, and 4) the long axis length of the bulbous segment is 145 Å with a mean cross-sectional diameter of 108.1 Å. These reported observations are compatible with the author's findings.

In reviewing the literature, there are no descriptions or electron microscopic visual recordings of the specific electron-dense

structures observed by the author. Further, there has been no direct visual demonstration of the pear-shaped structures within the cell cytoplasm.

The observations of the suggested tropocollagen molecules in the hyaloplasm lends visual evidence to the previously suggested accumulation of precursory collagenic material in the cytoplasm. The discernible presence of these pear-shaped structures in both the organizing fibril and the differentiated fibril is evidence that the collagen fibril is composed of these basic units. Their method of incorporation and organization within the fibril is unknown at this time and serves as a basis for further investigation.

CHAPTER V

SUMMARY

The growth, development, and cellular activity of specially induced fibrocollagenous tissue complexes in humans have been studied during the time course of fibroplasia utilizing both light and electron microscopic procedures.

The developed fibrocollagenous tissue complexes reflects the essential size and shape of the induction device. Tissue complexes retrieved after two to three weeks of development consists primarily of fibroblasts. The tissue complexes reclaimed after four to sixteen weeks of development demonstrates ordered lamellations consisting of zones composed predominantly of fibroblasts or zones consisting primarily of collagenous fibers. The development of the lamellations is referable to the specific fibrillogenic activities by the constituent fibroblasts.

The sequence of the morphological aspects of fibrillogenesis occurring in the cellular zones is indicative of an initial apocrine-like role by the fibroblast. Continued fibrillogenesis by the fibroblast eventually terminates in a holocrine-like role resulting in cytodestruction and concurrent formation of a predominantly collagenic fibrous zone.

Fibroblasts active in fibrillogenesis exhibit sequential changes in the cytoplasm involving hypertrophy of the hyaloplasm either locally in the apocrine-like role or generally in the holocrine-like role. The

hypertrophy is presumably due to the accumulation of precursory collagenic material within the hyaloplasm. Subsequently, the plasma membrane disappears either locally or generally and overt collagenic fibrils form in the hypertrophied hyaloplasm.

The plasma membrane reforms internal to the hypertrophied hyaloplasmic mass in the apocrine-like function depositing the organizing collagenic fibrils and the hyaloplasmic mass extracellularly. The plasma membrane is not reformed in the holocrine-like function. The deposition of hyaloplasm and organizing fibrils is continuous as the cell terminates itself by cytodestruction. The organizing collagenic fibrils continue to grow and differentiate intercellularly presumably by the incorporation of precursory collagenic material in the interfibrillar hyaloplasm.

The organizing collagenic fibrils are related to argyrophilic fibrils supporting the concept that certain reticular fibers are collagenic in nature. The morphological aspects of fibrillogenesis supports the concept that the fibroblast functions in the synthesis and organization of collagenic fibrils. Further, both the intra- and intercullular concepts of fibrillogenesis is elucidated.

The structural relationship of the hypertrophic fibroblast cytoplasm and the emerging collagen fibrils supports and extends the broad concept that fibroblast contact is essential to fibrillogenesis.

Light and electron microscopic studies of human fibrocollagenous connective tissue complexes induced <u>in vivo</u> indicate that the formation of the highly ordered connective tissue complex and the functional role of the fibroblasts in fibrillogenesis are fundamentally the same as in those induced in the dog.

BIBLIOGRAPHY

Abercrombie, M., Heaysman, J. E. M., Karthauser, H. M. 1957 Social behavior of cells in tissue culture. III. Mutual influence of sarcoma cells and fibroblasts. Exp. Cell Res., 13:276-291.

Alfejew, S. 1926 Über die embryonale histogenese der kollogenen und reticularen fasern des bindegewebes bei. Z. Zellforsch, 3: 149-168.

Arey, L. B. 1936 Wound healing. Physiol. Rev., 16:327-406.

Armed Forces Institute of Pathology. 1960 Manual of Histologic and Special Staining Techniques, 2nd edition, the Blakiston Division, McGraw-Hill Book Co., Inc., New York, pp. 28, 62, 91, 132.

Bairati, A., Amonte, L., Pernis, B. 1964 Studies on the ultrastructure of the lymph nodes. I. The reticular network. Z. Zellforsch, 63:644-672.

Baitsell, G. A. 1916 The origin and structure of a fibrous tissue formed in wound healing. J. Exp. Med., 23:739-756.

Baitsell, G. A. 1917 A study of the clotting of the plasma of frog's blood and the transformation of the clot into a fibrous tissue. Amer. J. Physiol., 44:109-131.

Barton, A. A. 1962 Tumors of nerve. An electron microscopic study. Brit. J. Cancer, 16:466-472.

Bennett, H. S. and Luft, J. H. 1959 S-dollidine as a basis for buffering fixatives. J. Biophys. Biochem. Cytol., 6:113-114.

Berrens, L. and Vandriel, L. M. J. 1962 Comparative studies of human collagen and reticulum. Naturwissenschaften, 49:608.

Bollet, A. J., Goodwin, J. F., Simpson, W. F., and Anderson, D. V. 1958 Mucopolysaccharides, protein, and desoxyribose nucleic acid concentration of granulation tissue induced by polyvinyl sponges. Proc. Soc. Exp. Biol. Med., 99:418-421.

Bouteille, M., Kalifat, S. R., and Dlarue, J. 1967 Ultrastructural variations of nuclear bodies in human diseases. J. Ultrastruct. Res., 19:474-486.

Bradbury, S. and Meek, G. A. 1958 A study of fibrogenesis in the leech, Hirudo Medicinalis. Quart. J. Micro. Sci., 99:143-148.

Brooks, R. E. and Siegel, B. V. 1967 Nuclear bodies of normal and pathological human lymph node cells. An electron microscopic study. Blood, 29:269-275.

Chapman, J. A. 1961 Morphological and chemical studies of collagen formation. I. The fine structure of guinea pig granulomata. J. Biophys. Biochem. Cytol., 9:639-651.

Chapman, J. A. 1962 Fibroblasts and collagen. Brit. Med. Bull., 18: 233-237.

Dalton, A. J. and Zeigel, R. F. 1960 A simplified method of staining thin sections of biological material with lead hydroxide for electron microscopy. J. Biophys. Biochem. Cytol., 7:409-410.

Dugan, K. H. 1970 The ultrastructural localization of adenosine triphosphatase in sebaceous glands and associated extrasebaceous structures. (Unpublished Ph.D. Dissertation, University of Oklahoma).

Dumphy, J. E. and Udupa, K. N. 1955 Chemical and histochemical sequences in the normal healing of wounds. New Eng. J. Med., 253:847-851.

Fernando, N. V. P. and Movat, H. Z. 1963 Fibrillogenesis in regenerating tendon. Lab. Invest., 12:214-229.

Fernando, N. V. P., VanErkel, G. A., and Movat, H. Z. 1964 The fine structure of connective tissues. IV. The intercellular elements. Exp. Molec. Path., 3:529-545.

Florey, H. W., Greer, S. J., Kiser, J., Poole, J. C. F., Telander, R., and Werthessen, N. T. 1962 The development of the pseudointima lining fabric grafts of the aorta. Brit. J. Exp. Path., 43: 655-660.

Foot, N. C. 1925 Studies on endothelial reactions. IX. The formation of reticulum in the lesions of experimental tuberculosis in rabbits. Amer. J. Path., 1:341-350.

Foot, N. C. 1927 On the origin of reticulin fibrils. Amer. J. Path., 3:401-412.

Gersh, I. and Catchpole, H. R. 1949 The organization of ground substance and basement membrane and its significance in tissue injury, disease, and growth. Amer. J. Anat., 85:457-521.

Godman, G. C. 1961 Are there races of fibroblasts and, if so what are their distinguishing characteristics? In: The Biology of Connective Tissue Cells. Arthritis and Rheumatism Foundation, New York, pp. 131-140.

Godman, G. C. and Porter, K. R. 1960 Chondrogenesis studied with the electron microscope. J. Biophys. Biochem. Cytol., 8:719-760.

Goldberg, B. and Green, H. 1964 An analysis of collagen secretion by established mouse fibroblast lines. J. Cell Biol., 22:227-258.

Gross, J. 1950 A study of the aging of collagenous connective tissue of rat skin with the electron microscope. Amer. J. Path., 26 (2):708.

Guyton, A. C. 1963 A concept of negative interstitial pressure based on pressures in implanted perforated capsules. Cir. Res., 12: 399-414.

Hansen, Fr. C. C. 1899 Über die genese einiger bindegewebs-grundsubstanzen. Anat. Anz., 16:417-438.

Harkness, R. D., Marko, A. M., Muir, H. M., and Neuberger, A. 1954 The metabolism of collagen and other proteins of the skin of rabbits. Biochem. J., 56:558-569.

Hartwell, S. W. 1955 The Mechanisms of Healing in Human Wounds. Charles C. Thomas, Springfield.

Hay, E. D. 1958 The fine structure of blastema cells and differentiating cartilage cells in regenerating limits of Amblystoma larvae. J. Biophys. Biochem. Cytol., 4:583-592.

Hay, E. D. 1961 Fine structure of blastema cells and differentiating cartilage cells in regenerating limits of Amblystoma epidermis. J. Biophys. Biochem. Cytol., 10:457-463.

Hay, E. D. and Revel, J. P. 1963 Autoradiographic studies of the origin of the basement lamellae in Amblystoma. Develop. Biol., 7: 152-168.

Henry, K. and Petts, V. 1969 Nuclear bodies in human thymus. J. Ultrastruct. Res., 27:330-343.

Jackson, D. S. 1957 Connective tissue growth stimulated by carrageenin. I. The formation and removal of collagen. Biochem. J., 65: 277-284.

Jackson, S. F. 1957 Structural problems associated with the formation of collagen fibrils in vivo. In: Connective Tissue. R. E. Tunbridge, The Council for International Organizations of Medical Sciences, Charles C. Thomas, Springfield, pp. 77-83.

Jackson, S. F. and Smith, R. H. 1957 Studies in the biosynthesis of collagen. I. The growth of fowl osteoblasts and formation of collagen in tissue culture. J. Biophys. Biochem. Cytol., 3(2): 897-912.

Karasaki, S. 1964 An electron-microscopic study of Wolffian lens regeneration in the adult neut. J. Ultrastruct. Res., 11: 246-273.

Karnovsky, M. J. 1961 Simple methods for staining with lead at high pH in electron microscopy. J. Biophys. Biochem. Cytol., 11:729-732.

Karrer, H. E. 1958 The fine structure of connective tissue in the Tunica propria of bronchioles. J. Ultrastruct. Res., 2:96-121.

Karrer, H. E. 1960 Electron microscope study of developing chick embryo aorta. J. Ultrastruct. Res., 4:420-454.

Kay, D. H. 1965 Techniques for Electron Microscopy. 2nd edition. F. A. Davis Co., Philadelphia.

Knese, K. H. and Knoop, A. M. 1961a Uber den ortder bildung des mukopolysaccharide protein komplexes in knorpelgewebe. Z. Zellforsch., 53:201-258.

Knese, K. H. and Knoop, A. M. 1961b Condrogenese und osteogenese. Z. Zellforsch., 55:413-468.

Kornhauser, S. I. 1952 Orcein and elastic fibers. Stain Tech., 27: 131-134.

Krishan, A., Uzman, B. G., and Hedley-Whyte, E. T. 1967 Nuclear bodies: A component of cell nuclei in hamster tissues and human tumors. J. Ultrastruct. Res., 19:563-572.

Lafontaine, J. G. 1965 A light and electron microscope study of small spherical nuclear bodies in meristematic cells of plant cell nuclear bodies. J. Cell Biol., 26:1-18.

Lewis, M. R. 1917 Development of connective tissue fibers in tissue cultures of chick embryos. Contrib. Embryol., 6:45-60.

Lillie, R. D. 1954 Histopathologic Technic and Practical Histochemistry. The Blakiston Division, McGraw-Hill Book Company, New York.

Louisot, P., Ray, A., Fontanges, R., and Colobert, L. 1967 Etal moleculaire des acides ribonucleigues intraribosomiques au cours du vieillissment cellulaire. C. R. Acad. Sci. Paris, 264:1208-1210.

Lowther, D. A., Green, N. M., and Chapman, J. A. 1961 Morphological and chemical studies of collagen formation. II. Metabolic activity of collagen associated with subcellular fractions of guinea pig granulomata. J. Biophys. Biochem. Cytol., 10:373-388.

Mall, F. P. 1901-1902 On the development of the connective tissues from the connective tissue syncytium. Amer. J. Anat., 1:329-365.

Mallory, F. B. 1903-1904 A hitherto undescribed fibrillar substance produced by connective tissue cells. J. Med. Res., 10:334-341.

Mallory, F. B. and Parker, F. 1927 Reticulum. Amer. J. Path., 3:515-525.

Manner, G., Kretsinger, R. H., Gould, B. S., and Rich, A. 1967 The polyribosomal synthesis of collagen. Biochim. Biophys. Acta, 134:411-428.

Maximow, A. 1928 Development of argyrophile and collagenous fibers in tissue culture. Proc. Soc. Exp. Biol. Med., 25:439-442.

Millonig, G. 1961 A modified procedure for lead staining of thin sections. J. Biophys. Biochem. Cytol., 11:736-739.

Moore, R. D. and Schenberg, M. D. 1960 Studies on connective tissue: The cytoplasm of the fibroblast. Exp. Cell Res., 20:511-518.

Movatt, H. Z. and Fernando, N. V. P. 1962 The fine structure of connective tissue. I. The fibroblast. Exp. Molec. Path., 1: 509-534.

Muir, R. 1964 Muir's Textbook of Pathology. Revised by: D. F. Cappell. 8th edition. The Williams and Wilkins Co., Baltimore. p. 24.

Nicander, L. 1964 Fine structure and cytochemistry of nuclear inclusions in the dog epididymus. Exp. Cell Res., 34:533-541.

Noble, N. J. and Boucek, R. J. 1955 Lipids of the serum and connective tissue of the rat and rabbit. Cir. Res., 3:344-350.

Odland, G. and Ross, R. 1968 Human wound repair: I. Epidermal regeneration. J. Cell Biol., 39:135-151.

O'Neal, R., Jordon, G. L., Rabin, E. R., DeBakey, M. E., and Halpert, B. 1964 Cells grown on isolated intravascular dacron hub. An electron microscopic study. Exp. Molec. Path., 3:403-412.

Papaconstantinou, J. 1967 Molecular aspects of lens cell differentiation. Sci., 156/3773:338-346.

Peach, R., Williams, G., and Chapman, J. A. 1961 A light and electron optical study of regenerating tendon. Amer. J. Path., 38: 495-513.

Peters, R. A. 1956 Hormones and cytoskeleton. Nature (London), 177: 426.

Popoff, N. and Stewart, S. E. 1968 The fine structure of nuclear inclusions in the brain of experimental golden hamsters. J. Ultrastruct. Res., 23:347-361.

Porter, K. R. 1953 Observations on a submicroscopic basophilic component of cytoplasm. J. Exp. Med., 97:727-750.

Porter, K. R. 1954 Electron microscopy of basophilic components of cytoplasm. J. Histochem. Cytochem., 2:346-355.

Porter, K. R. 1964 Cell fine structure and biosynthesis of intercellular macromolecules. Biophys. J., Suppl., 4:167-196.

Porter, K. R. and Pappas, G. D. 1959 Collagen formation by fibroblasts of the chick embryo dermis. J. Biophys. Biochem. Cytol., 5: 153-166.

Porter, K. R. and Vanamee, P. 1949 Observations on the formation of connective tissue fibers. Proc. Soc. Exp. Biol. Med., 71: 513-516.

Revel, J. P. and Hay, E. D. 1963a An autoradiographic study of collagen synthesis by cartilage cells. Anat. Rec., 145:367.

Revel, J. P. and Hay, E. D. 1963b An autoradiographic and electron microscopic study of collagen synthesis in differentiating cartilage. Z. Zellforsch., 61:110-144.

Reynolds, E. S. 1963 The use of lead citrate at high pH as electron-opaque stain in electron microscopy. J. Cell Biol., 17:208-212.

Richter, K. M. 1970 Personal communication, July, University of Oklahoma Medical Center, Oklahoma City.

Richter, K. M., Schilling, J. A., and Shurley, H. M. 1964a Electron microscopic studies on the morphologic relations of fibrocytes and collagen fibers in developing experimental scar structures. Amer. Zool., 4:343.

Richter, K. M., Schilling, J. A., and Shurley, H. M. 1964b Ultrastructure of experimentally induced scar tissue. J. Appl. Phys., 35:3099.

Richter, K. M., Schilling, J. A., and Shurley, H. M. 1964c Assay of ultrafiltrates of the dog pre-wound-serum, -plasma, and 7-day-wound-fluid, -serum and -plasma on L-strain fibroblast proliferation. Proc. 15th. Ann. Mtg. Tissue Cult. Assoc., p. 51.

Richter, K. M., Schilling, J. A., and Shurley, H. M. 1966 A possible electron microscopic demonstration of part of the intramacromolecular character of the tropocollagen molecule. Electron Microsc., 2:131.

Richter, K. M. and Schilling, J. A. 1969 The fine structure of an experimentally induced fibrocollagenous tissue complex. Ann. Surg., 169:10-34.

Richter, K. M. and Schilling, J. A. 1970 A dimensionally characterized corpuscular subunit of solid state collagen and its equivalence to the collagen macromolecule of solution. 7th International Congress of Electron Microscopy, Grenoble, France, Vol. 1, 2. (in press).

Robb-Smith, A. H. T. 1945 Tissue changes induced by Cl. Welchie type filtrates. Lancet, 2:362.

Ross, R. and Benditt, E. P. 1961 Wound healing and collagen formation. I. Sequential changes in components of guinea pig skin wounds observed in the electron microscope. J. Biophys. Biochem. Cytol., 11:677-700.

Ross, R. and Benditt, E. P. 1962a Wound healing and collagen formation. II. Fine structure in experimental scurvy. J. Cell Biol., 12: 533-551.

Ross, R. and Benditt, E. P. 1962b Wound healing and collagen formation. III. A quantitative autoradiographic study of the utilization of Proline-H in wounds from normal and scorbutic guinea pigs. J. Cell Biol., 15:99-108.

Ross, R. and Odland, G. 1968 Human wound repair. II. Inflammatory cells, epithelial-mesenchymal interrelations, and fibrillogenesis. J. Cell Biol., 39:152-168.

Schilling, J. A., Favata, B. V., and Radakovich, M. 1953a Studies of fibroplasia in wound healing. Surg. Gynec. Obstet., 96:143-149.

Schilling, J. A., Radakovich, M., and Favata, B. V. 1953b The relationship of vitamin C and ACTH in experimental wounds. Surg. Gynec. Obstet., 97:434-438.

Schilling, J. A., Joel, W., and Shurley, H. M. 1959 Wound healing. A comparative study of the histochemical changes in granulation tissue contained in stainless steel wire mesh and polyvinyl sponge cylinders. Surg., 46:702-710.

Schilling, J. A., Shurley, H. M., Joel, W., Richter, K. M., and White, B. N. 1961 Fibrocollagenous tubes structured in vivo. Arch. Path., 71:548-553.

Shetlar, W. R., Lacefield, E. G., White, B. N., and Schilling, J. A. 1959 Wound healing: Glycoprotein of wound tissue. I. Studies of hexosamine, hexose, and uronic acid content. Proc. Soc. Exp. Biol. Med., 100:501-503.

Silberberg, R., Silberberg, M., Vogel, A., and Wettstein, W. 1961 Ultrastructure of articular cartilage of mice of various ages. Amer. J. Anat., 109:251-275.

Sterns, M. L. 1940a Studies on the development of connective tissue in transparent chambers in the rabbits ear. Amer. J. Anat., 66: 133-176.

Sterns, M. L. 1940b Studies on the development of connective tissue in transparent chambers in the rabbits ear. Amer. J. Anat., 67: 55-97.

Stevens, B. J. 1965 The fine structure of the nucleus during mitosis in the grasshopper neuroblast cell. J. Cell Biol., 24:349-368.

Studnicka, F. K. 1903 Schematiche darstellungen zur entiwicklungsgeschicte einingergewebe. Anat. Anz., 22:537-556.

Studnicka, F. K. 1907 Uber einige Grundsubstanzgewebe. Anat. Anz., 31: 497-552.

Tomlin, S. G. 1953 Reticulin and collagen. Nature (London), 117:302.

Venable, J. H. and Coggeshall, R. 1965 A simplified lead citrate stain for use in electron microscopy. J. Cell Biol., 25:407-408.

Wassermann, F. 1954 Fibrillogenesis in the regenerating rat tendon with special reference to growth and composition of the collagenous fibril. Amer. J. Anat., 94:399-438.

Watson, M. L. 1958 Staining of tissue sections for electron microscopy with heavy metals. II. Application of solutions containing lead and barium. J. Biophys. Biochem. Cytol., 4(2):727-731.

Weber, A. F. and Frommes, S. P. 1963 Nuclear bodies: Their prevalence, location, and ultrastructure in the calf. Sci., 141:912-993.

Weiss, P. and Ferris, W. 1956 The basement lamella of amphibian skin: Its reconstruction after wounding. J. Biophys. Biochem. Cytol., 2(suppl.):275-282.

Welsh, R. A. and Meyer, A. T. 1967 Intracellular collagen fibers in human mesenchymal tumors and inflammatory states. Arch. Path., 84:354-362.

White, B. N., Shetlar, M. R., Shurley, H. M., and Schilling, J. A. 1959 Wound healing: Investigation of proteins, glycoproteins, and lipids of experimental wound fluid in the dog. Proc. Soc. Exp. Biol. Med., 101:353-356.

White, B. N., Shetlar, M. R., Shurley, H. M., and Schilling, J. A. 1961 The glycoproteins and their relationship to the healing of wounds. Ann. N. Y. Acad. Sci., 94:297-307.

Williams, G. 1957 A histological study of the connective tissue reaction to carrageenin. J. Path. Bact., 73:557-563.

Windrum, G. M., Kent, P. W., and Eastoe, J. E. 1955 The constitution of human reticulin. Brit. J. Exp. Med., 36:49-59.

Wolbach, S. B. 1933 Controlled formation of collagen and reticulin. A study of the source of intercellular substance in recovery from experimental scorbutus. Amer. J. Path., 9(2):689-700.

Yardley, J. H. 1961 The biology of connective tissue cells. In: Transactions of the Conference at the Princeton Inn. Arthritis and Rheumatism Foundation, Princeton. pp. 179-192.

Yardley, J. H., Heaton, M. W., Gaines, L. M., Jr., and Shulman, L. E. 1960 Collagen formation by fibroblasts. Johns Hopk. Hosp. Bull., 106:381-393.

Zelander, T. 1959 Ultrastructure of articular cartilage. Z. Zellforsch., 49:720-738.

APPENDIX

FIGURE LEGEND

The figures are micrographs of induced human connective tissue complexes representing 2, 3, 4, 8, 12, and 16 weeks of development.

The ligh micrographs were recorded with a Leica camera with a Micro-Ibso unit in conjunction with an AO Spencer research microscope using a Bausch and Lomb illuminator with a number 58 Wrattan filter, or a Wild M Ka4 camera in conjunction with a Wild M20 research microscope using a Number 8002 Wild daylight filter.

The electron micrographs were recorded with an RCA model EMU-4A electron microscope.

The figures for reproduction are reduced 20 per cent from the basic magnification indicated in the figure legends.

List of Abbreviations

A, A_1	point designations
a_1	apocrine-like phase 1
a_2	apocrine-like phase 2
a_3	apocrine-like phase 3
a_4	apocrine-like phase 4
ar	argyrophilic fibers
B, B_1	point designations
c	collagenic fibrils
cc	cortical condensation

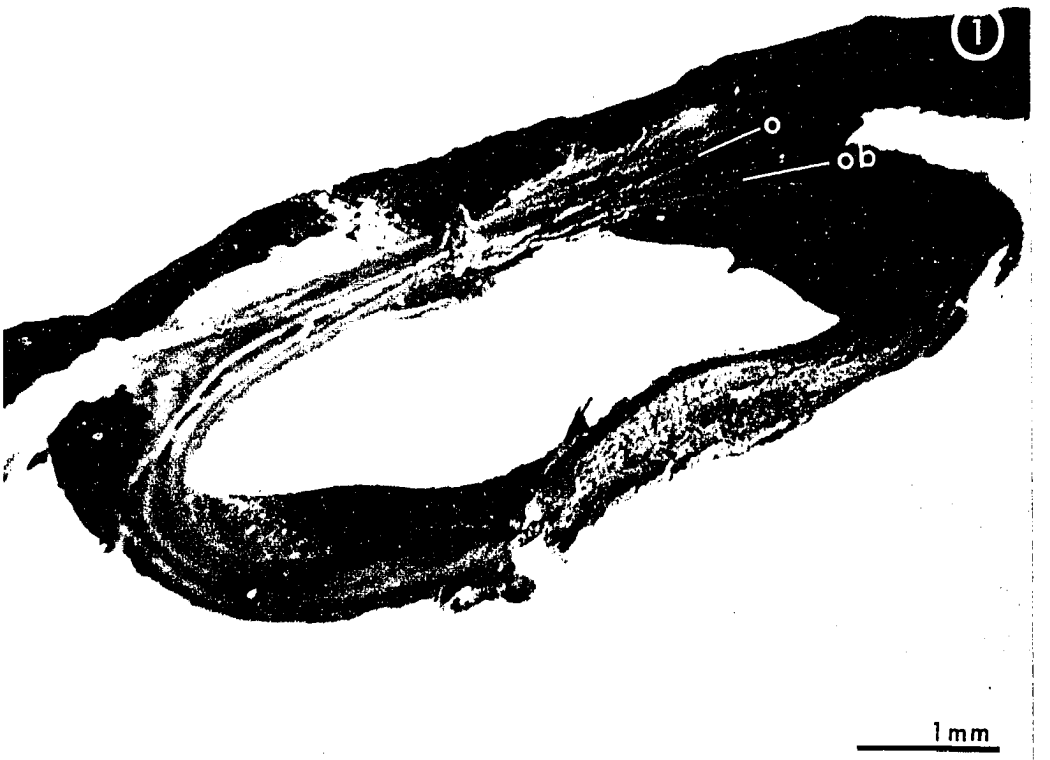
cd	cable diameter
cf	collagen fiber
cp	cell process
cr	common region of organizing collagenic fibrils
cu	corpuscular unit
cy	cell undergoing cytodestruction
dc	dilated cisternae
dh	electron-dense hyaloplasm
dia.	diameter
e	endoplasm
F	fibroblast
ff	filamentous cytoskeletal fibers
fz	fibrous zone
G	Golgi complex
g	granule
h_1	holocrine-like deterioration phase 1
h_2	holocrine-like deterioration phase 2
h_3	holocrine-like deterioration phase 3
l	localized region of organizing collagenic fibrils
lr	luminal region
ls	large electron-dense structure
m	mitochondrion
Mp	macroperiod
mr	mid-region
N	nucleus

nb	nuclear body
np	nuclear pore
o	osmiophilic zone
ob	osmiophobic zone
pr	peripheral region
R_1	row 1
R_2	row 2
R_3	row 3
rer	rough endoplasmic reticulum
ss	small electron-dense structure
ts	tissue fluid space
X	magnification

Figure 1--A 12-week-old osmicated fibrocollagenous tissue complex cut free-hand exhibits cylindrical conformity to the induction device. The osmiophilic (o) and osmiophobic (ob) zones impart a lamellated appearance to the tissue complex. The osmiophobic zones measure from 30 microns in width near the lumen to 110 microns in width near the periphery. (Stained with osmium tetroxide, X22.8).

Figure 2--By 4 weeks of development, the fibrocollagenous tissue complex has developed a luminal region (lr), a mid-region (mr), and a peripheral region (pr). The peripheral region is predominantly cellular and exhibits cell-lined tissue fluid spaces (ts). The mid-region consists of alternating fibrous zones and cellular zones. A minor amount of hemmorhage which occurred during reclamation is evident in the luminal region. (Gomori's reticulum stain, X159.6).

Figure 3--A 12-week-old tissue complex demonstrates the cellular character of the luminal region (lr). Cell-lined tissue fluid spaces are present in both the peripheral region and the cellular zones. One fibrous zone (fz) is readily apparent, a second is developing adjacent to the luminal region. (Stained with Heidenhain's "Azan", X111.6).


1
o
ob
1 mm


2
mr
ir
pr
ts

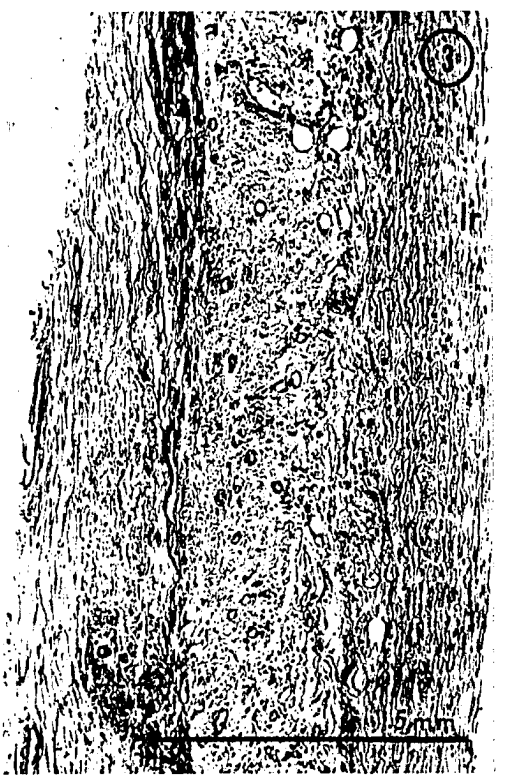

3
ir
5 mm

Figure 4--A healthy, active fibroblast of the peripheral region adjacent to a fibrous zone of a 16-week-old tissue complex has demonstrable collagen fibers associated with the cell surface. (Stained with hematoxylin and eosin).

Figure 5--A fibroblast of a 4-week-old tissue complex fibrous zone has a long attenuated configuration. The nucleus is spindle-shaped and collagen fibers (cf) appear to blend with the scanty cell cytoplasm. The collagen fibers range from very fine filamentous fibers to approximately 2.8 microns in diameter. (Stained with hematoxylin and eosin).

Figure 6--The nuclear configuration of fibroblasts in a fibrous zone developed adjacent to the luminal region ranges from long spindle-shaped nuclei with multiple nucleoli to remnants and fragments of cells undergoing cytodestruction. The tissue complex is 16-weeks-old. (Stained with hematoxylin and eosin).

Figure 7--Foamy histiocytes were observed in the peripheral region of a 16-week-old tissue complex. These cells were generally found in clusters rather than occurring as single cells. (Stained with hematoxylin and eosin).

Figure 8--A foreign body giant cell observed in the peripheral region of a 3-week-old tissue complex is indicative of the type observed in other tissue complexes. (Stained with hematoxylin and eosin).

Figure 9--A reticulum stain demonstrates argyrophilic fibers (ar) blending with or in close apposition to the cell surface. The differentiated collagen fibers (cf) did not exhibit argyrophilia. The micrograph is of the peripheral region adjacent to a fibrous zone of a 12-week-old tissue complex. (Stained with Gomori's reticulum stain). Note: (The magnification of all figures is X1206).

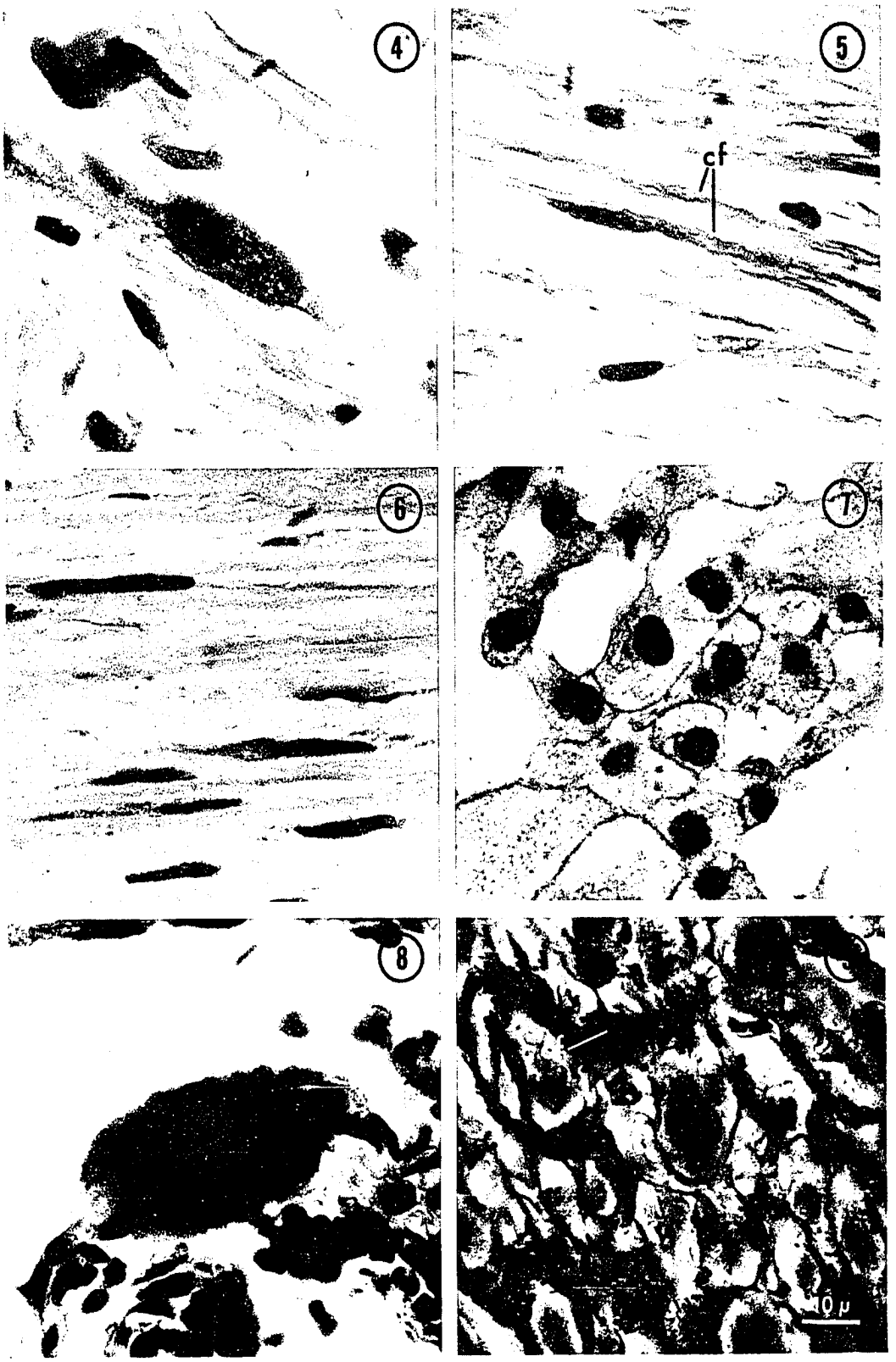

4
5
cf
6
7
8
10 μ

Figure 10--The peripheral region of the tissue complexes is highly cellular with a small amount of collagenic fibrillar material (cf). Cells of this region exhibit apocrine-like secretory deposition of collagenic fibrils. The primary cell type identified for this region is the structurally normal, healthy fibroblast (F). Some of the fibroblasts of this region possess a rough endoplasmic reticulum (rer) delimiting large dialated cisternae (dc). A cell-lined tissue fluid space is located in the lower right corner of the micrograph. The tissue complex is 8-weeks-old. (Stained with aqueous uranyl acetate and lead citrate, X11,842).

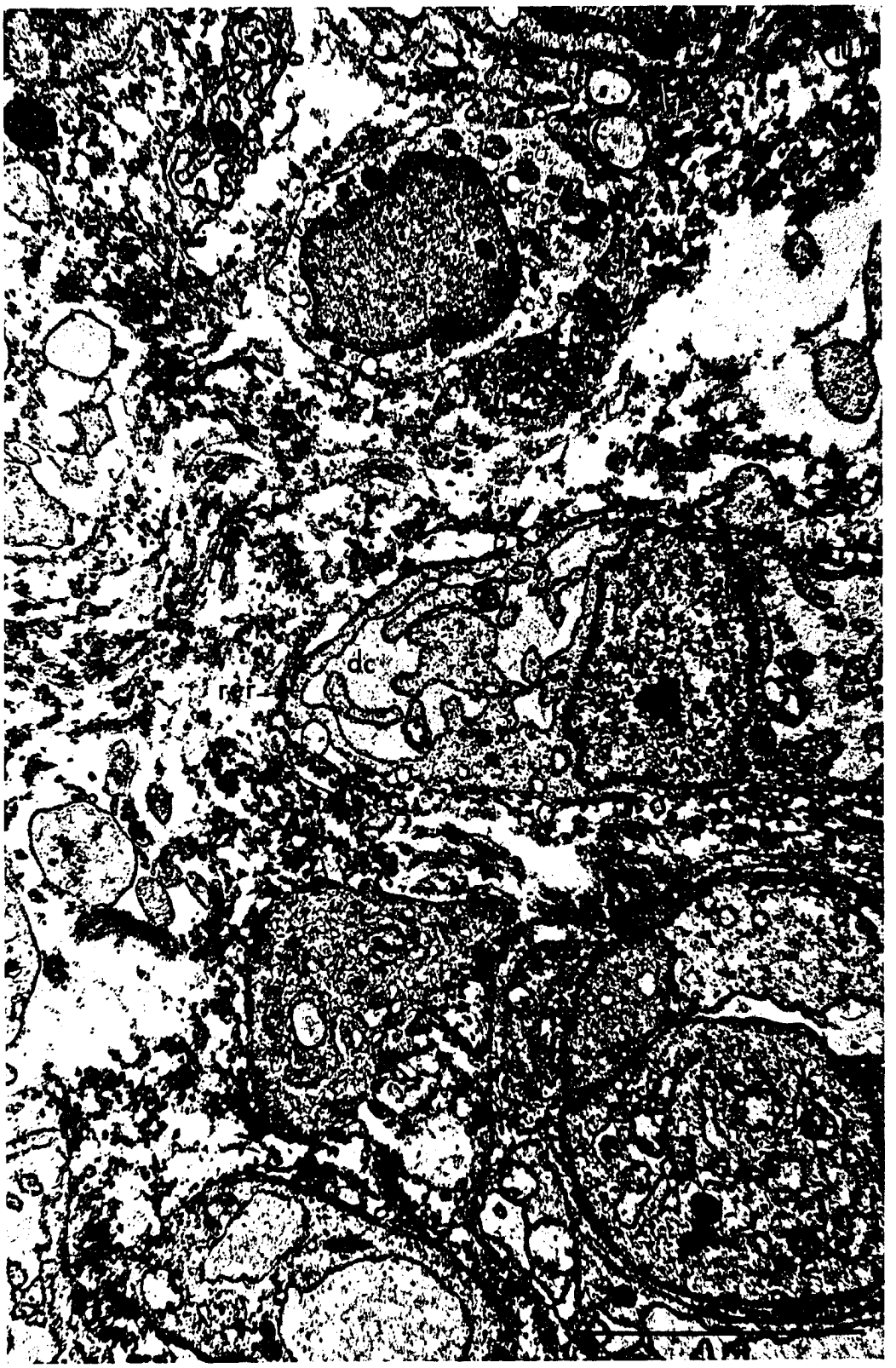

dc
rer

Figure 11--A fibrous zone of a 16-week-old tissue complex demonstrates a predominantly fibrous character. The electron-dense cytoplasmic processes (cp) and the fibroblast undergoing cytodestruction (cy) are common to this zone. The vast amount of collagenic fibrils taken as a whole comprise the osmiophobic zones of Figure 1. (Stained with 1 per cent phosphotungstic acid, X13,176).

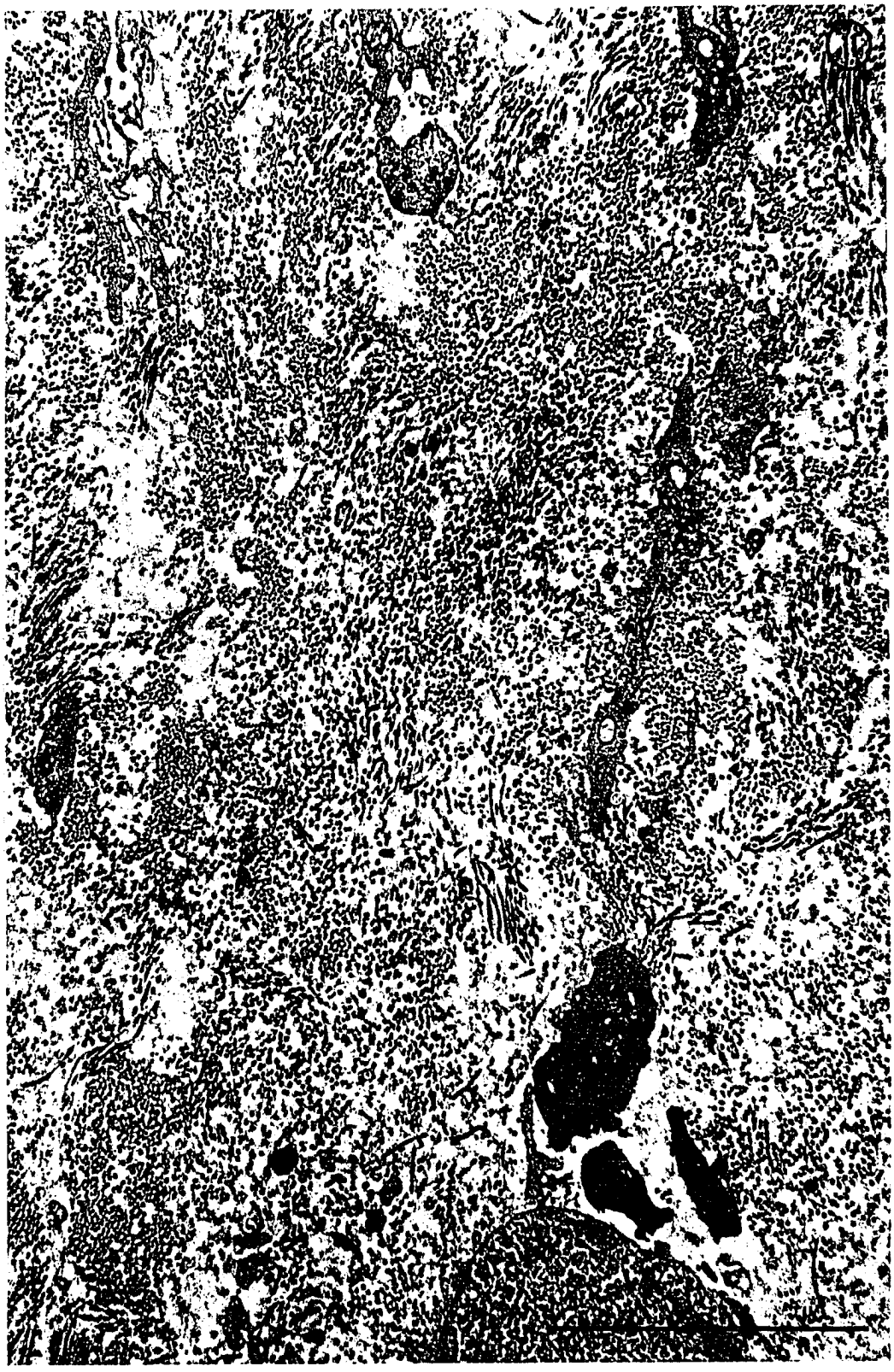
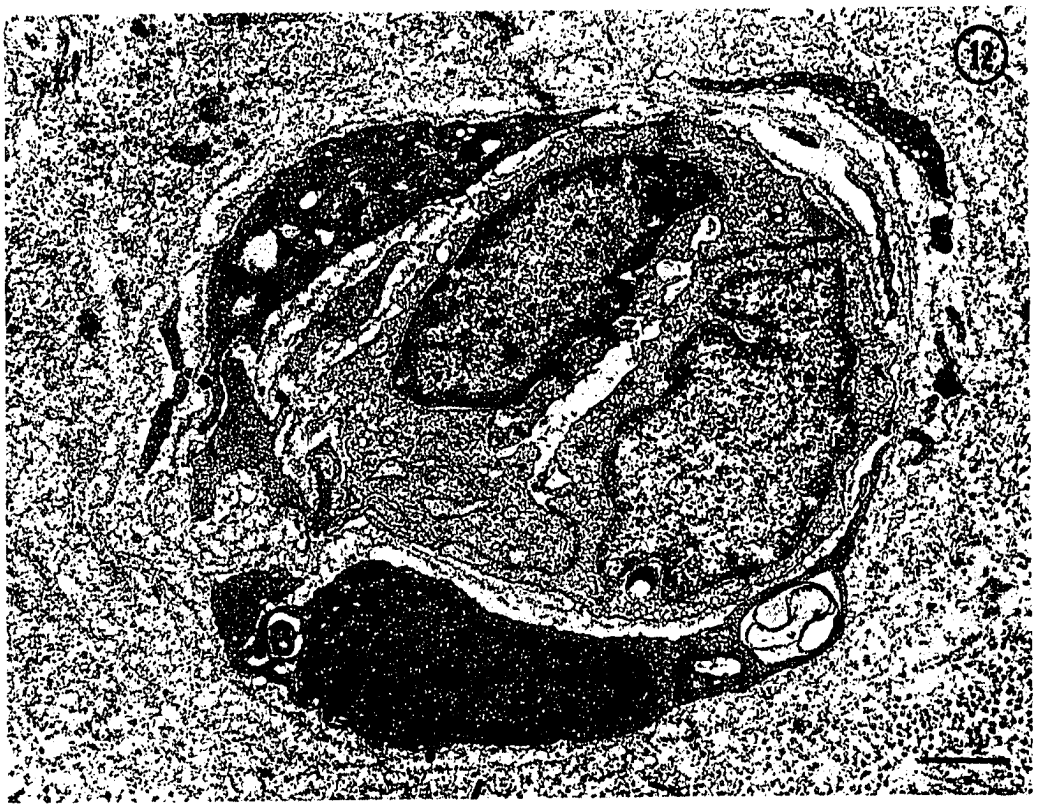


Figure 12--A cell-lined tissue fluid space is positioned at the junction of a cellular and fibrous zone. The fibroblasts immediately lining the space structurally appear healthy and normal. The fibroblasts peripherally located show changes associated with cytodestruction or holocrine-like deterioration. The tissue fluid space is irregularly shaped with small cytoplasmic projections extending into the fluid space. This is a 16-weeks-old tissue complex. (Stained with uranyl acetate and lead citrate, X15,552).

Figure 13--The fibroblasts lining the tissue fluid space (ts) have a wide spectrum of organelle variation which is generally in cells in the apocrine-like phases of collagen deposition. Observe the close apposition of the adjoining cell membranes, the electron-dense cortical hyaloplasm (dh), and the stacked, flattened membranes of the Golgi complex (G). (Stained with uranyl acetate and lead citrate, X25,434).


12

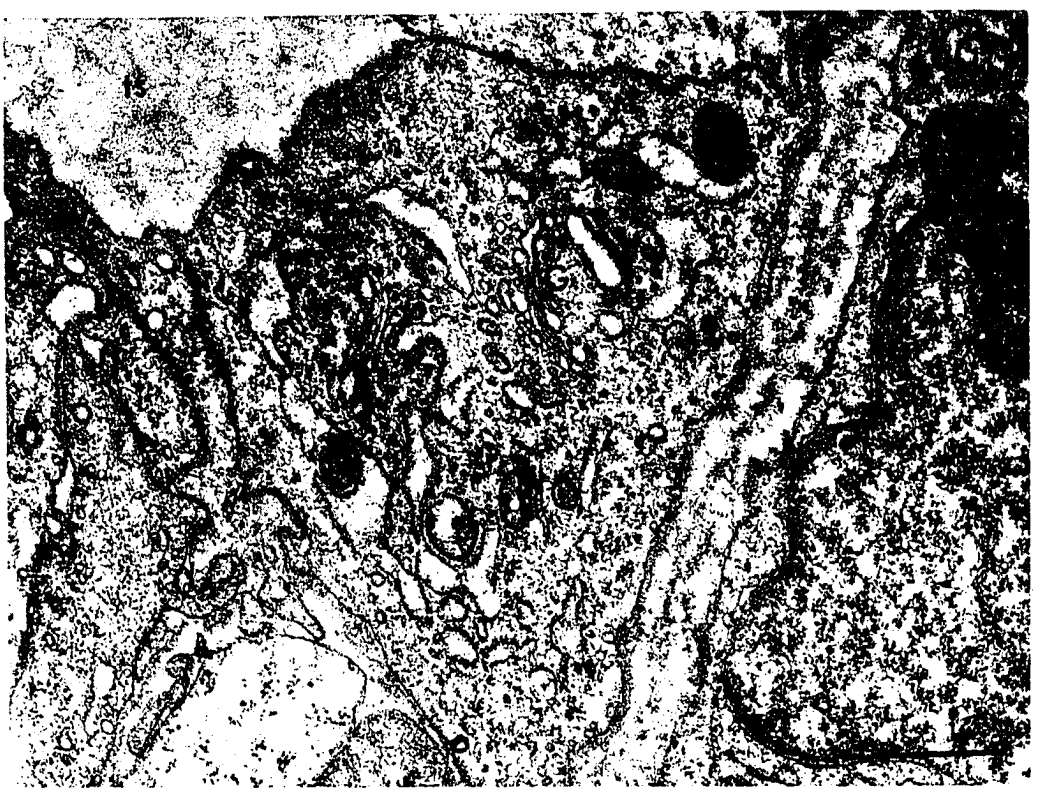


Figure 14--A fibroblast of the cellular zone of an 8-week-old tissue complex demonstrates the various cell surface changes related to the apocrine-like cyclic phases of fibrillogenesis. In the first phase (a_1), the cell surface consists of an intact plasma membrane with little or no cortical hyaloplasmic zone. The second phase (a_2) is characterized by an intact plasma membrane and a cortical condensation consisting of electron-dense cortical hyaloplasm and condensed cytoskeletal fibrils. In the third phase (a_3), the plasma membrane disrupts and the organization of overt collagenic fibrils is observed in the cortical condensation. The plasma membrane is reestablished in the fourth phase (a_4) internal to the cortical condensation and organizing collagenic fibrils. With the excision of the region of organizing collagenic fibrils and the integrity of the plasma membrane renewed, the apocrine-like cycle may recur.

The undifferentiated collagenic fibrils in the established regions of organizing fibrils as a whole correspond to the argyrophilic fibers (ar) seen at the light microscopic level (compare to Figure 9). The regions of undifferentiated collagenic fibrils measure from .4 microns to 1.0 microns in this micrograph.

The endoplasm of the fibroblast has an abundant amount of rough endoplasmic reticulum delimiting tubular cisternae. The nucleus contains nuclear bodies (nb) and a single nucleolus. (Stained with a 1 per cent aqueous uranyl acetate and lead citrate, X19,353).

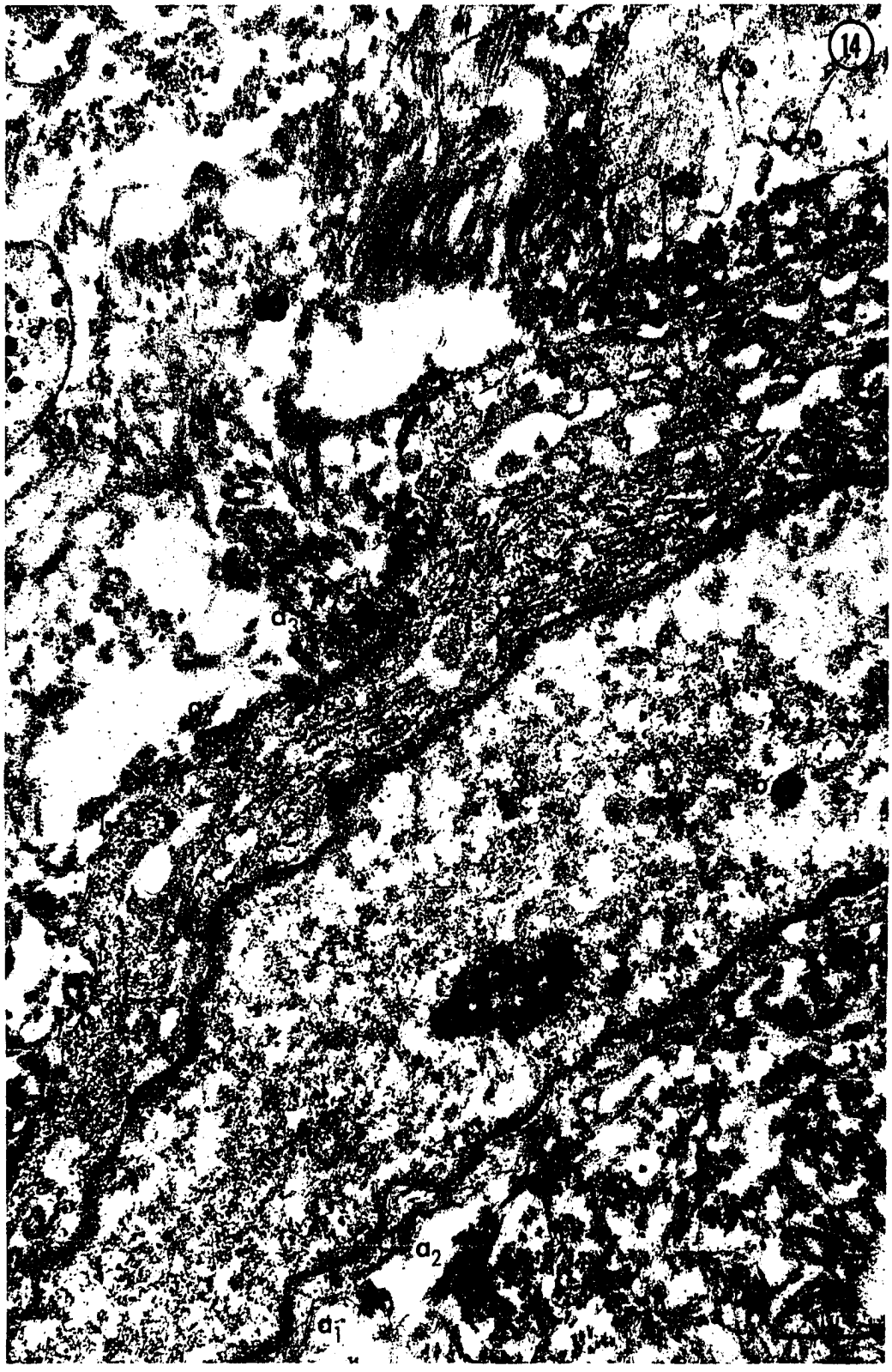

14
a2
a2
a1

Figure 15--The deposition of collagenic fibrils in the apocrine-like secretory phases is accomplished in such a manner as to form the collagenic fibers seen at the light microscopic level.

In the third apocrine-like phase (a_3) the cortical condensation consisting of the electron-dense cortical hyaloplasm and the condensed fibers of the cytoplasm establishes a localized region (1) of organizing collagenic fibrils. With excision of this region by reestablishment of the plasma membrane (apocrine-like phase a_4), the region of organization becomes intercellular. As adjacent cells undergo the same process, the homologous regions blend to form a common region of organization (cr). The individual contributions to the common region are unidentifiable. This common region of organization would correspond to the intercellular collagenic fibers seen in Figure 9. The tissue complex is 8-weeks-old.

Note: Apocrine-like phase (a_1), mitochondria (m). (Stained with aqueous uranyl acetate and lead citrate, X33,726).

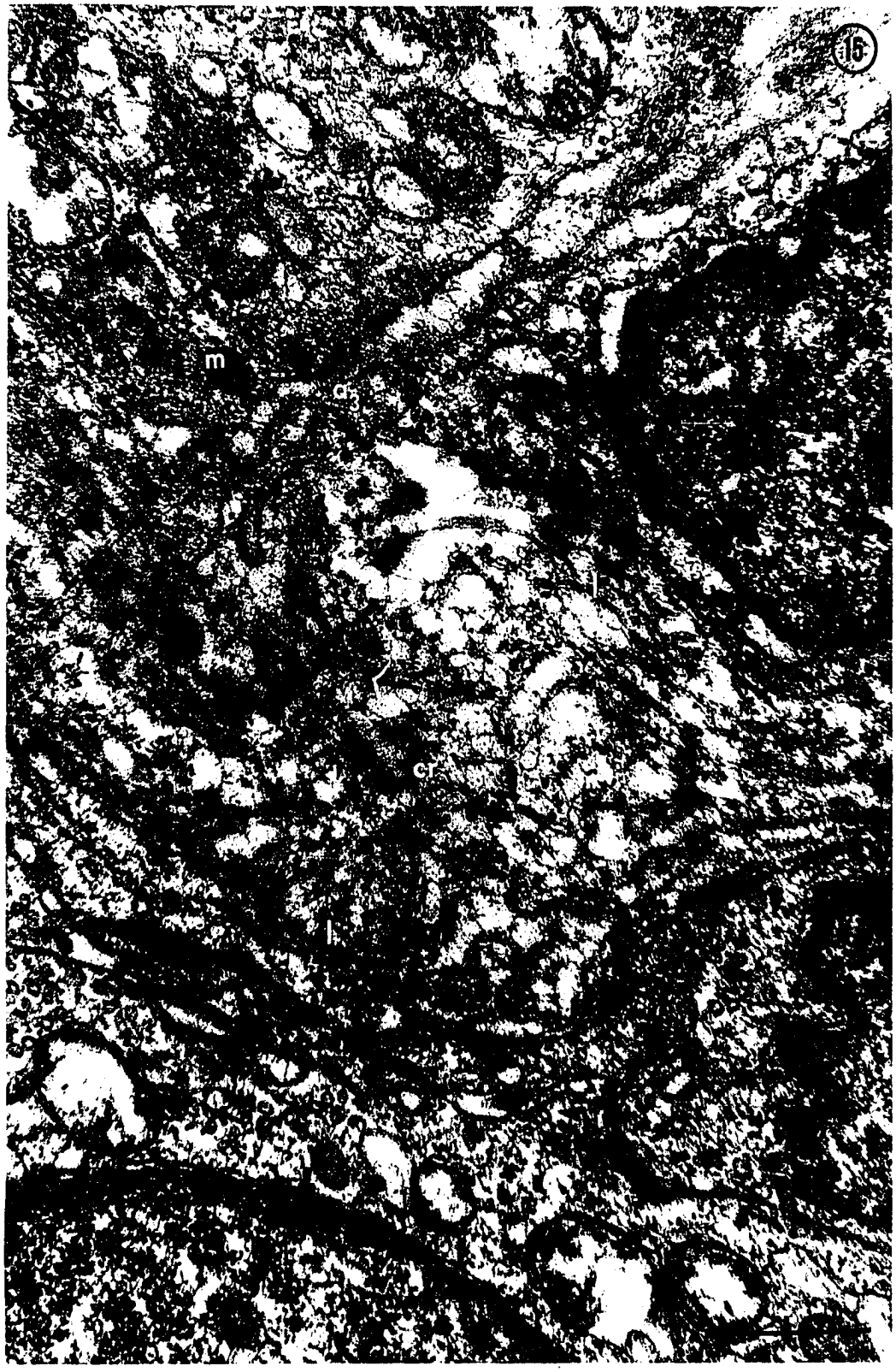

15
m
a
I
cr
I

Figure 16--Filamentous nuclear bodies (nb) are demonstrated in a fibroblast of the cellular zone of an 8-week-old tissue complex. The circular arranged, closely packed filaments measure from 39.3 Å to 72.0 Å. Note: Nuclear pore (np). (Stained with aqueous uranyl acetate and lead citrate, X50,868).

Figure 17--A fibroblast of the cellular zone of an 8-week-old tissue complex contains a filamentous nuclear body with a clearly visable granule (g). (Stained with aqueous uranyl acetate and lead citrate, X62,100).

Figure 18--Four relatively dense granules are present in the center of the circularly arranged filaments of the nuclear body. The fibroblast is from the cellular zone of an 8-week-old tissue complex. (Stained with aqueous uranyl acetate and lead citrate, X54,960).

Figure 19--The granules of a nuclear body of a 4-week-old tissue complex fibroblast of the cellular zone are clustered, and the filaments are not as closely packed as the filaments of Figure 16 and Figure 17. (Stained with aqueous uranyl acetate and lead citrate, X152,604).

Figure 20--The nuclear body of a cellular zone fibroblast of a 16-week-old tissue complex contains a large dense central body which appears to be comprised of a condensation of granules. (Stained with aqueous uranyl acetate, phosphotungstic acid, and lead citrate, X55,954).

Figure 21--A nuclear body in a condensed chromatin type nucleus identified to be in the h_2 phase of holocrine-like deterioration. The nuclear body exhibits a marked decrease in affinity for stain; however, granules and filaments are still recognizable. The fibroblast is from a 12-week-old tissue complex. (Stained with aqueous uranyl acetate and lead citrate, X76,464).

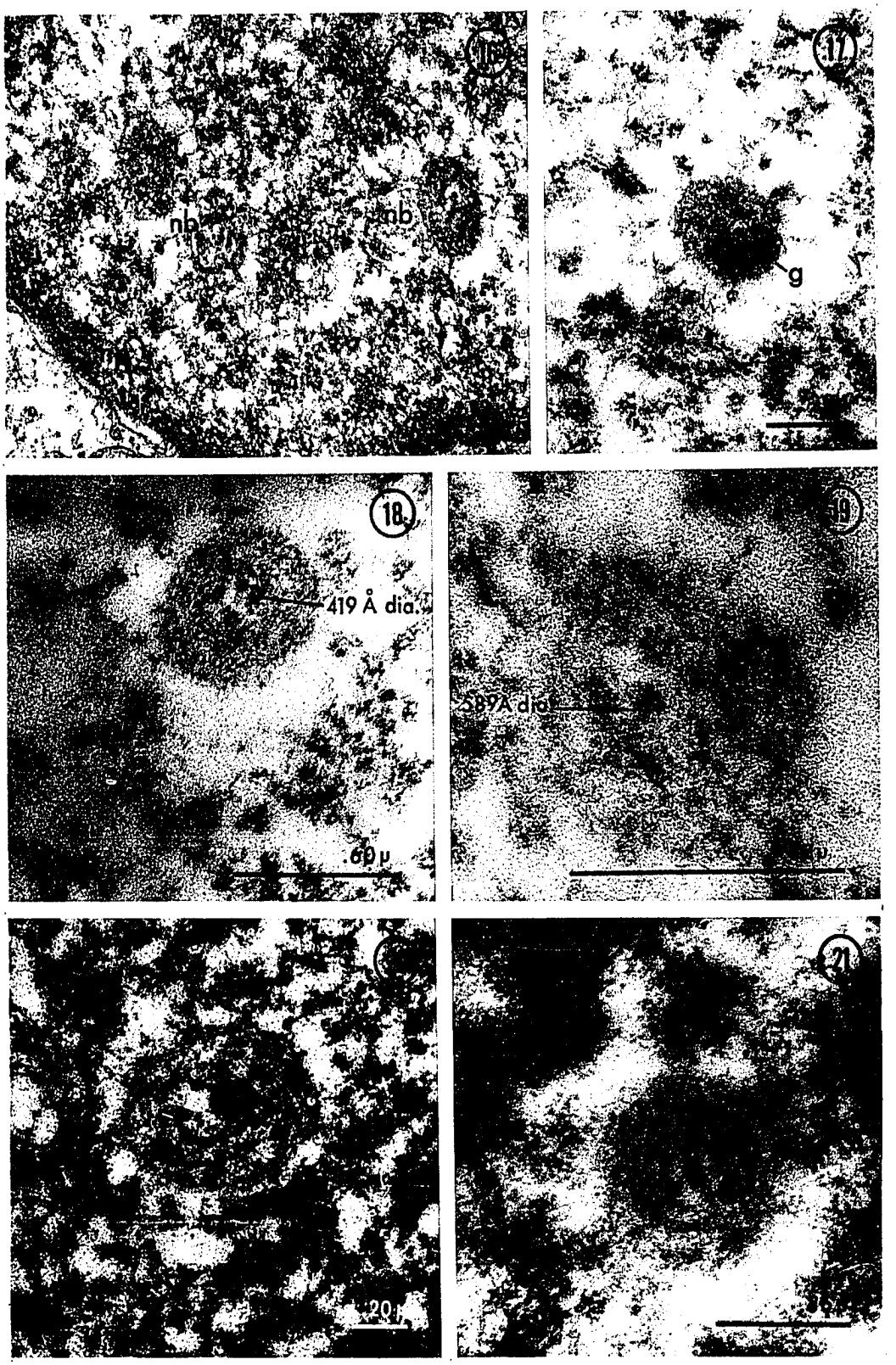

16
nb
nb
17
g
18
419 Å dia.
.60 μ
19
589Å dia
20
21

Figure 22--A fibroblast in the apocrine-like phases of collagenic fibril deposition demonstrates the filamentous fibers (ff) of the cytoplasm which comprise a cytoskeleton. The condensed fibers form a part of the cortical condensation (cc) and with the emergence of overt collagenic fibrils (cf), the fibers of the cytoskeleton maintain a close relationship. In the excised cortical condensation, the fibers appear as separate entities but are in close apposition to and ordered parallel to the overt collagenic fibrils. The fibrils measure from 50 Å to 90 Å in diameter. The fibroblast is from a cellular zone of an 8-week-old tissue complex. (Stained with aqueous uranyl acetate and lead citrate, X89,508).

Figure 23--The cytoskeletal fibers may be masked due to the density of the cortical hyaloplasm. A fibroblast of a 12-week-old tissue complex in holocrine-like deterioration demonstrates faintly discernible cytoskeletal fibers within the electron-dense hyaloplasm. Extracellularly, the fibers (ff) are readily discernible as in Figure 22. Note the variation in mitochondria (m) and the lack of nuclear membranes delimiting the nucleus (N). (Stained with aqueous uranyl acetate and lead citrate, X55,890).


22
c
ff
ff
c
.1μ

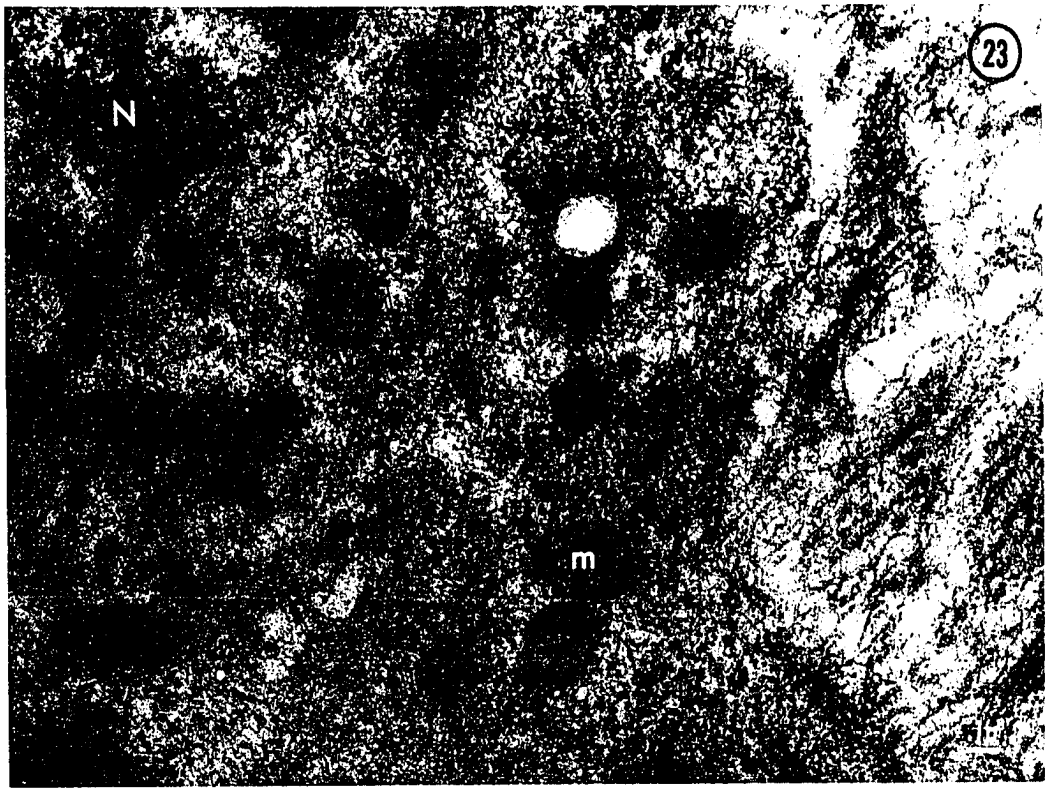

23
N
m

Figure 24--A fibroblast from the junctional area between a cellular zone and a fibrous zone demonstrates the early phases (h_1 and h_2) of the terminal holocrine-like deterioration. In the first phase (h_1) the plasma membrane is intact. The fibers of the cytoskeleton condense in the cortical hyaloplasm and the cortical hyaloplasm becomes electron-dense. This cortical condensation (cc) hypertrophies. The plasma membrane disrupts (phase h_2) and overt collagenic fibrils organize in the cortical condensation. With continued hypertrophy, the cortical condensation may extend to a juxtanuclear position.

In the cytoplasm of this cell, the organelles show various changes. The mitochondria (m) contain a dense mitochondrial matrix. The endoplasmic reticulum and Golgi complex are not in evidence. Ribosomes are free in the cytoplasm. The chromatin material is condensed peripherally in the nucleus and the outer nuclear membrane is observable as an occasional remnant. The tissue complex is 12-weeks-old. (Stained with aqueous uranyl acetate and lead citrate, X26,281).

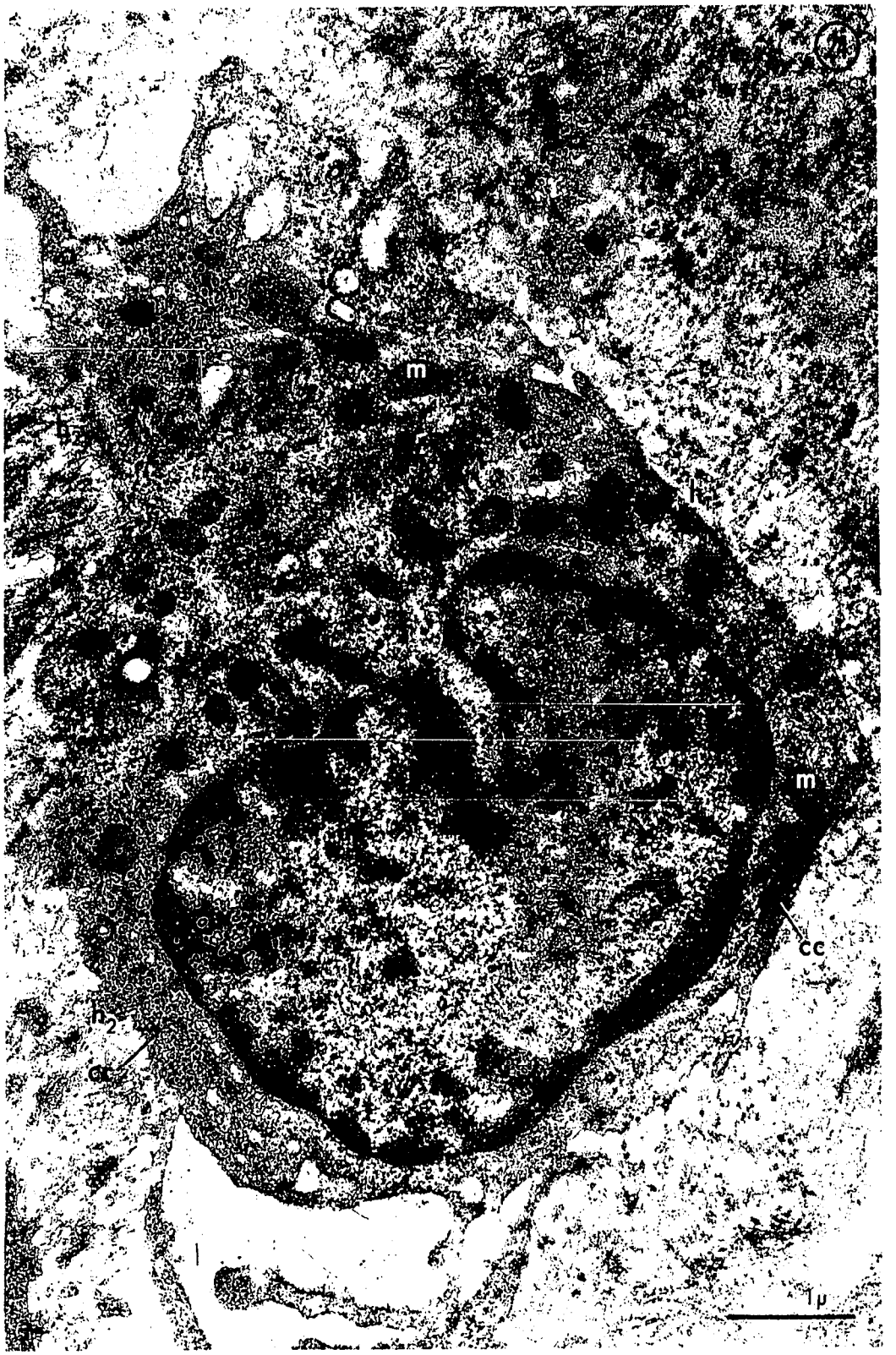

m
m
cc
1μ

Figure 25--A fibroblast at the junction of a cellular and fibrous zone of a 12-week-old tissue complex demonstrates the h_2 phase of holocrine-like deterioration. Only scant remnants of the plasma membrane remain. The cell surface is represented by the electron-dense cortical condensation (cc) and organizing collagenic fibrils. The electron-dense cortical hyaloplasm is greatly hypertrophied and extends in some areas from the arbitrary cell surface to a juxtanuclear position. Small areas of endoplasm (e) remain in which fragmented endoplasmic reticulum can be seen along with highly modified mitochondria (m). In the nucleus, the chromatin is becoming dense and clumped. The outer nuclear membrane exists as only faintly visable remnants. (Stained with uranyl acetate and lead citrate, X48,679).

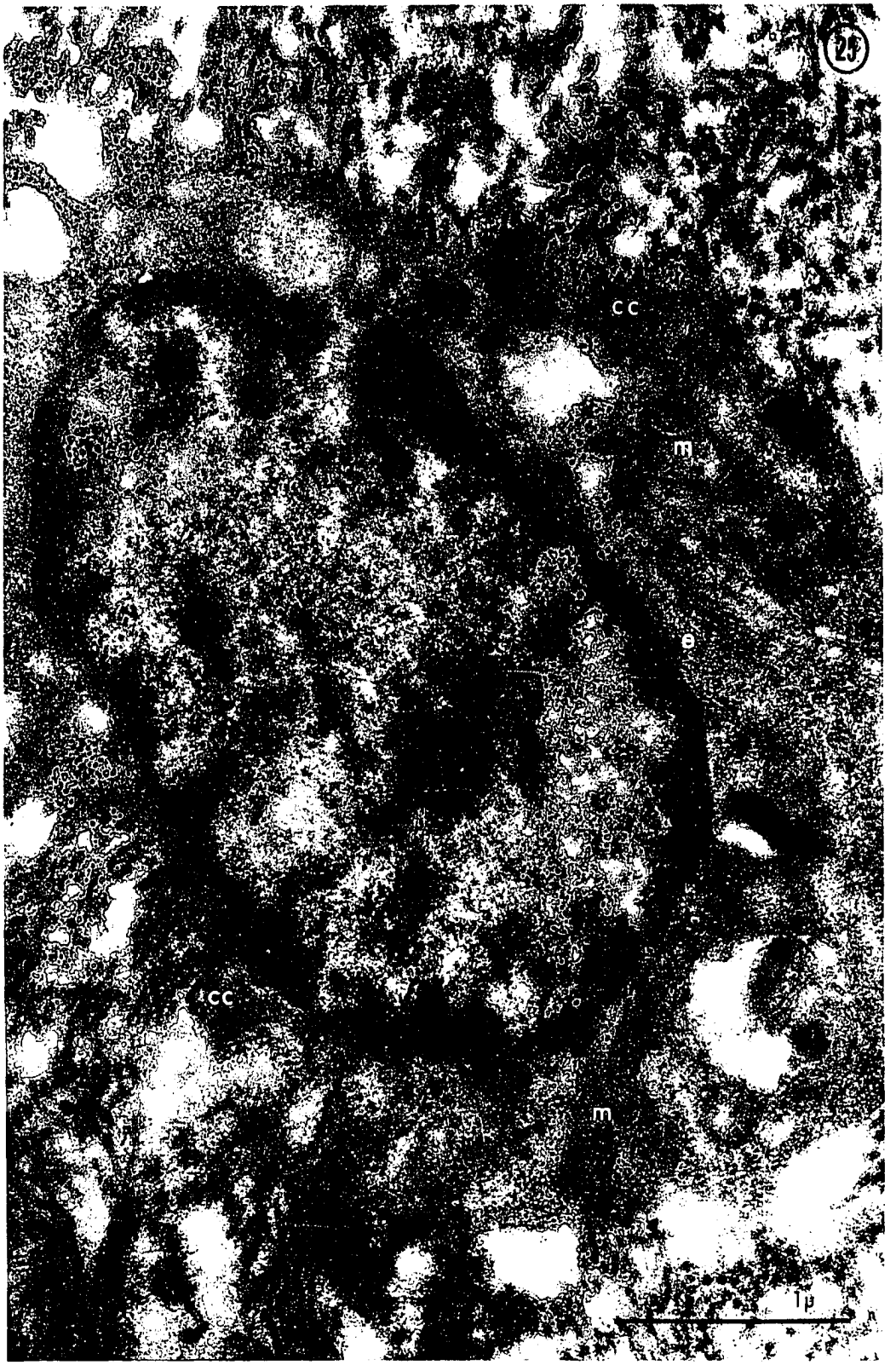

25
cc
m
e
cc
m
1μ

Figure 26--A fibrous zone of a 12-week-old tissue complex demonstrates the terminal or h_3 phase of holocrine-like deterioration. The fragmented cell demonstrated is in the terminal phase of cytodestruction (cy). Cytoplasmic extensions consisting of non-membrane bounded electron-dense cortical condensation (cc) exist among the already formed collagen fibrils (c). The cell extensions represent regions of organizing collagenic fibrils. (Stained with aqueous uranyl acetate and lead citrate, X7,764).

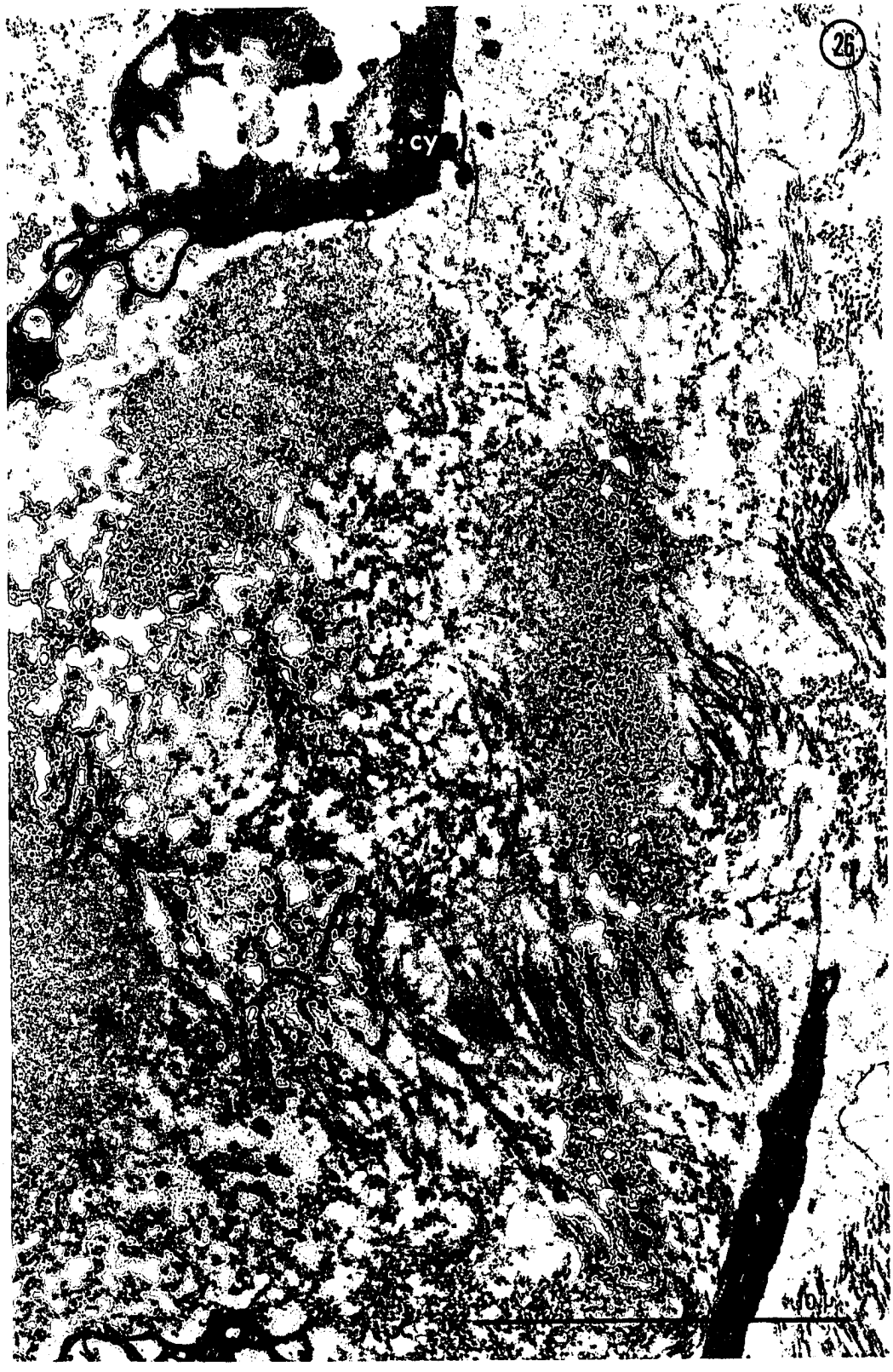

26
cy

Figure 27--With the continued formation of overt collagen fibrils and the concomitant regression of the cytoplasm extensions demonstrated in Figure 26, the electron-dense cortical condensation (cc) is reduced to scant remnants. The cortical condensation comes to reside in an interfibrillar position in which collagenic fibrils continue to organize and differentiate.

The established common regions of collagen deposition are demonstrated. This common tract of collagenic deposition would be delimited by a line between points A to A_1 and B to B_1. The transverse measurements of the common region, points A to B (1.6 microns) and A_1 to B_1 (2.8 microns), roughly correspond to the range of diameter measurements of the collagenic fibers in Figure 5.

Notice the small attenuated cell process (cp) undergoing holocrine-like deterioration. The tissue is from the fibrous zone of a 12-week-old tissue complex. (Stained with aqueous uranyl acetate and lead citrate, X46,035).

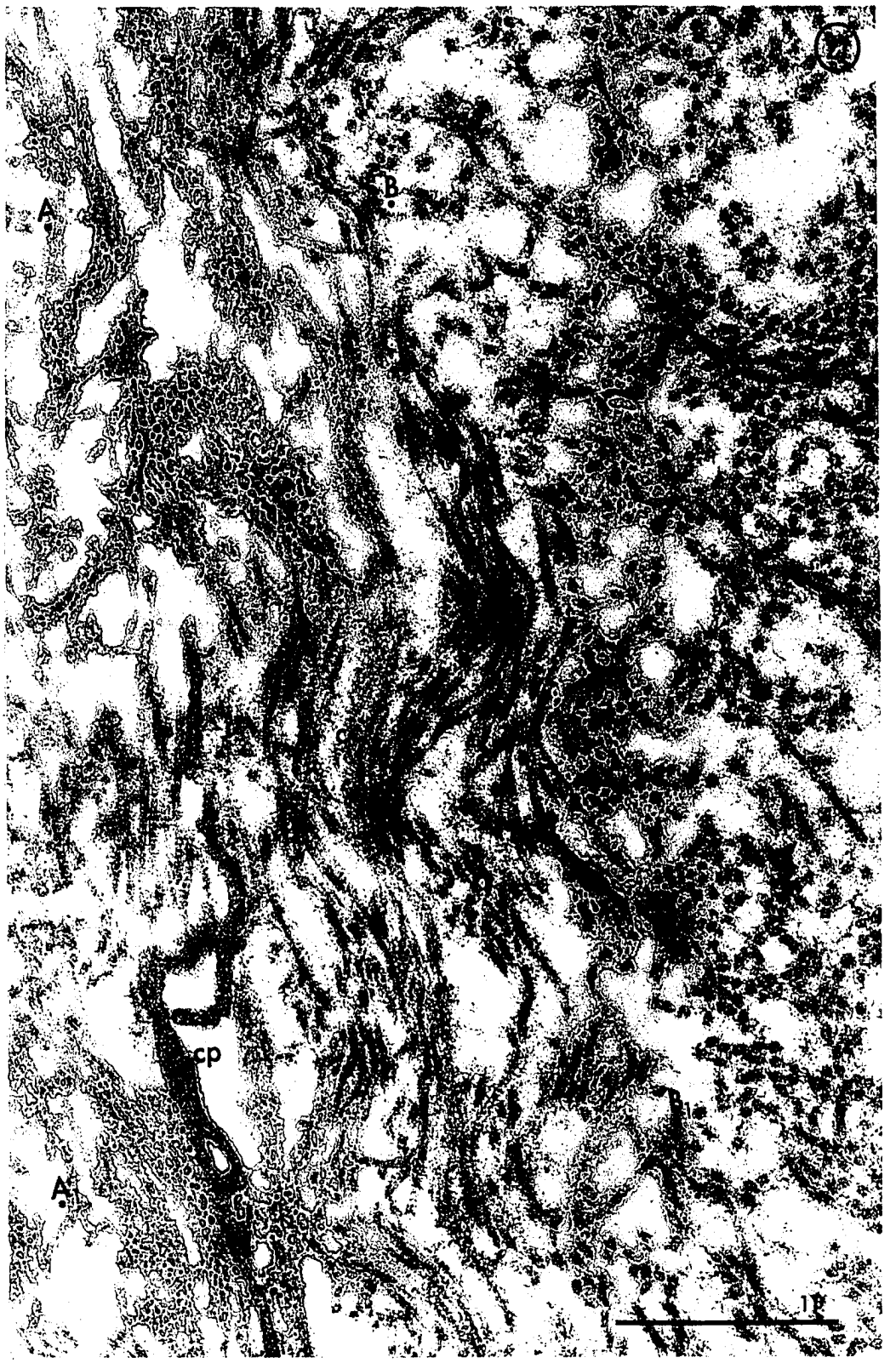
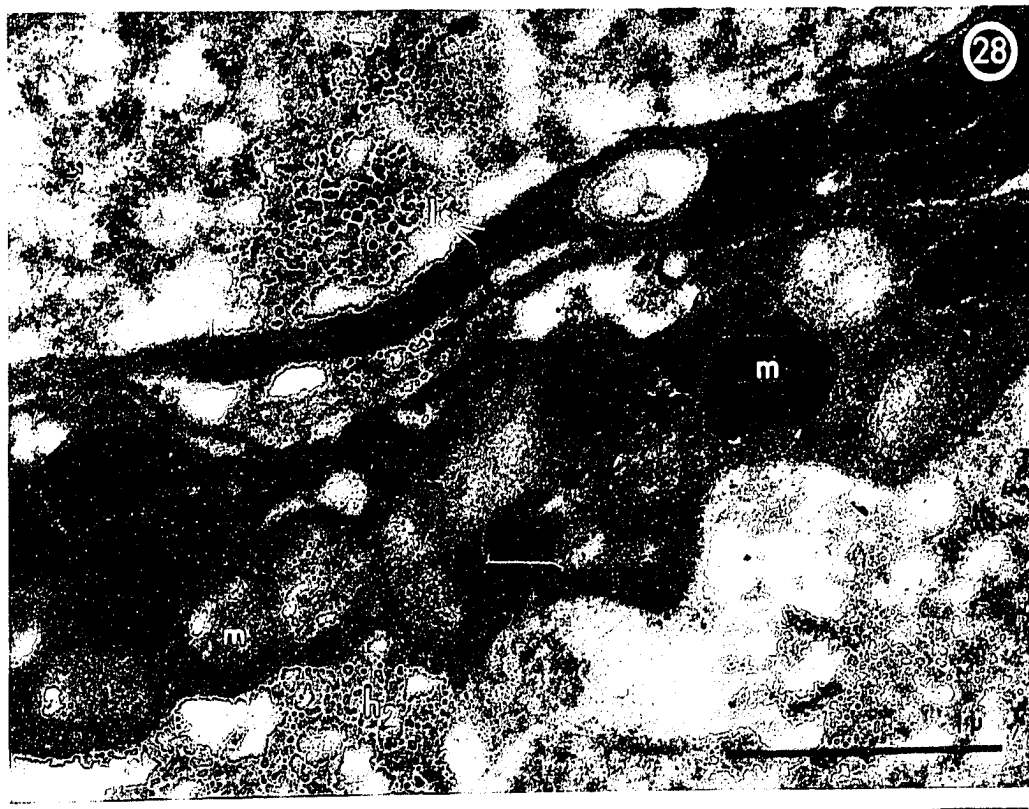

A
B
cp
A
1μ

Figure 28--A cell process extending into a fibrous zone demonstrates changes related to the holocrine-like deterioration phases. The cytoplasm consists wholely of electron-dense cortical hyaloplasm and fibers of the cytoskeleton.

Note: Mitochondria (m), dense granule-like structures appearing in the cytoplasm (ls), holocrine-like phases (h_1 and h_2). The fibrous zone is from a 12-week-old tissue complex. (Stained with aqueous uranyl acetate and lead citrate, X48,438).

Figure 29--A cell process demonstrates holocrine-like deterioration. The cytoplasmic matrix consists of electron-dense hyaloplasm and cytoskeletal fibers. Within the cytoplasm, two types of small, spherical, electron-dense structures are observed. The first type is a larger, exceptionally dense structure (ls). The larger dense structure has no apparent organization in the cytoplasm and may occur as aggregates. The second type is smaller and less electron-dense. The smaller structure (ss) appears in ordered rows or free in the cytoplasm. Neither structure has apparent membranes associated with them. The tissue is from a fibrous zone of a 12-week-old tissue complex. (Stained with aqueous uranyl acetate and lead citrate, X99,403).


28
m
m
h


SS
SS
S

Figure 30--Three ordered rows (R_1, R_2, R_3) of the small electron-dense structures (ss) described in Figure 29 are demonstrated. The small structures are found to be comprised of circular or corpuscular units (cu) of varying size; ranging from 53.1 Å to 129.1 Å. Internally the corpuscular unit is comprised of a rope or cable-like structure which is coiled around a central axis to form the corpuscular units. The mean cable diameter (cd) is 24.9 Å.

Figure 31--The larger electron-dense structures (ls) described in Figure 29 appear to be aggregates of the small structures of Figure 30, and would therefore be dense aggregates of corpuscular units (cu).

Figure 32--A section through the long axis of a corpuscular unit demonstrates the configuration to be pear-shaped. The total length of the pear measures 246 Å in length. The limits of the lower bulbous portion of the pear is not readily discernible due to superimposition of adjacent structures; however, the lower portion measures approximately 140 Å.

Note: Stained with aqueous uranyl acetate and lead citrate, X1,780,380).

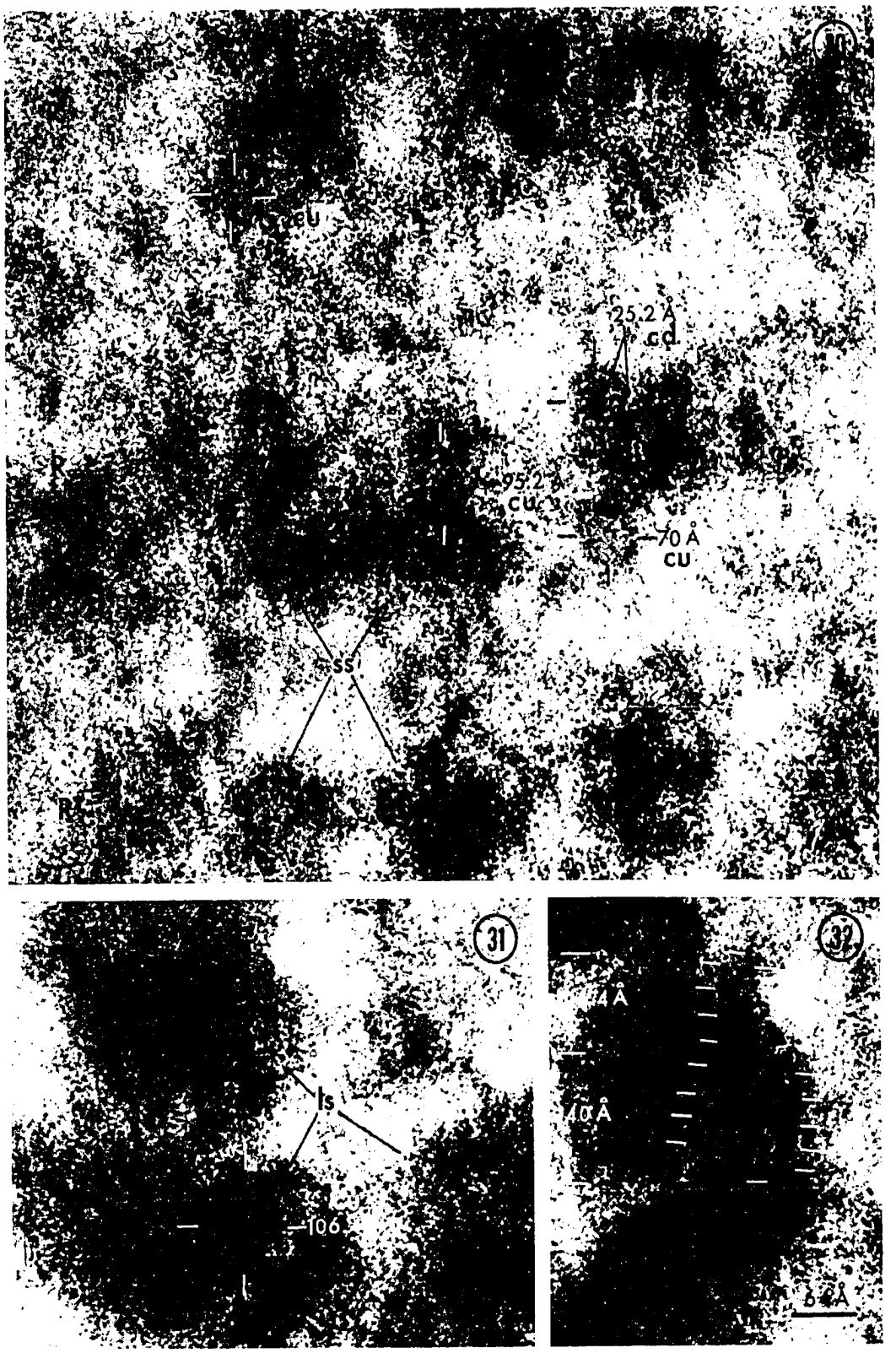

30
25.2 Å
CU
95.2 Å
CU
70 Å
CU
Ss
31
Is
106
32
44 Å
40 Å
8 Å

Figure 33--Within the cytoplasm of a cell process in holocrine-like deterioration a collagenic fibril (c) is organizing in the electron-dense cortical hyaloplasm. The undifferentiated fibril demonstrates a macroperiodicity (mp); however, the a, b, c, d, and e bands within the macroperiod are not discernible as discrete entities. The mean macroperiod measurement for the immature fibrils was found to be 490.1 Å.

The larger electron-dense structures (ls) described in Figure 29 and Figure 31 are present. Their incorporation or relationship to the organization of an overt fibril is incompletely understood at this time. The cell process is in a fibrous zone of a 12-week-old tissue complex. (Stained with aqueous uranyl acetate and lead citrate, X661,284).

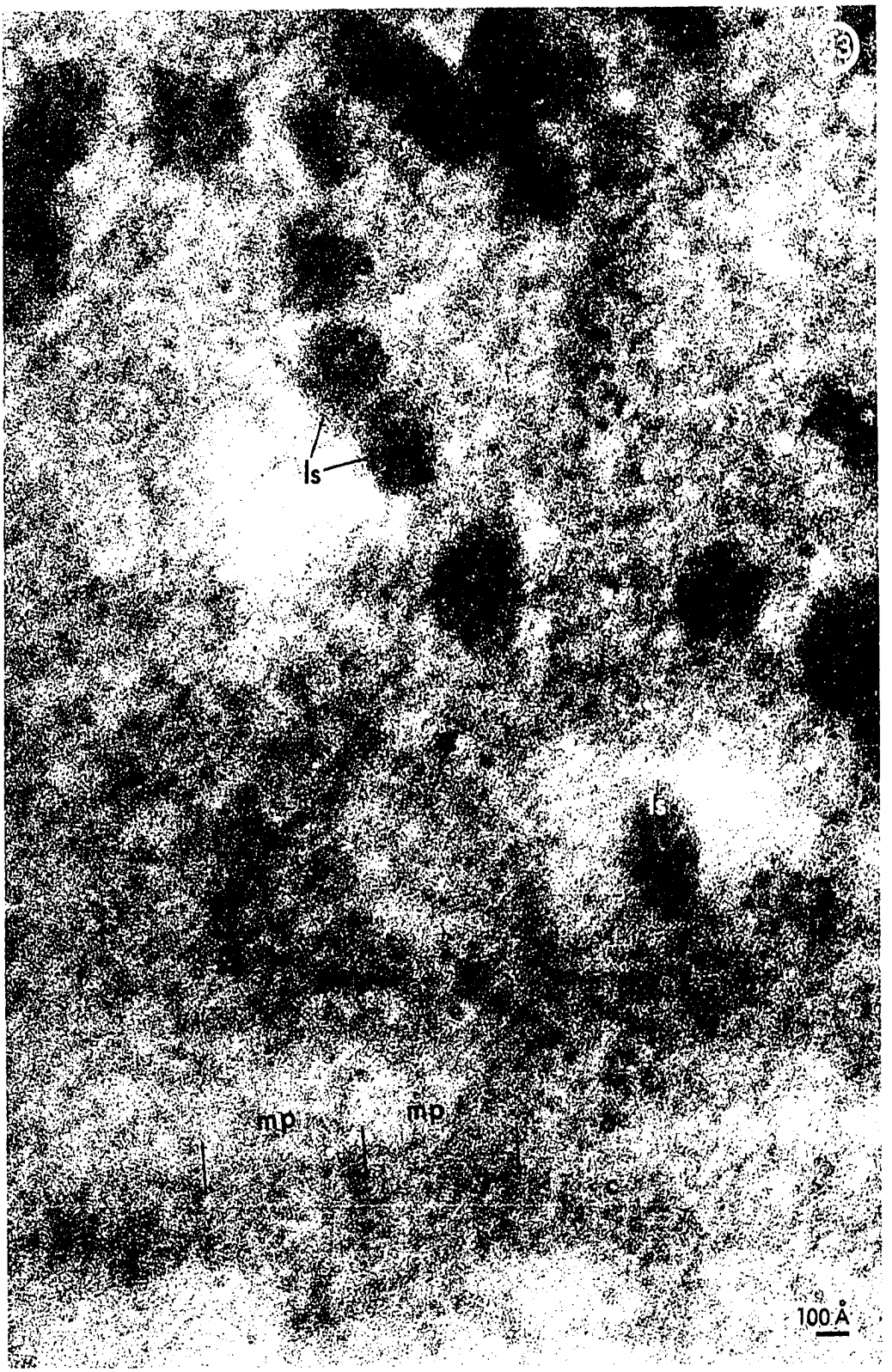

ls
s
mp
mp
c
100 Å

Figure 34--The accumulation and organization of collagenic precursory material within the electron-dense cortical hyaloplasm is demonstrated by corpuscular units (cu) of varying size within the structure of an immature collagenic fibril (c) and a large electron-dense structure (ls) in a cell process undergoing holocrine-like deterioration.

The macroperiodicity of the undifferentiated collagenic fibril is obscure. The tissue is from the fibrous zone of a 12-week-old connective tissue complex. (Stained with aqueous uranyl acetate and lead citrate, X2,103,494).

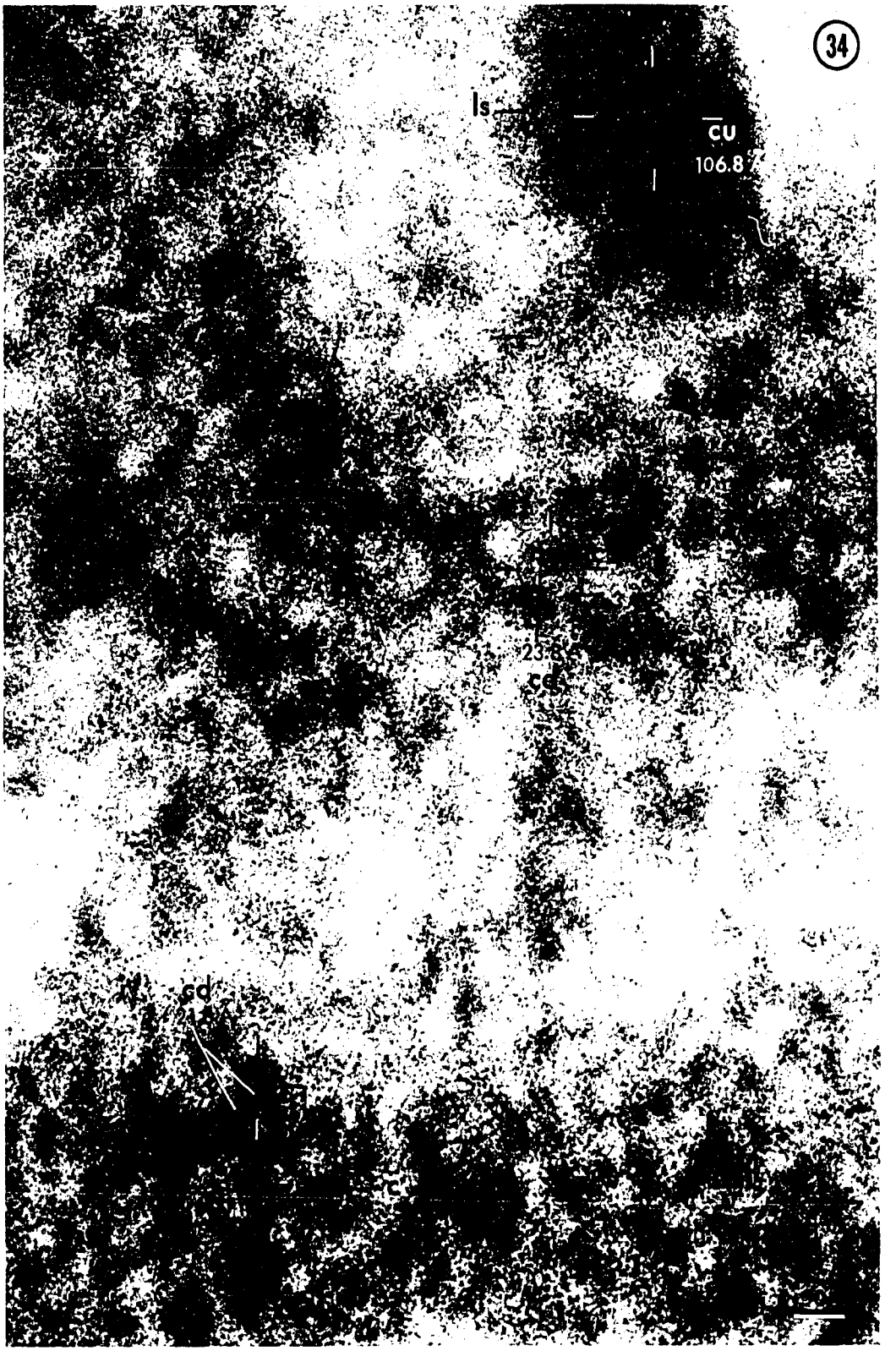
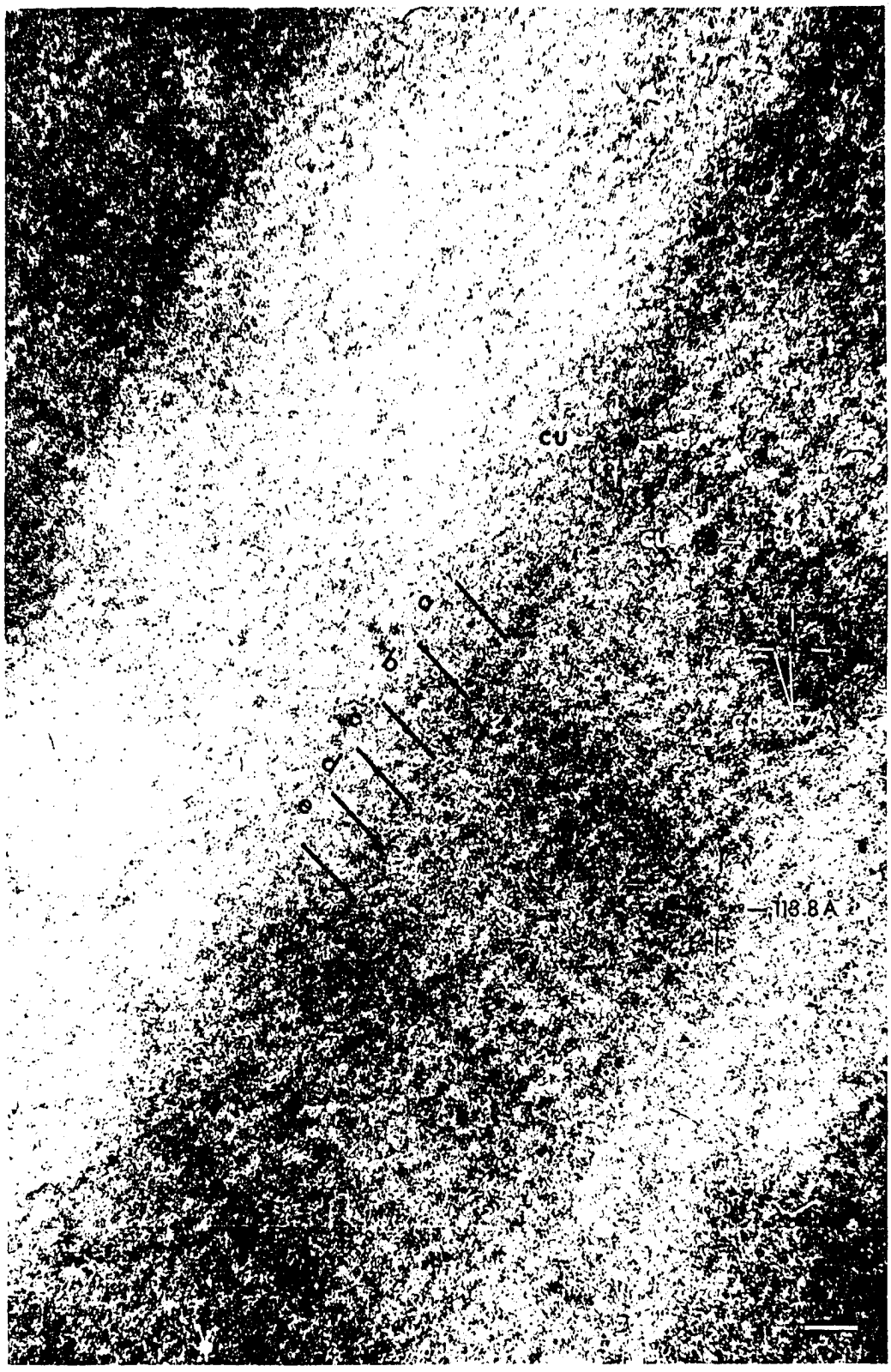

34
ls
cu
106.8
cd
cd

Figure 35--The differentiated collagenic fibrils of the fibrous zone of a 16-week-old tissue complex demonstrate the periodicity associated with collagenic fibrils. Within the macroperiod the conventional a, b, c, d, and e bands are present. The length of the macroperiod marked is 540.9 Å. Corpuscular units (cu) of varying size (52.2 Å to 132.8 Å) are observed within the macroperiod. The corpuscular units have an internal structure which is rope or cable-like. This cable-like structure is coiled to form the corpuscular unit. The cable-like structures have a mean diameter of 23.1 Å.

Note: Cable diameter (cd), bonds of the macroperiod (a, b, c, d, and e). (Stained with 1 per cent aqueous phosphotungstic acid, X1,053,000).


CU
CU
a
b
d
b
d
b
d
118.8 Å