THE UNIVERSITY OF MANITOBA

ULTRASTRUCTURAL FEATURES OF THE HARDING-PASSEY MELANOMA FOLLOWING MAXIMAL, MINIMAL AND INTERMITTENT COLCHICINE CHEMOTHERAPY

by

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ABSTRACT

This research programme was designed with a two fold objective: Firstly to establish whether colchicine induced morphologic changes in the Harding-Passey melanoma, reported in earlier studies, were transitory or more enduring. Secondly to compare the effects of maximal, minimal and intermittent forms of colchicine chemotherapy on the morphology of the tumor cells.

Optical and electron microscopic studies were initially carried out on untreated tumor to establish the normal morphology. This was followed by similar observations of tumors of experiments A, B and C.

EXPERIMENT A - MAXIMUM DOSAGE

Tumor bearing Strain A mice received daily intraperitoneal injections of colchicine in doses of 0.5 mgm/kgm for seven days, followed by 1.0 mgm/kgm daily for seven more days. Animals were sacrificed 24 hours after the last injection. One-half of the tumor was fixed for electron microscopy and was referred to as "Treated" tumor. The remaining tumor, without further treatment, was passed through five successive transfers of host mice at two week intervals. At the time of each transfer, small portions of tissue were fixed for electron microscopy and were referred to as Generations I, II, III, IV, and V. "Treated" tumor cells presented as the most significant observation the appearance of filaments 35-50Å in diameter in both mitotic and interphase cells, a feature not seen in control animals. In interphase cells filaments were generally perinuclear in position and closely related to ribosomes. In addition an increase in melanin

granules with tendency to marginate was evident in interphase cells. Electron microscopic observations on cells of colchicine treated tumor - Generation I-V, revealed the same features, viz. presence of filaments and increase in pigment granules. Desmosome-like adhesions were sometimes found between cell membranes.

EXPERIMENT B - MINIMUM DOSAGE

Tumor bearing mice received daily intraperitoneal injections of colchicine in doses of 0.5 mgm/kgm for seven days, one week following transplantation. Tumor tissue was fixed, referred to as Treated tumor, and remaining tissue transferred to new host animals. This procedure of transplantation took place every two weeks for 10 more weeks without further treatment, tissues referred to as Generations I, II, III, IV and V.

Fine structure observations revealed the presence of filaments 35-50Å in diameter, in smaller bundles and with less frequency than observed in experiment A. Less pronounced melanin accumulation and desmosomal junctions were seen in later generations as well. By generation V many cells had to be observed in order to find a cell possessing filaments.

EXPERIMENT C - INTERMITTENT DOSAGE

Strain A mice bearing tumors one week old received daily intraperitoneal colchicine injections in doses of 0.5 mgm/kgm for seven days. Treated animals were sacrificed 24 hours after the last injection, part of the tumor was fixed for electron microscopy and is referred to as 1^{o} Treated-tumor, part transplanted to a host animal. Tumors were transplanted every two weeks as in experiments A & B.

Alternating chemotherapy was performed at 1, 5 and 9 weeks following initial transplantation with successive fixed tissues being described as 1^{o} Treated-tumor; Generation I; 2^{o} Treated-tumor; Generation III; 3^{o} Treated-tumor; and Generation V.

Ultrastructurally the 1^{o} Treated tumor revealed filamentous bundles, usually perinuclear in position, and smaller in size than those observed in Experiment A. Generation I cells also demonstrated small amounts of filaments (closely associated with ribosomes), permeating the cytoplasm.

'2^{o} Treated tumor' cells were marked by increase in filaments in both interphase and mitotic cells. More cells revealed greater quantities of filaments than in any generations of the previous two experiments. Generation III; 3^{o} Treated tumor; and Generation V presented features almost identical to those observed in cells of '2^{o} Treated tumor.'

In summary these studies revealed that colchicine administration resulted in the formation of microfilaments as well as an increase in number of melanin granules in tumor cells of Harding-Passey melanoma. The effects were not transitory but more enduring than initially supposed to be, and persisted for 10 weeks through five generations of transplantation. These studies further showed that intermittent administration of colchicine resulted in more striking morphologic differentiation in tumor cells than that observed after maximal dosage.

The formation of microfilaments which resemble tonofilaments and junctional attachments in the tumor cell, suggested a possible attempt of the melanocyte to change into an epidermal-like cell.

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INTRODUCTION

INTRODUCTION

Treatment of neoplasms is principally in the domain of surgery and irradiation. For the past couple of decades, a new method that is gaining some degree of acceptance as a mode of treatment is chemotherapy. Chemotherapy of human cancers is used extensively in leukemias and other conditions where surgery is impossible or of limited value.

The new field of chemotherapy is one of the major areas of research. The effects of chemical analogues on experimental animal tumors are being investigated extensively with the hope of obtaining information that may be applicable to the treatment of human neoplasms. It is generally considered that the beneficial effect of chemotherapy and irradiation on tumors is not always necessarily mediated through necrosis of tumor cells, but also by alteration of the cellular cycle of growth, division and differentiation.

Amongst the tumors, the melanomas belong to a class that are generally radioresistant. Several substances such as antibiotics, alkaloids, and alkylating agents are being investigated as to their effects on animal melanomas. Some of these substances have the effect of either potentiating radiation effects or making tumors more susceptible to irradiation.

Previous studies on the Harding-Passey melanoma of mouse have been done at the light microscopic level by Harding and Passey (1930), Grand (1935), Luck (1956), Friedman and Drutz (1958), Sugiura (1963), Menon and Haberman (1970), and El-Fiky et al (1971). Electron microscopic observations on the Harding-Passey melanoma by Nathaniel

and Friedman (1963) and Nathaniel et al. (1968) were directed to the establishment of normal morphology of this tumor and its morphologic alteration following colchicine treatment. The study of Novikoff et al. (1968) was primarily directed to the elucidation of the origin of melanosomes.

The most relevant work to the present study was done by Nathaniel and Friedman (1963) and Nathaniel et al. (1968) in which they reported that administration of colchicine to mice bearing the Harding-Passey melanoma, resulted in the formation of microfilaments, 35-50Å in diameter, within the cytoplasm of both mitotic and interphase tumor cells.

The purpose of this study was three fold. First to determine whether the morphologic changes found in tumor cells of Harding-Passey melanoma by Nathaniel et al. (1968) were transitory, or would persist through several generations of transplantation. The second objective was to compare the fine structural changes in tumor cells resulting from maximal continuous and minimal continuous dosages of colchicine. Lastly, to study the effects of continuous versus intermittent therapy of Harding-Passey melanoma.

The problems related to cancer are many, most of which are little understood. However, the objective of all cancer researchers is to find ways and means of slowing down cell duplication or multiplication and/or in some way aid in cellular differentiation by altering the morphology of the tumor cell. In this way, it is hoped to stop the unending relentless progression of a tumor, or make it more amenable to other methods of treatment.

II

REVIEW OF LITERATURE

THE MORPHOLOGY OF CANCER CELLS

A tumor in general is composed of two basic components: 1. proliferating neoplastic cells which comprise the parenchyma of the tumor and 2. supporting elements made up of connective tissue and blood vessels. The parenchymal cell is by far the most important since it constitutes the bulk of the tumor. The stroma provides the structural support and nutritive blood supply for the parenchyma.

Little is known about the fundamental molecular change or changes that convert normal cells to tumor cells, but there is no doubt that neoplastic parenchymal cells have undergone some alteration that either promotes unrestricted growth or releases them from normal control mechanisms.

Tumor cells may morphologically resemble normal cells and is termed differentiation, on the other hand they may bear no resemblance to normal cells when it is known as dedifferentiation. In anaplasia, there is not only intracellular dedifferentiation but also an organizational structural alteration.

Light microscopic evaluation of a tumor is based on the cytoplasmic and nuclear morphology of the neoplastic cell. The cytoplasmic features indicate the degree of differentiation of the neoplasm and provide information as to its precrusor cell. The nuclear characteristics of a tumor cell are well known and include nuclear hypertrophy, irregularities in shape and structure, chromatin abnormalities and nuclear inclusions. To these criteria must be added the number of mitoses as an index of growth rate.

With the advent of the electron microscope, oncologists had

hoped that this new dimension of visualization would provide some criteria which would characterize a neoplastic cell and hopefully earmark the malignant one. This hope has not been realized fully. However, electron microscopy has not only reconfirmed all the lesions observed by the light microscope, but also considerably extended our knowledge of fine structure morphology of the nucleus and the cytoplasm of the tumor cells.

An exhaustive review of the fine structure morphology of tumors is not within the scope of this dissertation. Excellent reviews dealing with the ultrastructure of tumor cells are offered by Oberling and Bernhard (1961) and Bernhard (1963). It is therefore proposed to present a brief resume of the nuclear and cytoplasmic cytology of normal and tumor cells.

FINE STRUCTURE OF THE NUCLEUS:

In the classic sense, the nucleus of a cancer cell is characterized by its large size, irregularity in shape, conspicuous clumps of intensely staining chromatin, and presence of one or several large nucleoli.

Irregularities in nuclear outline caused by deep invagination of the nuclear membrane have been reported to be a common feature of cancer cells (Koller, 1963). Bernhard (1963) observed the presence of large nucleoli at the bottom of several nuclear invaginations in Reed-Sternberg cells of Hodgkins disease and suggested that it may represent a mechanism of self-regulation of nucleo-cytoplasmic exchange, faciliating the transfer of nucleolar material into the cytoplasm. On the other hand studies of Chambers and Wiser (1964) showed that the nuclei in Sarcoma I were spherical and located slightly eccentric in position. The fine structure of the nuclear membrane in normal and

malignant cells appeared similar, particularly with respect to the number and shape of its pores (Haguenau and Bernhard, 1955; Dalton and Felix, 1956). Bernhard and Granboulan (1963) observed in cancer cells an increase in the number and size of chromatin clumps which they suggest corresponds to chromosomal polyploidy.

It is a classic statement that cancer cells contain a very hypertrophic nucleolus. Cowdry and Paletta (1941) reported that the nucleoli of cancer cells frequently increase in number as compared with normal homologous tissues. Investigations of Caspersson (1950) and his school, and those of Brachet (1950) definitely demonstrated the importance of the nucleolus in the synthetic activity of the cell. Since the number and size of the nucleoli bear no constant relation to malignancy, the significance of the hypertrophic nucleolus as a specific phenomenon related to the cancer process is far from clear. It may merely indicate a manifestation of metabolic disturbance to which the cell is subject.

FINE STRUCTURE OF THE CYTOPLASM

With the advent of the electron microscope our knowledge of the cytoplasmic structures in normal and neoplastic cells greatly increased. The cytoplasm of cells in general, whether they be normal or cancer cells, contain formed bodies which are often classified into two categories: organelles when they are composed of living, differentiated cytoplasm; and inclusion bodies when they are metabolic or ingested substances. The first group includes (1) ribosomes, (2) endoplasmic reticulum with or without ribosomes, (3) Golgi complexes and lysosomes (4) mitochondria, (5) central body and centrioles, (6) microtubules and (7) filaments. The second group include (1) yolk, (2) fat and carbohydrate

deposits (3) secretion granules and (4) pigment granules.

In the following pages an attempt will be made to briefly review some of the organelles and inclusions encountered in normal and tumor cells.

RIBOSOMES AND ENDOPLASMIC RETICULUM:

Since free ribosomes and endoplasmic reticulum are closely related, they shall be discussed together. In the early part of this century, it was found that the cytoplasm of many cells contained material which stained with basic dyes and was called "chromophilic substance" or 'ergastoplasm' when it occurred in glandular cells. These areas gave a negative Feulgen reaction for DNA. Electron microscopic observations of Palade (1961) established that the chromophilic regions of the cell contained numerous, small dense granules, averaging about 150 Å in diameter, termed ribonucleoprotein or ribosomes.

Electron microscopic studies also showed that the cytoplasm of cells contained a delicate network of channels which came to be accepted as an organelle and was termed "endoplasmic reticulum". The term granular or rough surfaced endoplasmic reticulum was given to these system of channels which were associated with ribosomes, and 'agranular reticulum' or smooth surfaced reticulum to those without ribosomes. The degree of development of the reticulum varied in different cells and in different phases of physiological activity of the same cell type. The granular endoplasmic reticulum corresponds to the "ergastoplasm" of the light microscopist.

Reviews on ribosomes and endoplasmic reticulum are those of Haguenau (1958), Porter (1961) and Rich (1963).

It is well known that cytoplasmic basophilia often increases in

tumor cells and corresponds to an increase in free ribosomes (Bernhard, 1963) and only rarely associated with an increase in granular endoplasmic reticulum as in Rous sarcoma (Epstein, 1957), mouse hepatomas (Fawcett and Wilson, 1955) and in some primary cancers of human liver (Rouiller, 1957). In a study related to the forms of endoplasmic reticulum in squamous cell carcinoma, Klehr and Klingmuller (1972) observed different forms of granular endoplasmic reticulum ranging from scanty or diffuse tubes to lamellae in the form of "whorls" or "finger prints" in neoplastic keratinocytes. Studies up to date indicate that there is a trend in the cancer cell towards a decrease in organized granular endoplasmic reticulum (Oberling and Bernhard, 1961).

Rapidly growing embryonic cells and normally growing cells resemble tumor cells in exhibiting an increase in free ribosomes (Howatson and Ham, 1955). Cells such as the pancreatic acinar cells concerned with protein synthesis have an elaborate system of granular endoplasmic reticulum.

It has been suggested by Munger (1958), Siekevitz and Palade (1958) and Slautterback and Fawcett (1959) that for enzyme synthesis, the ribonucleoprotein (RNP) granules apparently must be associated with endoplasmic reticulum. Palade (1961) revised his original hypothesis and conceded that RNP particles themselves were capable of synthesizing proteins for local consumption such as growth and repair without being related to membranes. It may therefore be summarized that free ribosomes are principally concerned with the formation of proteins, including the thousand or so enzymes necessary for cell activity; while ribosomes associated with membranes are involved in the process of synthesizing proteins for export from the cell such as the digestive enzymes secreted

by the pancreatic acinar cell (Palade, 1966).

THE GOLGI COMPLEX AND LYSOSOMES:

The Golgi complex was first described by Camillio Golgi in 1898 in the cells of the nervous system and was given the name internal reticular apparatus. In the routine histological preparation , it is sometimes identified as a negative image in an unstained juxtanuclear area. In tissues subjected to prolonged impregnation with silver or osmium the area is demonstrated as a blackened network in the juxta-nuclear region.

The early investigations of Sjostrand and Hanzon (1954) and Dalton and Felix (1956) established the fine structure of the Golgi apparatus. The electron microscope revealed that the Golgi complex is a ubiquitous cell organelle composed of parallel arrays of flattened sacs or cisternae that were often expanded at their ends, associated with vesicles and vacuoles. The vesicles were generally located close to the convex outer surfaces of the stacks, sometimes referred to as the "forming face", and the vacuoles found within the concave inner surfaces of the cisternal stacks sometimes designated the "maturing face".

The Golgi complex has been shown to be concerned with concentration and packaging of secretory products, the synthesis of complex poly-saccharides, and the production of lysosomes. Excellent reviews on the ultrastructural morphology of the Golgi complex have been offered by Dalton (1961), Beams and Kessel (1968), Novikoff (1968) and Neutra and Leblond (1969).

The Golgi complex is present in all neoplasms. In general it is hypertrophied in tumors which are under hormonal stimulation such as mammary tumors, and in those which show secretory activity as in

melanomas. In highly dedifferentiated tumors such as the ascites tumors of Yoshida and Ehrlich, the Golgi complex is greatly reduced in size (Wessel and Bernhard, 1957). In his review, Bernhard (1958) states that neither hypertrophy or hypotrophy of the Golgi are characteristic for all neoplasms.

The Golgi complex has also been considered as the site of origin of melanin granules in several mammalian tumor cells (Birbeck et al., 1956; Dalton, 1959; Wellings and Siegel, 1959). Rose and Stehlin (1961) observed an association between the Golgi complex and melanin elaboration. Variations in size and nature of the Golgi complex were described in comparing B-16 and S-91 mouse melanomas (Demopoulos, 1965). It was suggested that one of the possible reasons for a prominent Golgi complex in a fast growing melanoma such as the B-16 melanoma is the necessity for production of lysosomes. These lysosomes then formed the catabolic vacuoles which enclosed old melanized melanosomes.

MITOCHONDRIA:

In 1890 Altmann using fuchsin demonstrated particles which he called "elementary living particles, bioblasts, present in all cells". These thread-like, elongated bioblasts, seen following staining with alizarian and crystal violet were termed "mitochondria" by Benda (1898). With the development of adequate methods of fixation and thin sectioning for electron micorscopy, Sjostrand (1953) and Palade (1953) described independantly the fine structure of the internal membranes of the mitochondrion. It is now well established that mitochondria serve as mobile "power plants" furnishing the energy required for the several functions of the cell through a process termed 'oxidative phosphorylation'.

The extent to which this process occurs varies as to the stage of the cell cycle and to the instantaneous requirements of the cell for special situations. This energy, linked with adenosine triphosphate (ATP) is required for many different processes, including ionic transport across cell membranes, synthesis of protein, and muscle contraction. Mitochondrial structure and function has been the subject of several reviews (Novikoff, 1961; Lehninger, 1964; Parsons, 1965; Racker, 1968 and Roodyn, 1968).

Mitochondrial number and size vary considerably in tumor cells as they do in the normal. The general impression is that cancer cells have fewer mitochondria which tend to be smaller than normal cells (Oberling and Bernhard, 1961). However, it is easy to find exceptions to this general rule. Mitochondrial shape varies considerably in tumor cells as well as in the normal and is considered to be more dependant on the metabolism of the cell than on the origin of the tumor.

Mitochondria whose shape varied from a sausage to oval to circular have been observed in mammary adenocarcinomas (Mehard at al., 1971). Tubular mitochondria characterized by longitudinal configuration of cristae mitochondrialis have been described in normal tissues such as adrenal medulla and liver (Belt and Pease, 1956) and in macrofollicular tumors of the theca-granulosa series (Hamlett et al., 1971). Tubulo-vesicular mitochondria have been reported in normal steroid secreting organs such as ovaries, corpus luteum and adrenal cortex (Belt and Pease, 1956), and in Harding-Passey melanoma of mouse (Nathaniel el al., 1968).

As in the normal cells the location of the mitochondria in neoplastic cells was highly variable, as seen in rat squamous cell carcinomas

(Bernhard, 1958; Pierce and Wallace 1971), Sarcoma I (Chamber and Weiser, 1964), and human mammary carcinoma (Ozzello, 1972). Novikoff (1968), Seiji et al., (1971) in Harding-Passey melanoma and Demopoulos et al., (1965) in B-16 and S-91 mouse melanomas agree that mitochondria from one set of tumors generally resemble those of other tumors.

Swelling of mitochondria was often seen in neoplastic cells (Selby et al., 1956) and resembled mitochondrial swelling seen in various injuries such as cloudy swelling (Gansler and Rouiller, 1956). The significance of swollen mitochondria in some tumors, whether they are related to the malignant process and represent a primary lesion or the result of rapid aging and degeneration of these cells or of poor nutrition and respiration, is far from clear.

Condensation of mitochondria resulting in small dense mitochondria without internal structure have been reported by Oberling et al., (1951), Porter and Kallmann, (1952), and Selby and Berger (1952) in rapidly growing cells such as embryonic, neoplastic and inflammatory cells. These structures have been called growth granules (Porter and Kallmann, 1952), ultrachondrioma (Oberling et al., 1951) and microbodies, and are considered to represent stages in mitochondrial regeneration.

CENTRAL BODY, CENTRIOLES:

The central body is the specialized zone of cytoplasm which contains the centrioles. Its role in mitosis is well known. Investigations of Amano (1957), Yamada (1958) and De Harven (1968) established the fine structure of the centriole as a hollow cylinder whose walls were composed of nine evenly-spaced, triplet, hollow tubules, embedded in an amorphous matrix. Though earlier light microscopic investigations claimed that centrioles becomes hypertrophic in tumor cells, electron

microscopic studies of De Harven (1956) did not reveal any ultrastructural features of the centriole in cancer cells that would distinguish it from the normal.

MICROTUBULES:

Electron microscopic studies of glutaraldehyde fixed tissues revealed the existence of long, slender, cylindrical structures, the microtubules, in diverse cells. Microtubules have a diameter of 200-270Å and are straight or slightly curved. In cross section they appear circular, composed of on the average 13 globular subunits, each about 40-50Å in diameter. Microtubules resemble muscle protein, actin, in having binding sites for nucleotides. The proteins of microtubules contain specific binding sites for colchicine which explains the disruption of microtubules in the presence of this agent resulting in metaphase arrest.

Microtubules besides playing a significant role in mitosis as spindle fibers, function as a resilient cytoskeleton in maintaining cell shape and as contractile elements of cilia and flagella.

Studies on microtubules are those of Slautterback (1963), Sanborn et al., (1964) and Porter (1966) to mention a few.

FILAMENTS:

Microfilaments measuring 30-100Å in diameter, indeterminate in length and in varying amounts have been observed in numerous cells. They have been observed in diverse cells as epithelial cells, smooth and striated muscle cells, endothelial cells, neuroglia, adrenal cortex, intersititial cells of testis, and many others. These filaments are said to subserve several functions: maintaining the shape of the cell,

functioning as a microskeleton, contributing to cell movement as in cytoplasmic streaming and in the contractile mechanism of muscle fibers.

The fine filaments observed in epithelial cells are designated tonofilaments. Aggregations of tonofilaments constitute tonofibrils of the histologist. Tonofilaments often terminate in the "desmosomes". The large numbers of filaments in epidermal cells and their apparent involvement in the process of keratinazation led to the general notion that tonofilaments may be keratin or its precursor. However, evidence for such a concept is not universally acknowledged.

Giroud and Leblond (1951) distinguished soft and hard keratin based on morphological and chemical criteria, the former being found in the epidermis, internal root sheath of hair follicles and medulla of hair; and the latter in nails, cortex and the cuticle of hair. Matoltsy (1962) distinguishes three forms of keratinization based on morphological criteria: one which is elaborated through the formation of amorphous cytoplasmic granules, another through production of cytoplasmic fibrils, and a third through manufacture of both cytoplasmic fibrils and granules.

The electron microscopic studies of Birbeck and Mercer (1957), Brody (1959 a, b) and Rogers (1959) significantly contributed to a better understanding of the finer details of the mechanism of keratinization. Suffice if is to say that both the cytoplasmic filaments and fine granules contribute to the formation of keratin, the relative proportions of these components determining the type of keratin. Based on histochemical studies in vertebrate keratins Barnett and Sognnaes (1962) stated that most keratinized tissues contain some free sulfhydryl groups and not all these are oxidized to disulfides in the process of

keratinization. Some of the regions concerned with the formation of fibrous keratins appear to contain disulfides in addition to sulfhydryls. Menefee (1957) stated that in keratinization tonofilaments have a spatial relationship to mitochondria and he was of the opinion that keratin sub-units could be synthesised in the ergastoplasmic system or elsewhere in the cell, while mitochondria might be involved in a later stage of polymerization into visible tonofilaments. Electron microscopic studies of Rhodin and Reith (1962) contributed significantly to our understanding of the formation of soft keratin. They postulated the following steps in the formation of soft keratin: The first step was the appearance of microfilaments 35Å in width, closely associated with free ribonucleoprotein (RNP) particles and presumably contributing to its formation. Continued growth and aggregation of tonofilaments takes place along with the appearance of keratohyalin granules under the influence of free RNP particles. Eventually the aggregated tonofilaments and keratohyalin granules associate as one of the last steps in the evolution of soft keratin (Fig. 1).

Mammalian keratins give us x-ray diffraction diagrams with a periodicity of 5.15Å which is characteristic of the alpha form (Astbury, 1933). Rudall (1953) in a series of studies established that x-ray diffraction patterns of epidermis were similar to those of alpha keratin.

An excellent monograph on the significance of sulfhydryl and disulfide groups in the epidermis has been offered by Montagna (1962) in which he discusses the theories of keratinization.

Microfilaments have been observed in tumor cells. Ozzello (1972) found fine filaments, perinuclear in position, in mammary carcinoma

Figure 1. This schematic diagram represents the changes associated with the formation of soft keratin in the esophagus of adult mice, and skin of newborn rats. The left side of the figure represents surface epithelium undergoing soft keratinization. The right schematic outlines the events which occur during the formation of soft keratin.

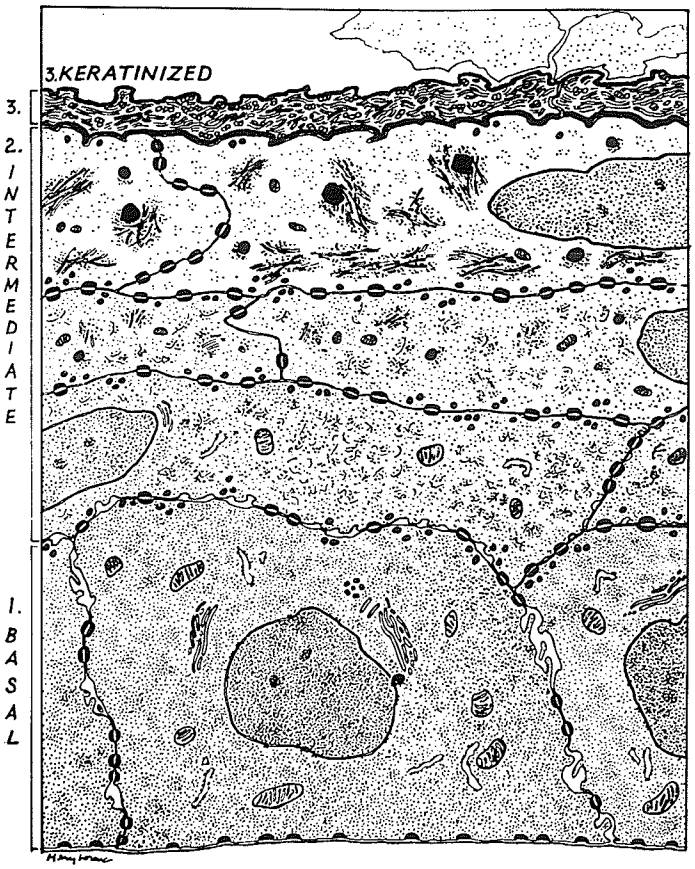
A. The first indication is the appearance of delicate tonofilaments closely associated with ribonucleoprotein particles.

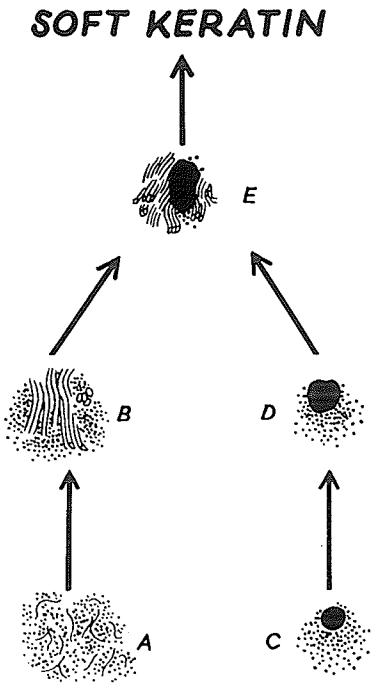
B. The tonofilaments grow and aggregate as more filaments are formed. Ribonucleoprotein particles are also closely associated with these events.

C & D. Represent formation of keratohylin granules.

E. The largest tonofilaments become associated with the irregular keratohyaline granules prior to stabilization and consolidation which results in soft keratin.

(after J.A.G. Rhodin and E.J. Reith, 1962)





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cells which he considered to be contractile proteins. Flaxman (1972) in a study of basal cell cancer reported the presence of bundles of cytoplasmic filaments 50-70Å in diameter. In a fine structure study of unincubated (control) Ehrlich ascites carcinoma, Molnar and Bekeski (1972) described conspicuous bundles of fine filaments which were perinuclear in position.

Occurrence of fine filaments has also been reported in tumors treated with anticancer drugs. Robbins and Gonatas (1964) using colchicine as a spindle inhibitor, noted the presence of filaments, 60-80Å in diameter, in interphase cultured HeLa cells. Nathaniel et al., (1968) also observed bundles composed of fine filaments in both interphase and mitotic cells of the Harding-Passey melanoma following colchicine treatment.

CELL MEMBRANE SPECIALIZATION:

Cells exhibit specializations of their plasma membranes along their free, lateral and basal surfaces suited to carry on the diverse functions required of them. These specilizations are best visualized in epithelia. The specializations of the surface for cell to cell attachment are reviewed by Weiss (1960) and Curtis (1962).

The plasma membranes of adjacent cells constituting the epidermis or for that matter any organ are usually parallel and are separated from one another by an intercellular space of 150-200Å in width. It is widely believed that the intercellular cleft is occupied by a mucopolysaccharide. Gasic and Berwick (1962) demonstrated histochemically the presence of a carbohydrate rich layer on the surface of some mammalian cells. A more extensive confirmation of the "cell coat"

at the surface of the mammalian cells was given by Rambourg and Leblond (1967). These investigators used periodic acid-silver methanamine, which was said to be fairly specific for glycoproteins and demonstrated in the tissues of adult rat that most cells were covered with a thin layer of stained material. They also observed that staining usually vanished in regions where two adjacent plasma membranes were fused to form "tight junctions". They concluded that the "cell coat" is located outside the plasma membranes and contained glycoproteins and some acidic residues.

The uniform spacing found between adjacent cell membranes suggests that some cohesive force is operating over the entire surface of cell to cell contact. To what degree is this cohesion due to the intercellular material and to what extent to long range forces attributable to the properties of the membranes themselves are not known. Coman and Anderson (1955) observed that tumor cells derived from epithelium have been shown to be deficient in calcium which may be an important factor in decreasing the cohesiveness between cancer cells thereby permitting invasiveness and wide spread dissemination.

The junctional complexes observed between various epithelial cells consist of three elements which are usually closely and sequentially arranged. However, they may also occur independantly of one another in other tissues. The three elements are: tight junctions (zonula occludens), intermédiate junctions (zonula adherens) and the desmosomes (macula adherens) (Fawcett, 1961; Farquhar and Palade, 1963). These three function as intercellular attachment devices, but the tight junction differs from the other two in that the apposed cell membranes come into contact resulting in fusion of their outer leaflets, thus

completely eliminating the intercellular space. This type of junctional complex is considered to function as a seal or barrier to diffusion as well as serving as a pathway of low electrical resistance permitting rapid spread of excitation from cell to cell.

Although intercellular attachments between cancer cells are not generally found, few authors have described them in neoplastic cells. Hamlett et al., (1971) reported the presence of "desmosome like" structures between cell membranes of macrofollicular tumor cells. Ozzello (1972) also observed desmosomes in mammary carcinoma. Nathaniel et al., (1968) described the occurrence of junctional complexes in tumor cells of Harding-Passey melanoma of mouse following colchicine administration.

VIRUS PARTICLES:

There have been a number of review articles on viruses and tumors (Bernhard, 1958, 1960; Dmochowski, 1960; and Dalton and Haguenau, 1962). Tumor viruses have diameters which occupy the whole size range from the pox group measuring between 220-260 mμ in diameter to the polyoma-papilloma type measuring 30 mμ. Viruses may be associated with nucleus such as the polyma and Shope papilloma agent, or may be found in the cytoplasm as in leukemias and chicken viruses. Some viruses may be present in both the nucleus and cytoplasm. Examples of this kind are Lucke virus in the frog kidney and agents of the herpes group which exhibit a complex maturation of the particles starting in the nucleus and continuing in the cytoplasm.

Bernhard (1960) described three types of cytoplasmic virus particles based on their size and shape and designated them as A, B and C. Type A

particles measured about 70 mμ, doughnut shaped, and were found in a variety of mouse tumors as melanomas, sarcomas and ascites tumors. These particles were found in relation to the endoplasmic reticulum (Dalton and Felix, 1956; Sobin, 1964) or in the Golgi zone. Nathaniel et al., (1968) described the evolution of the type A virus particle from the endoplasmic reticulum in the Harding-Passey melanoma of mouse. They observed the thickening of the membrane of the granular endoplasmic reticulum as the earliest sign in the formation of the virus particle. The thickened portion of the endoplasmic reticulum was gradually invaginated into the lumen of the endoplasmic reticulum. As it protruded into the lumen, it acquired a well defined stalk, which was eventually lost and the doughnut shaped virus-like particle comes to be free in the cistern. Novikoff et al., (1968) described the virus particles in Harding-Passey melanoma to be of the C type. The possibility exists that variations in technique have led the two groups of investigators to differ in their interpretation of the virus particles encountered in the Harding-Passey melanoma. In a study of mammary cancer Sarkar and Moore (1972) described the staining difficulties in trying to differentiate type B from type C virus particles.

Type B virus particles were predominantly located extracellularly and measured about 150 mμ in diameter with an eccentric nucleoid and represent the Bittner Virus (Bernhard et al., 1955).

Type C virus particles have been associated with leukemias. There is some experimental evidence that the C-particles represent the infective agent in Friend's leukemia (Harven and Friend, 1958).

Banks et al., (1971) in a study of mouse 6C3HED ascites tumor, described type C virus particles within the cytoplasm but did not indicate

their origin. Michaels et al., (1971) in a study of Rowson-Parr virus observed type C virus particles in the intercellular space.

Dalton (1972) proposed the formation of B and C type virions from mammary-tumor-virus either by the incorporation of an intracytoplasmic A particle into the forming bud, or by development of two layers of the nucleoid beneath the plasma membrane during bud formation. Volkman et al., (1971) observed a progressive modification of cellular morphology in the early passages of a newly induced murine myeloma tumor. This was accompanied by an apparent increase in intracellular A-type virus particles associated with the endoplasmic reticulum, but with immunoglobulin production remaining constant.

MELANOSOMES AND MELANIZATION

The presence of melanin granules within a tumor cell is the most significant criterion for identifying the neoplasm as a melanoma. Observations by light and electron microscopy have led to the development of three conflicting theories as to the origin of melanin pigment found within melanomas. The theory of nuclear origin (Meirowsky and Freeman, 1951) states that the granules are supposed to be composed of extruded nuclear material.

The second theory proposes that the Golgi complex is the organelle responsible for the evolution of the melanin granules (Birbeck, 1963; Seiji et al., 1963; Wellings and Siegel, 1963). Birbeck and Barnicot (1959) from an electron microscopic study of melanin formation in human hair follicles stated that the Golgi apparatus initiated the gradual melanization process. Wellings and Siegel (1963) based on studies in several animal and human melanomas proposed a hypothesis

of melanin granule formation. Briefly stated, tyrosinase rich protein, visible as minute particles, is synthesised in the granular endoplasmic reticulum under the influence of cytoplasmic ribonucleic acid with the participation of cell energy producing processes. The minute particles accumulate within the ergastoplasmic sacs and travel to the Golgi apparatus where they are concentrated to form a premelanin granule. Karasek and Hultin (1962) in their studies on the Harding-Passey melanoma observed that the formation of melanin granules in melanocytes occurs in the part of the cell associated with membranes of the Golgi complex. Zelickson (1962) in his studies on the human malignant melanoma acknowledged the presence of melanin granules in the region of the Golgi complex, and further stated that the granules in this area appear to be more immature.

The mitochondrial origin of melanin granules has been put forward by du Buy et al., (1949) based on biochemical data which showed that many enzyme systems are common to both mitochondria and melanin granules. Woods (1959) subscribed to this theory due to the presence in both structures of cytochrome oxidase, succinic oxidase, cytochrome C and tyrosinase (the essential element of melanin formation).

Dalton and Felix (1959), Seiji et al., (1963) and Wellings and Siegel (1963) found no ultrastructural evidence for mitochondrial derivation of melanin. The electron microscopic evidence agrees with the enzyme studies carried out on particles obtained from fractionated cells suggesting that melanin granules and mitochondria are different morphological and chemical entities (Baker et al., 1960). However, the available data does not rule out the possibility that mitochondria and melanin granules may arise from identical precursor material

(Woods and Hunter, 1959).

The process of melanization has been well put forth by Seiji et al., (1963) and is shown in fig. 2. Nomenclature used in describing melanin formation start with pre-melanosomes, the smallest partially darkened units having substructure, ending as fully pigmented melanin granules from which substructure has been effaced. The features of melanization as proposed by Seiji et al., (1963) have been generally accepted. The amount of tyrosine present within the granule appears to be the determining factor as to the stage of development of the melanin granule (Fig. 3).

Compound melanosomes, which contain mitochondria and endoplasmic reticulum, as well as a number of premelanosomes, were considered by Novikoff et al., (1968) to be autophagic vacuoles. Seiji and Otaki (1971) in a recent paper agreed with this statement in their studies on the Harding-Passey melanoma. Wolff and Honigsman (1972) conclude that since lysosomes labelled with a tracer (thorotrast) fused and emptyed into melanosome complexes, the melanosome complexes within keratinocytes, represent secondary lysosomes.

THE MITOTIC CELL

Mammalian cells reproduce by a process known as mitosis which consists of an equal division of nuclear material (karyokinesis) followed by a division of the cell body (cytokinesis) in which each daughter cell receives one of the daughter nuclei. Mitosis is divided into four stages: prophase, metaphase, anaphase and telophase (Fig. 4). Excellent reviews on mitosis are offered by Leblond and Walker (1956), Mazia (1961) and Levin (1963) to mention a few. Ultrastructural studies

Figure 2. This figure demonstrates the stages in the development of melanin granules. Three sequential time periods have been described categorizing the stages of development, they being:

I. biosynthesis of protein

II. biosynthesis of organelle

III. biosynthesis of melanin.

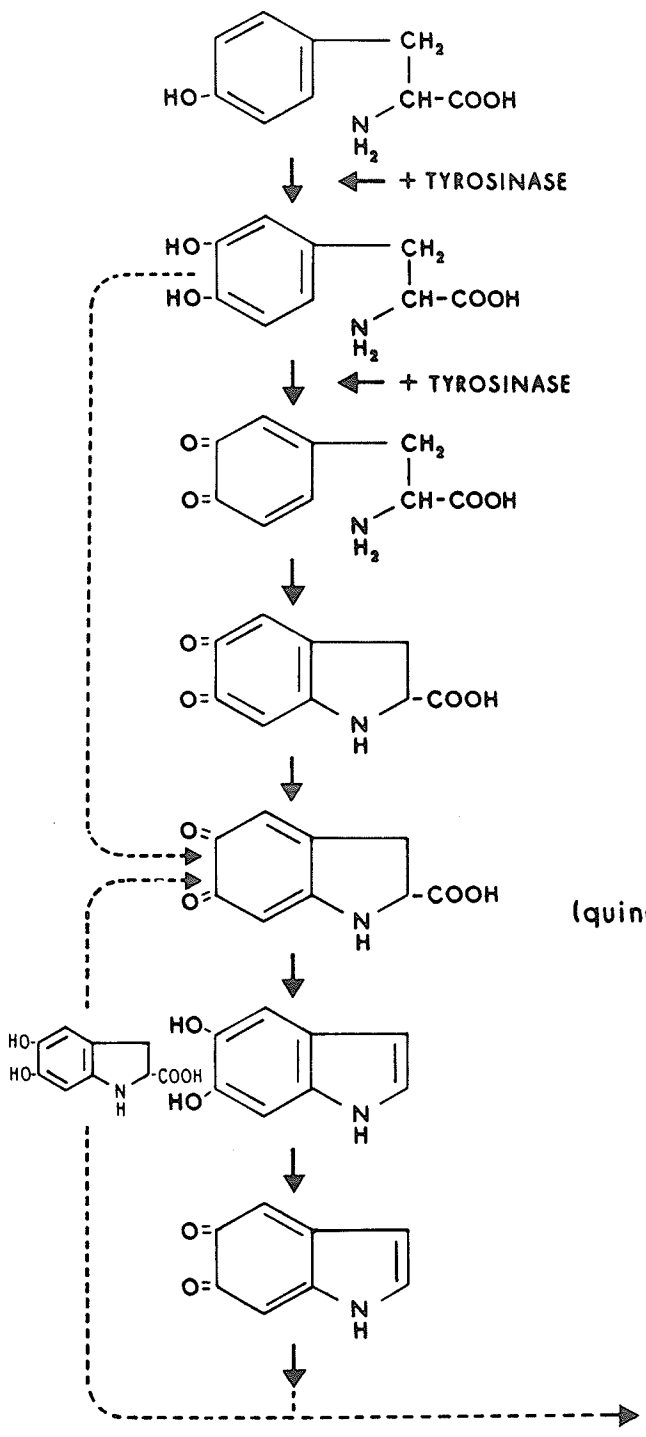
(after Seiji et al, 1963)

NOMENCLATURE	MORPHOLOGY	BIOCHEMICAL COMPOSITION		
RIBOSOME	100-150 Å	RNA + PROTEIN	Site of polypeptide biosynthesis	I
GOLGI VESICLE	0.05 μ	PHOSPHOLIPID + PROTEIN	Condensation (?)	
INTERMEDIATE VESICLE	 0.5 μ	PHOSPHOLIPID + PROTEIN	Stage of "Pro-Tyrosinase" arrangement in a structural form	II
PREMELANOSOME	 0.7 x 0.3 μ *	"PRO-TYROSINASE"	End stage of "Pro-Tyrosinase" arrangement and final product in albino melanocyte	
MELANOSOME	 0.7 x 0.3 μ *	TYROSINASE + MELANIN	Specific site of melanin formation	III
MELANIN GRANULE	 0.7 x 0.3 μ *	MELANIN + NO MEASURABLE TYROSINASE ACTIVITY	Final product of melanocyte	

* *Typical values for human brown melanin granules.*

Figure 3. This figure depicts the stepwise procedure in the biochemical synthesis of melanin from tyrosine. Since the fully pigmented melanin granule is virtually free of tyrosinase enzymes, the elimination of tyrosine through subsiduary steps eventuates in the completely tyrosine free melanin granule.

(after J.N. Attie and R.A. Khafif, 1964)



TYROSINE
(4 hydroxyphenylalinine)

DOPA
(3, 4 dihydroxyphenylalinine)

DOPA QUINONE
(3, 4 dioxyphenylalinine)

LEUCO COMPOUND
(REDUCED HALLOCHROME)
(5,6 dihydroxydihydroindole carboxylic acid)

HALLOCHROME
(DOPACHROME [RED])
(quinone of 5,6 dihydroxydihydroindole carboxylic acid)

5, 6 dihydroxyindole

indole 5, 6 quinone

MELANIN

Figure 4. This diagrammatic representation features the major events taking place during the cell cycle with the different phases of the division cycle.

G_1 - post-mitotic

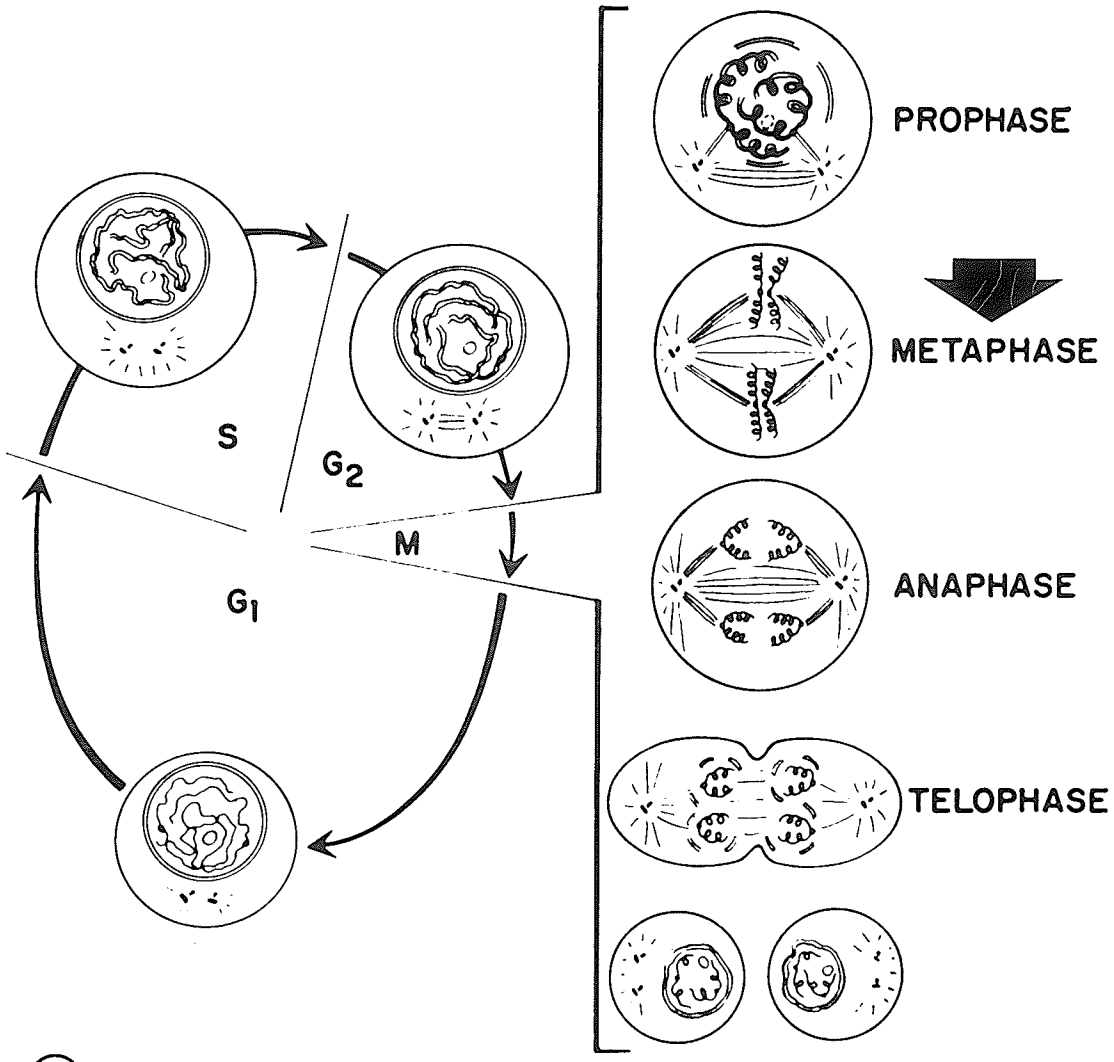
S - DNA synthesis

G_2 - pre-mitotic

M_1 - mitotic

The arrow indicates the site of action during the mitotic cycle in which colchicine acts upon the cell, causing arrest and the so called "colchicine metaphases".

(after D.A. Karnovsky and B.C. Clarkson, 1963)



4 MITOTIC CYCLE

MITOSIS

on mitosis are those of Harris and Mazia (1963); Robbins and Gonatas (1964); Brinkley et al., (1967) and Inoue and Sato (1967).

Electron microscopy has not been able to augment our knowledge of abnormal chromosomal structures in cancer cells and the light microscope is still a more satisfactory tool for the investigation of mitosis. The ultrastructure of the achromatic apparatus consisting of the centriole, spindle and kinetochore in the tumor cells has not been found different from that observed in normal cells.

CHEMOTHERAPY OF TUMORS

The chemotherapy of cancers is an intriguing field. Recent advances in molecular biology have furthered our understanding of the action of anticancer agents. Conversely several antitumor agents like actinomycin-D have become valuable tools in the study of the mechanism of action of hormones and other substances.

It is to be conceded that the traditional forms of treatment of cancer such as surgery and radiation are principally useful in circumscribed types of neoplasms. The surgeon operates on a comparatively small area. The patient can tolerate cancerocidal radiation to only a limited area. Cancer chemotherapy is a form of generalized treatment, especially suited to a disease, which is all too often disseminated when the cancer patient is first seen by the clinician.

Chemotherapy has been advocated as the method of treatment for many types of tumors, especially those said to be radio-resistant (Knock, 1967). Among this group are the sarcomas, gliomas and melanomas.

CLASSIFICATION OF CHEMOTHERAPEUTIC AGENTS:

The chemotherapeutic drugs most often used have been divided into

alkylating agents eg., nitrogen mustard, ethylene imines and alkylmethane sulfonates; alkaloids eg., vincristine and vinblastine; antibiotics eg., actinomycin-D and mitomycin-C; and a chemically unrelated group of alkaloids including colchicine and colcemid.

Antimitotic agents for cancer chemotherapy can also be divided into chromosome poisons and spindle poisons (Dustin, 1963). The spindle poisons include the sulfhydryl inhibitors such as arsenose, iodacyl, and meleimide derivatives, as well as the inorganically unrelated substances such as colchicine, vinblastine, and vincristine. Many substances such as nitrogen and the sulfurs can poison both chromosomes and the achromatic apparatus.

SITE OF ACTION OF CHEMOTHERAPEUTIC DRUGS:

Many cancer drugs are aimed at specific parts of the cell, such as deoxyribonucleic acid (DNA) of chromosomes; or several ribonucleic acid (RNA) types; or essential sulfhydryl (SH) - containing residual proteins bound to DNA in chromosomes; or the sulfhydryl (SH) - containing protein of cell membranes (Knock, 1967).

It may be stated that the majority of anticancer agents attack either (1) at the level of large cellular polymers such as DNA, RNA or protein or (2) at the level of small chemical units or monomers such as purines, pyrimidines and aminoacids from which macromolecules of the cancer cell are built. The great bulk of drugs used in the treatment of cancer attack at the level of the large polymers.

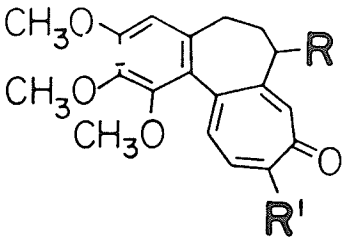
COLCHICINE AND ITS EFFECTS:

Colchicine, a mitotic inhibitor, is an alkaloid derived from the plant colchicum autumnale (Fig. 5). Eigsti and Dustin (1955) in their

Figure 5. This figure illustrates the structural chemical formulas of plant alkaloids commonly used in cancer chemotherapy.

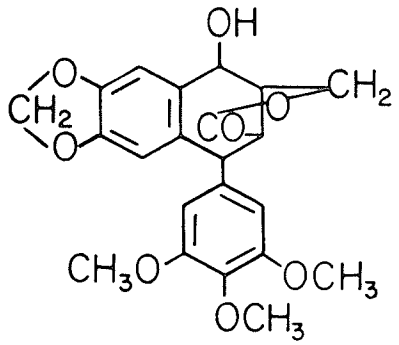
(after W.H. Cole, 1970)

COLCHICINE

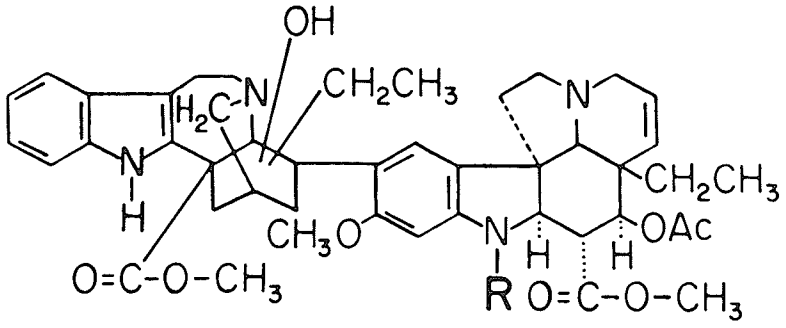


	R	R'
Colchicine	$NHCOCH_3$	OCH_3
Demecolcine	$NHCH_3$	OCH_3
N-Desacetyl-thiocolchicine	NH_2	SCH_3

PODOPHYLLOTOXIN



VINCA ALKALOIDS



Vinblastine R = CH_3

Vincristine R = CHO

classical monograph on colchicine, stated that colchicine causes chromosomal aberration and arrest for up to 10 hours; after which the cell either recovers or degenerates. Levan (1942) felt that colchicine reacts with the molecular system of spindle precursors. Mazia (1955) concluded that colchicine might produce its effect by interfering with establishment of secondary bonding which he regarded as responsible for the orientation and geometry of the fully formed spindle.

The studies of Mazia (1955, 1957) on the isolated mitotic apparatus demonstrated that (1) the spindle fibers are real structures, (2) the spindle protein has a characteristic composition, (3) the linear orientation of the spindle is probably maintained by means of -SS- linkages, and (4) the particular kind of protein from which the spindle is organized is present at prespindle stages. Lettre (1952) further suggested that the effects of colchicine on mitosis resulted from inhibition of reaction between ATP and a contractile mechanism of the actinomysin type. Investigations of Inoue (1952) and Swann (1953) using the polarization microscope, showed the absence of anerrition of spindle fibers after colchicine administration.

Brinkley et al., (1967) also proposed that colchicine (as well as other alkylating agents such as podophyllin, vincristine, vinblastine and colcemid), (Fig. 5), inhibit mitosis by preventing the formation of certain elements of the mitotic apparatus, perhaps the assembly of continuous spindle filaments necessary for centriole movement.

Recently it has been proposed that colchicine not only produces dissolution of microtubules, but also inhibits secretion by exocrine organs (Porter et al., 1970). They further reported that colchicine

inhibits migration of pigment granules in fish dermal melanocytes.

Studies on the stathmokinetic effect of colchicine, vincristine and vinblastine on L1210 leukemia cells by Cardinale et al., (1963) in DBA/2 mice produced a metaphase block of bone marrow cells which lasted from 1-16 hours. It was found further that the duration of colchicine mediated arrest was shorter than that of vincristine.

Davidson et al., (1966) have stated that the effects of colchicine do not wear off for several hours, even after shorter treatments, and arrest of metaphase continues to occur. They further suggested that increases in the mitotic index caused by the arrest of metaphase is maintained after treatment has ceased. Taylor (1963) observed that colchicine arrested mitosis of human carcinoma KB cells in vitro, but reported no inhibition of DNA, RNA, or protein synthesis until long after the mitotic block was complete. Kleinfeld and Sisken (1966) however, using time lapse cinemicrography on HeLa cells following colcemid treatment observed no effects on interphase cells.

OTHER CHEMOTHERAPEUTIC AGENTS:

Many drugs tested by investigators have influential effects on tumor activity; some effecting the amount of tumor growth, others causing cytoplasmic changes within the tumor cells observable at the light and electron microscopic levels. As in the case of most cancers to date, total tumor regression with long-term survival is in-frequently obtained. Jewell (1972) suggests that the role of chemotherapy is to provide palliation of tumors.

Luck (1956) observed a marked growth inhibition in Harding-Passey melanoma following L-phenylalanine mustard treatment. Sugiura (1963) using chemotherapy methods on the Harding-Passey melanoma found that

among 178 compounds and antibiotics, none produced a complete inhibitory effect, although some gave moderate to marked inhibition. Sugiura (1963) was of the opinion that both alkylating agents and antibiotics were equally effective against the Harding-Passey melanoma. Belkin and Hardy (1957) reported that chlorpromazine had an inhibitory effect on the growth of the mouse Sarcoma 37 tumor; however, toxic levels of the drug were necessary to produce this significant inhibition of growth. Van Woert and Palmar (1969) also using chlorpromazine on the Harding-Passey melanoma found a 38% decrease in tumor weight in animals injected with the drug. Blois (1965) reported a higher accumulation of chlorpromazine in melanoma than in non-melanized tissue.

Others, including Chihara et al., (1970), have reported that polysaccharides, lentinan and pachymaran, strongly inhibit the growth of Sarcoma 180 transplanted in mice. Hamuro et al., (1971) further demonstrated that carboxymethyl pachymaran injected intraperitoneally for 10 days in mice starting 24 hours after transplantation resulted in striking tumor regression in Sarcoma 180 and MM-1-2 adeno-carcinoma tumors.

Bekeski et al., (1969) and Molnar and Bekeski (1972) have reported that D-glucosamine and D-mannoseamine added to cells or tumors of Ehrlich ascites and Sarcoma 180, resulted in tumors losing their viability and transplantability with cells showing striking cytoplasmic and nuclear changes. Fjelde et al., (1956) observed that glucosamine caused marked inhibition of the growth of human epidermoid carcinoma cells in tissue culture.

Apple and Greenberg (1968) observed the effects of a propanol, oxopropanol, on animal tumors including Ehrlich ascites carcinoma;

L1210, and L496 leukemias, and Sarcoma 180 in mice; and noted significant inhibition and prolonged survival time of the animals.

Cohen and Carbone (1972) found that Vitamin A alcohol enhanced the anti-tumor effect of 1, 3-bis (2-chlorethyl) - 1-nitrosurea (BCNU) to a considerable extent, and cyclophosphamide to a lesser degree. Therman (1972) found in the Ehrlich ascites tumor, chromosomal translocations occurred following 1-methyl-2-benzyl-hydrazine. The investigator further stated that substances (derivatives of MBH) resemble alkylating agents in their action, but that possibly their mode of action involves some other mechanism than that already known to be incurred by alkylation.

Vadlamudi and Goldin (1971) using demecolcine and vinblastine in combination with ara-C observed an enhancement of therapy against leukemia L1210 when administered at the appropriate time during the cell cycle.

Numerous studies using chemotherapeutic agents have been done on human malignant melanoma. The tendency among many investigators and clinicians has been to apply combination chemotherapy in the treatment of tumors. Combination chemotherapy using demecolchicine or vinblastine followed by mephalin treatment on VX2 carcinoma showed increased survival times of patients (Mashima et al., 1972). Whitecar et al., (1972) reported significant remission results using cyclophosphamide, vincristine, ara-C, and prednisone in combination chemotherapy for acute leukemic adults. Canellos et al., (1972) reported complete remission in 76% of patients treated for Hodgkins disease using nitrogen mustard, vincristine, prednisone and procarbazine for 6 monthly cycles in combination chemotherapy following extensive radiation.

TMCA (tri-methyl colchicinic acid methyl ether d-tartrate) has been used in the treatment of metastatatic malignant melanoma. Johnson and Jacobs (1971) reported significant remission results in patients treated with this drug, while Stolinsky et al., (1972) concluded from their results that patients did not respond clinically well enough for TMCA to be used as a treatment of malignant melanoma.

DTIC (Dimethyl-triazeno-imidazole-carboxamide) either alone or in combination with another drug resulted in remissions in malignant melanoma (Larsen and Hill, 1971; Sarlov et al., 1971; Wagner et al., 1971 and Gardere et al., (1972), and in L1210 leukemia (Carter and Friedman, 1972).

Recent clinical investigations indicate greater beneficial effect resulting from low dosage intermittent therapy rather than a large dosage, non-intermittent therapeutic regime. Kenis and Stryckmans (1972) using mitomycin-C on human solid tumors reported that patients showed better responses when treated with intermittent doses than those given one large dosage. Helson et al., (1972) found significant remission results in patients suffering from disseminated nueroblastoma who received intermittent chemotherapy using vincristine, cyclophosphamide and daunomycin C following surgery.

RADIATION THERAPY

The general concensus is that human malignant melanoma, and melanomas in general, are only moderately sensitive to radiation and drug therapy. Barranco et al., (1971) suggested that the "resistence" may be due to differences at the cellular level such as an extremely efficient repair system, or a change in length or absence of a particular

phase of the cell cycle. Friedman and Drutz (1958) observed a decrease in the size of tumors of Harding-Passey melanoma following irradiation. Madoc-Jones and Mauro (1970), studying murine lymphoma cells found that with dose-fractionation using x-rays, the cell allowed repair of sub-lethel damage at certain positions in the cell cycle. Klein et al., (1966) using lasers on experimental melanomas observed results which ranged from complete regression to accelerated deterioration of tumors depending on the stage of tumor development and dosage of radiation.

ORIGIN AND NATURE OF HARDING-PASSEY MELANOMA

Harding and Passey (1930) first described the presence of an unusual spontaneous tumor in the ear of a uniform chocolate brown mouse. The presence of black pigment in the tumor led them to identify the tumor as a melanoma. They found that one strain of the spontaneous tumor suddenly assumed a very rapid growth, so active that transplantation became a necessity every six or seven weeks. The remaining strains of the melanoma required transplantation every ten to sixteen weeks. The present status of the tumor is such that two to three weeks remains the limit for successful viability and transplantability.

LIGHT AND ELECTRON MICROSCOPIC STUDIES ON MELANOMAS

There have been several studies at the light and electron microscopic level of mouse melanomas. Previous studies on Harding-Passey of mouse have been done at the light microscopic level by Harding and Passey (1930), Grand (1935), Luck (1956), Friedman and Drutz (1958), Sugiura (1963), Menon and Haberman (1970) and El-Fiky et al., (1971). Bertalanffy and McAskill (1964) at the light, and Demopoulos et al., (1965) at the electron microscopic level studied the structure of B-16 mouse

melanoma. The fine structural studies of Dalton (1969) on the Cloudman S-91 mouse melanoma suggested the role of the Golgi apparatus in the evolution of the melanin granule. Demopoulos et al., (1965) carried out a comparative study of the ultrastructure of B-16 and S-91 mouse melanomas. Their findings showed certain ultrastructural differences between the rapidly growing B-16 melanoma and slower growing S-91 melanoma. The B-16 melanoma cells revealed a greater amount of granular endoplasmic reticulum, more melanosomes, greater degree of hypertrophy of the Golgi complex, and giant mitochondria.

Electron microscopic observations of Nathaniel, Friedman and Rychuk (1968) on the Harding-Passey melanoma revealed that the tumor cells were extremely pleomorphic, the nuclei were mostly irregular with moderate to heavy margination of chromatin. The cytoplasm contained abundant free and attached ribosomes, tubulo-vesicular mitochondria, prominent Golgi complexes and numerous melanin granules. The mitotic cell in these tumors consisted principally of a central portion composed of ribosomes in which lay the chromosomal bodies and a peripheral part made up of dilated cisternae of endoplasmic reticulum. Novikoff et al., (1968) in an ultrastructural and cytochemical study of B-16 and Harding-Passey mouse melanomas concluded that compound melanosomes arise within the melanoma cells by autophagy.

III

MATERIALS AND METHODS

GENERAL PLAN AND METHOD OF PROCEDURE:

The animals used throughout this study were six week old female BALB-cj Swiss mice procured from the Jackson Laboratories, Ann Arbor, Maine. The mice were fed Purina lab chow and water ad libitum.

The first phase of this research program was to re-establish the fine structural morphology of the Untreated Harding-Passey Melanoma as obtained from the Jackson Laboratories, to eliminate any possibilities of altered morphology due to repeated passages of the tumor.

The second phase was to study the morphology of this tumor, over an extended period, following different dosage regimes of colchicine administered intraperitoneally to tumor bearing mice. Tumor bearing animals were divided into three groups. Each group consisted of 6 animals. Group A animals received maximal dosage of colchicine. Group B animals were administered minimal dosage of the same drug. Group C animals were given minimal dosage of colchicine in an intermittent manner. Details of the experimental design are given below.

EXPERIMENTAL DESIGN:

Six mice in each experiment received sub-cutaneously a piece of non-necrotic Harding-Passey melanoma approximately 0.5 mm^3 in size after being previously anesthetized with ether. The wound was closed with suture clips.

EXPERIMENT A:

One day following initial transplantation, mice bearing the Harding-Passey melanoma received daily intraperitoneal injections of colchicine in doses of 0.5 mgm/kgm for seven days. The dosage was then doubled and the animals received 1.0 mgm/kgm daily for seven more days. All animals were sacrificed under ether anaesthesia 24 hours after the last injection. Tumor size was measured and recorded. Pieces of tumor were subdivided in saline with a razor blade. Small pieces of this treated tumor from each animal were transplanted to new host animals. The remaining tumor tissue from each animal was fixed in a mixture of aldehydes and processed for electron microscopic study. This fixed tumor will be designated as Treated tumor. The details of composition of the fixative and the procedural methods, being common to all the three experimental designs, are considered under methods of fixation and embedding for electron microscopy.

Following a two week period, the host mice were sacrificed, with part of the tumor being used for transplantation and part fixed for electron microscopy. This process of transplantation and fixation of the treated tumor without further treatment was repeated for four more generations. The tissues that were fixed during these transfers were designated Generation I, Generation II, Generation III, Generation IV and Generation V representing tissues fixed 2, 4, 6, 8 and 10 weeks after cessation of colchicine therapy.

EXPERIMENT B:

Seven days following the initial transplantation of untreated

tumor, six mice received daily intraperitoneal injections of colchicine in doses of 0.5 mgm/kgm for seven days consecutively. All animals were sacrificed under ether anaesthesia 24 hours after the last injection, and the tumors exposed by blunt dissection. Small pieces of the tumor were transferred to new host animals and the remainder of the tumor fixed for electron microscopy. This fixed tumor was designated Treated tumor.

As in experiment A, the treated tumor was carried by host mice for a period of two weeks without any further treatment at the end of which the animals were sacrificed. Pieces of the tumor were transplanted to new hosts and some pieces fixed for ultrastructural study. This method of transplantation and fixation of Treated Tumor was repeated four more times, all without any further colchicine treatment. The tumor tissues fixed for electron microscopy during the transfers were designated Generation I, Generation II, Generation III, Generation IV and Generation V representing tissues fixed at 2, 4, 6, 8 and 10 weeks after cessation of the colchicine therapy.

EXPERIMENT C:

Six mice carrying an untreated Harding-Passey melanoma tumor for one week were given intraperitoneal injections of colchicine in doses of 0.5 mgm/kgm daily for seven days. Twenty-four hours after the last injection the animals were sacrificed, part of the tumor being fixed for electron microscopic studies, and part transplanted to host animals. This fixed tumor was designated 1^{0} Treated tumor.

The host animals carried the treated tumor without any further

therapy for two weeks at the end of which the animals were sacrificed. Portions of this tumor were fixed and designated Generation I. Portions of the remaining tumor were transplanted to new hosts.

Seven days following this transplantation, the mice received daily intraperitoneal injections of colchicine in a dose of 0.5 mgm/kgm daily for seven days. Twenty-four hours after the last injection, the procedure of transplantation and fixation was carried out. The fixed tumor was designated as 2^{o} Treated tumor. This method of alternating chemotherapy every second generation took place once more. This resulted in three more groups of fixed tumors which were labelled as Generation III, 3^{o} Treated tumor and Generation V representing tissues 6, 8 and 10 weeks after the first colchicine treatment.

METHOD OF FIXATION

Tumor tissue was fixed in a mixture composed of 3% paraformaldehyde and 0.5% glutaraldehyde in 0.1 M cacodylate buffer at pH. 7.2 Small pieces of tumor were fixed for 1 hour in the above fixative. Following a rinse in 0.1 M cacodylate buffer containing 0.5% sucrose, tissues were post-fixed in a solution of 1% osmium tetroxide in 0.1 M cacodylate for an additional hour.

METHOD OF EMBEDDING

After fixation, tissues were dehydrated in ascending grades of ethanol. Final dehydration took place in propylene oxide, from which tissues were transferred to a mixture of propylene oxide and araldite. Tissues were passed through mixtures of varying compositions

of propylene oxide and araldite till a final concentration of 100% araldite was reached. Pieces of tissue were then embedded in 100% araldite in Beem capsules and polymerized in a laboratory oven at 55-60^{o}C for 3-4 days.

LIGHT MICROSCOPY

The araldite embedded tissue was trimmed and sectioned at 0.5 micron on a Reichert OM.U_2 ultramicrotome using glass knives. Sections were mounted on glass slides and stained with toluidine blue for 2 minutes.

One-half micron araldite sections were studied with the aid of an optical microscope to study tumor morphology of this level of resolution and to do a count of mitotic figures. Light microscopic study of these plastic embedded sections also permitted trimming away of necrotic parts of the tumor with a razor blade.

A wild M20 camera mounted photomicroscope was used to take photomicrographs on Panatomic X film using Kodak #58 green and red filters.

ELECTRON MICROSCOPY

The sections were cut from non-necrotic portions of the tumor using a Reichert OM.U_2 ultramicrotome with glass knives. Sections were picked up from the water boat on uncoated copper grids and stained with uranyl acetate for 1 hour followed by lead citrate for 5 minutes (Reynolds, 1965).

Thin sections were visualized with a Philips 300 electron microscope operated at 60 K.V. Electron micrographs were recorded

on Dupont Ortho Litho sheet film and developed for 3 minutes in Kodak D-19.

IV

OBSERVATIONS

LIGHT MICROSCOPY

MACROSCOPIC ANATOMY OF HARDING-PASSEY MELANOMA

A growth period of 2½ - 3 weeks in the mouse results in moderate growth and successful viability for transplantation. The tumor size of untreated melanomas was approximately 5 mm. x 10 mm. by the end of two weeks with only a small degree of central necrosis evident (Fig. 6). The skin adjacent to the front limb was found to be the most ideal location for tumor transplantation since movement of the animal did not traumatize the area of transplantation. As seen in Fig. 6, the area was highly vascular and enabled growth to ensue without restriction. Untreated tumors when allowed to continue growing, reached amazingly large diameters of approximately 20 x 30 mm. at the end of two months following transplantation (Fig. 7). The tumors appeared very vascular on the outside, but much central necrosis was evident. Some animals survived 2 - 2½ months, but most animals succumbed earlier due to poor general health while carrying the tumor. Often by this stage of growth, the tumors had become ulcerating necrotic lesions, and viability for transplantation decreased. Average animal weight before transplantation was approximately 21 gms.

LIGHT MICROSCOPIC OBSERVATIONS

PERCENTAGE OF MITOSES.

A light microscopic study was done in order to evaluate the number of cells which were undergoing mitosis at the time of transplantation. These observations were made on all tumor tissues fixed for electron microscopy in all three experimental regimes that were undertaken. One half micron sections of tumor stained with toluidine blue were used to count the cells. By utilizing the Whipple

Figure 6.

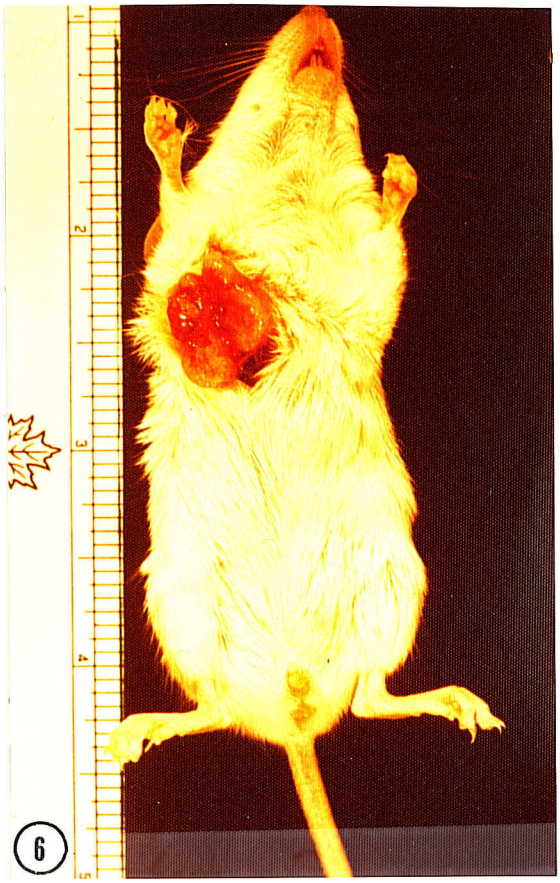
This photograph demonstrates tumor size after 2 weeks from transplantation. The tumor measures approximately 10 mm x 5 mm with only a small amount of central necrosis evident. The tumor is encapsulated and has a rich blood supply.

Figure 7.

This photograph shows tumor size 8 weeks after transplantation. The tumor had a diameter of 30 x 20 mm with necrotic lesions. The tumor was beginning to ulcerate through the skin. Animal viability is limited by the time tumors reach this size and the animals usually expire before tumors reach two and one-half months old.

grid, all cells found to be undergoing any form of mitosis were counted, and the number of these cells recorded as a percentage of 100 cells counted. Text-Fig. 1 graphically illustrates the three sets of experimental results. Percentage mitosis in un-treated tumor gave a mean value of 3.5. The results showed that in Experiment A, maximum dosage of 0.5 mg/kg of colchicine daily for seven days, followed by a further dosage of 1.0 mg/kg daily for seven more days, resulted in peaks of % mitosis as shown in Text-Fig. 1 with values of Treated, 7%; Generation I, 6%; Generation II, 3.8%; Generation III, 4.5%; Generation IV, 4.3% and Generation V, 4.1%. Mitotic indices obtained for Experiment B, minimum dosage of 0.5 mg/kg daily for seven days, followed by no further chemotherapy in the succeeding generations showed Treated, 5.9%; Generation I, 5.8%; Generation II, 3.7%; Generation III, 4.3%; Generation IV, 3.4% and Generation V, 3.8%. Mitotic values obtained for Experiment C, intermittent chemotherapy, of 0.5 mg/kg daily for seven days, on every other transplantation resulted in 1^{o} Treated, 5.9%; Generation I, 5.8%, 2^{o} Treated, 23.8%; Generation III, 7.6%; 3^{o} Treated, 9.8%; Generation V, 7.5% mitosis.

These observations indicated that intermittent chemotherapy of smaller doses of the drug, colchicine, caused metaphase block, as well as slowing down the number of cells passing through the cell cycle. Since the observations were made on tissues which had received colchicine on the day previous to the day of sacrifice in all experiments, the results may be cumulative after colchicine therapy. An explanation cannot be given for the large % mitotic results observed in Expt. C after 2^{o} Treated, and why the decrease in 3^{o} Treated tumor,



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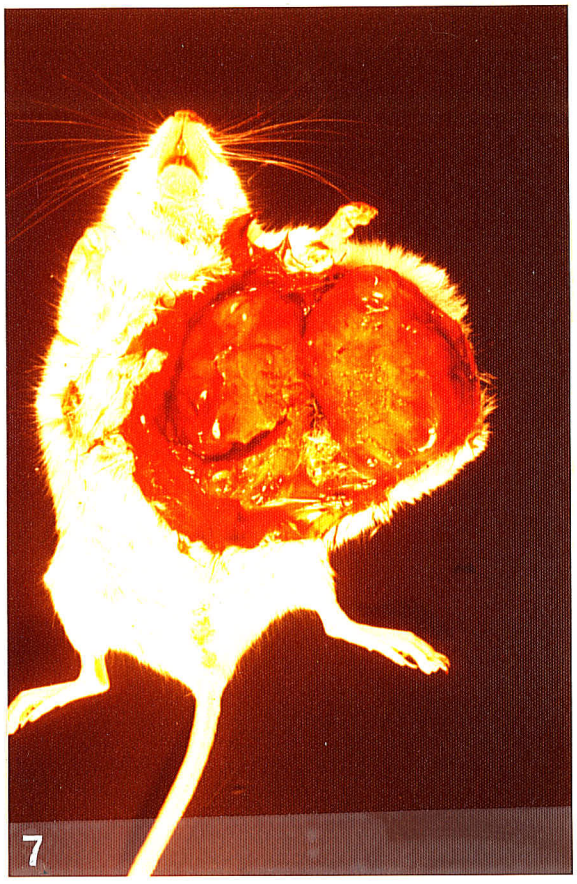

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Text Fig. 1.

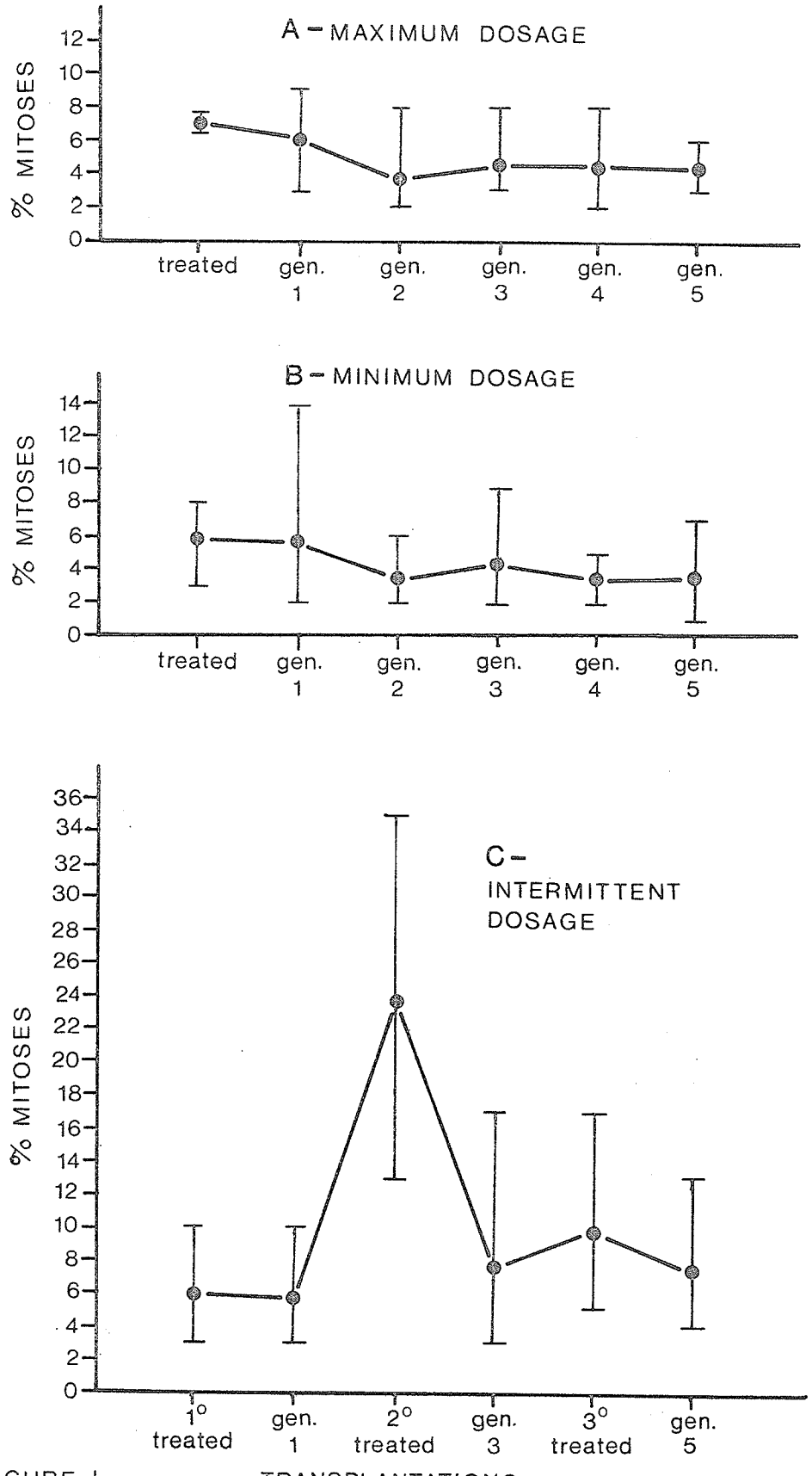
This graph illustrates the % mitoses observed in three experiments following varying doses of colchicine.

Experiment A - Maximum dosage gave mean values of 7.0% for Treated tumor cells, 6.0% for Generation I cells, 3.8% for Generation II cells, 4.5% for Generation III cells, 4.3% for Generation IV cells and 4.1% for Generation V cells.

Experiment B - Minimum dosage gave mean values of 5.9% for Treated tumor cells, 5.8% for Generation I cells, 3.7% for Generation II cells, 4.3% for Generation III cells, 3.4% for Generation IV cells, and 3.8% for Generation V cells.

Experiment C - Intermittent dosage gave mean values of 5.9% for 1^{o} Treated tumor, 5.8% for Generation I cells, 23.8% for 2^{o} Treated tumor cells, 7.6% for Generation III cells, 9.8% for 3^{o} Treated tumor cells, and 7.5% for Generation V cells.

Non-Treated tumor cells gave a mean value of 3.5% mitosis.



TEXT-FIGURE I.

but possible theories will be dealt with in the discussion.

HISTOLOGICAL OBSERVATIONS.

Optical microscopic studies of the untreated tumor showed the tumors to be very cellular and pleomorphic. The cells were so closely packed that there was no appreciable amount of extracellular space. The nuclear configuration of the tumor cells varied from an unimpressive oval configuration to a highly irregular pattern. Each nucleus possessed multiple nucleoli. The nucleus occupied a considerable portion of the cytoplasm. Few mitotic figures were visualized (Fig. 8 & 9). As to be expected in a melanoma which is a highly vascular tumor, blood vessels containing erythrocytes were often seen in the neoplasm (Fig. 9).

Examination of half micron toluidine blue stained sections from Treated tumor belonging to the group administered maximal dosage of colchicine, revealed the presence of numerous mitotic figures. While many mitotic figures revealed metaphase arrests typical of colchicine, several other mitotic configurations were also visualized (Fig. 10). However, by the end of 6 weeks after colchicine therapy, the cells comprising the tumor of Generation III exhibited fewer mitotic figures and appeared similar to that of the untreated tumor (Fig. 11).

Light microscopic studies of experimental series B - subjected to minimal colchicine treatment revealed the pleomorphic nature of the tumor cells in both Generation I (Fig. 12) and Generation III (Fig. 13). Capillaries with few blood cells and some colchicine mitoses were seen in these tumors.

Tumor cells of experiment C - subjected to repeated intermittent administration of colchicine exhibited a striking increase in number

of mitotic figures, especially in the 2^{o} Treated tumor of this series (Fig. 14). Features of 3^{o} Treated tumor cells were identical to those of 2^{o} Treated cells except that the number of mitotic figures were considerably less. The tumor cells in Generation V of this group revealed a progressive decrease in percent mitoses in comparison to 1^{o}, 2^{o}, and 3^{o} Treated tumors of this group (Fig. 15).

LIGHT MICROGRAPHS

FIGURES 8-15

Figure 8. NON-TREATED TUMOR

Photomicrograph of non-treated tumor cells, showing pleomorphic nature of Harding-Passey melanoma. A few mitotic figures are present, but the majority of cells are in interphase.

Half micron section. Toluidine blue stain.

X 640

Figure 9. NON-TREATED TUMOR

A photomicrograph of non-treated tumor cells showing the presence of a few mitotic figures. Note a few blood vessels (BV) containing red blood cells which permeate the tumor. In general cells are quite compact.

Half micron section. Toluidine blue stain.

X 640

Figure 10. EXPERIMENT A - MAXIMUM DOSAGE - TREATED TUMOR

This figure shows cells of the Harding-Passey melanoma treated with maximum dosage of colchicine. Many mitotic metaphases are evident, as well as other mitotic figures. Interphase cells remain pleomorphic in nature.

Half micron section. Toluidine blue stain.

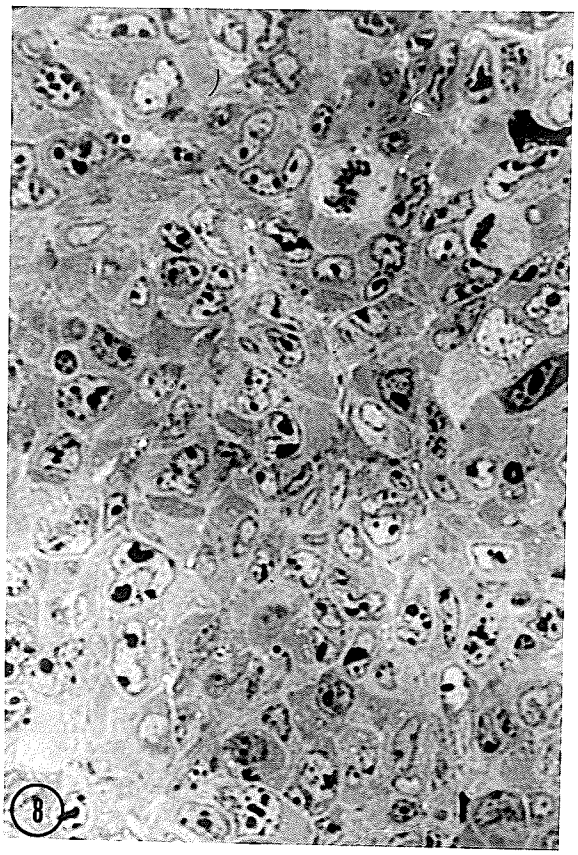
X 640

Figure 11. EXPERIMENT A - MAXIMUM DOSAGE - GENERATION III

This figure shows tumor cells from generation III, 6 weeks after cessation of colchicine treatment. Very few mitotic figures are evident by this stage of transplantation and the cells resemble those of the non-treated tumor.

Half micron section. Toluidine blue stain.

X 640



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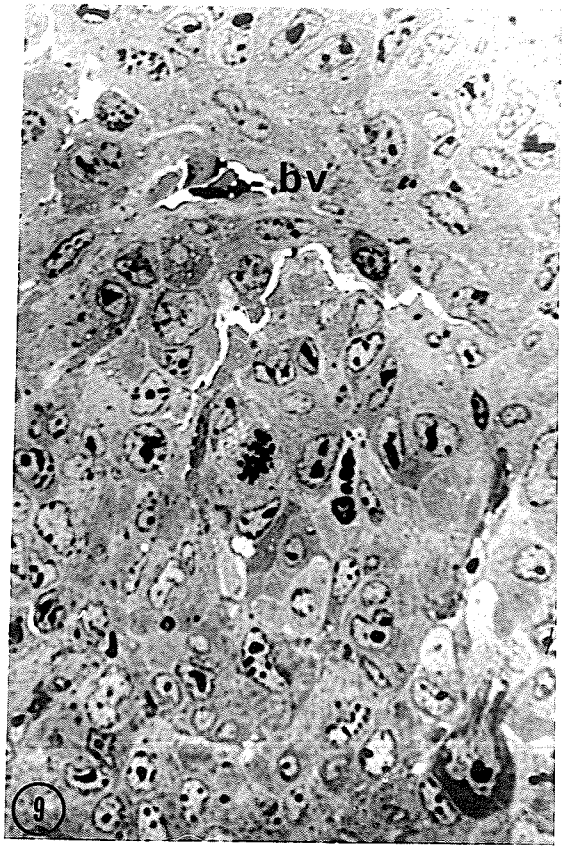

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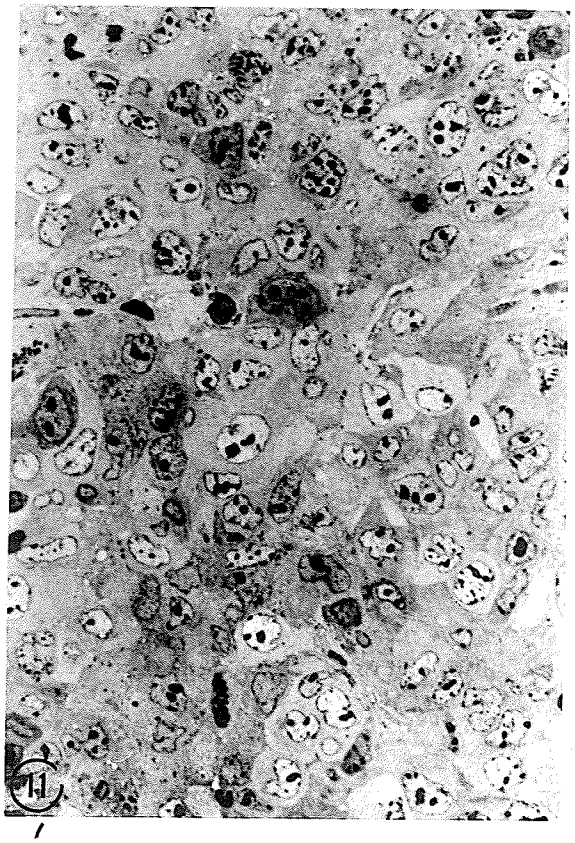

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Figure 12. EXPERIMENT B - MINIMUM DOSAGE - GENERATION I

Photomicrograph showing the cellular morphology of generation I following minimum dosage of colchicine chemotherapy. A few mitotic figures are evident. Cells appear somewhat similar to those of untreated tumor.

Half micron section. Toluidine blue stain.

X 640

Figure 13. EXPERIMENT B - MINIMUM DOSAGE - GENERATION III

A photomicrograph demostrating cells at generation III, six weeks after cessation of colchicine treatment. Pleomorphism is still evident, with small capillaries permeating the cytoplasm as seen in other generations. In general fewer mitotic figures are evident towards the latter three or four generations after cessation of colchicine treatment.

Half micron section. Toluidine blue stain.

X 640

Figure 14. EXPERIMENT C - INTERMITTENT DOSAGE - 2^{o} TREATED

This figure depicts numerous large mitotic cells with centrally located chromosomes and dilated peripheral cytoplasm. The increase in the number of mitotic figures is due to a second series of injections of colchicine six weeks after initial transplantation, and 5 weeks after initial colchicine injection.

Half micron section. Toluidine blue stain.

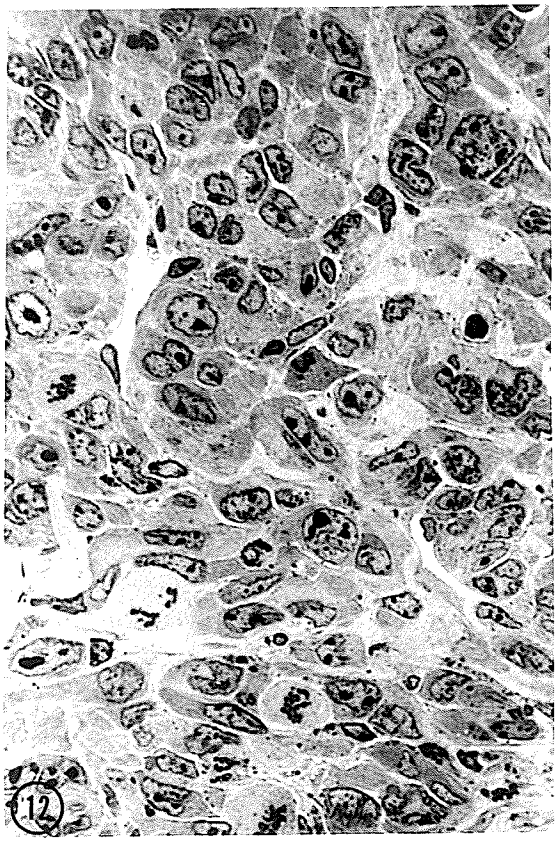
X 640

Figure 15. EXPERIMENT C - INTERMITTENT DOSAGE - GENERATION V

This figure shows generation V cells following intermittent chemotherapy. The number of mitotic figure has decreased considerably in comparison to 1^{o}, 2^{o}, or 3^{o} Treated tumors. The cellular morphology is not unlike that seen in latter generations of the other two experiments (at least at the light level).

Half micron section. Toluidine blue stain.

X 640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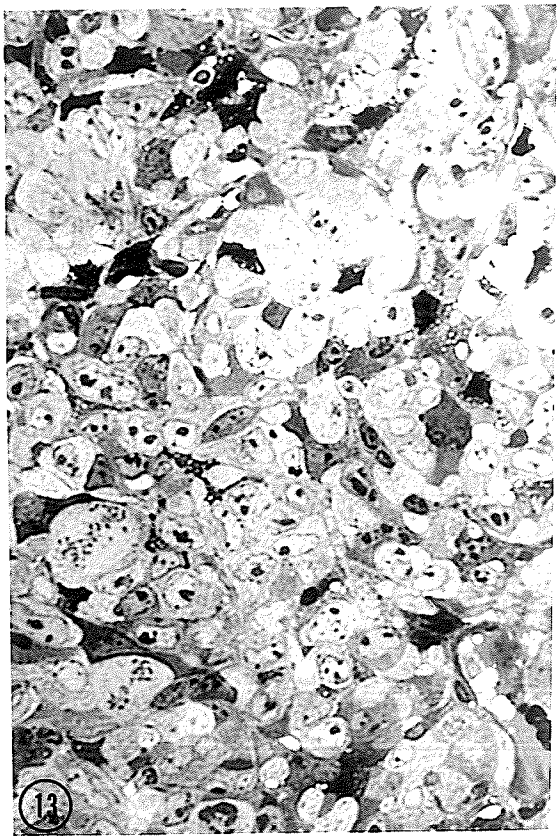
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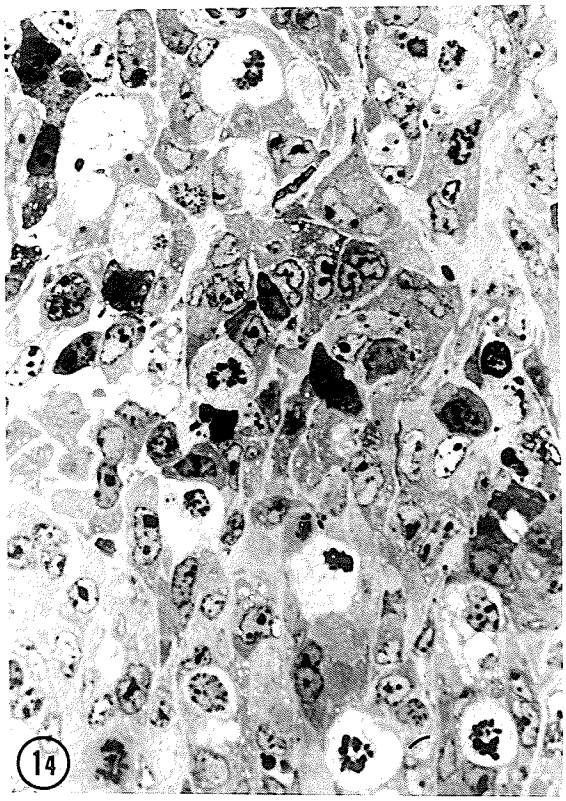
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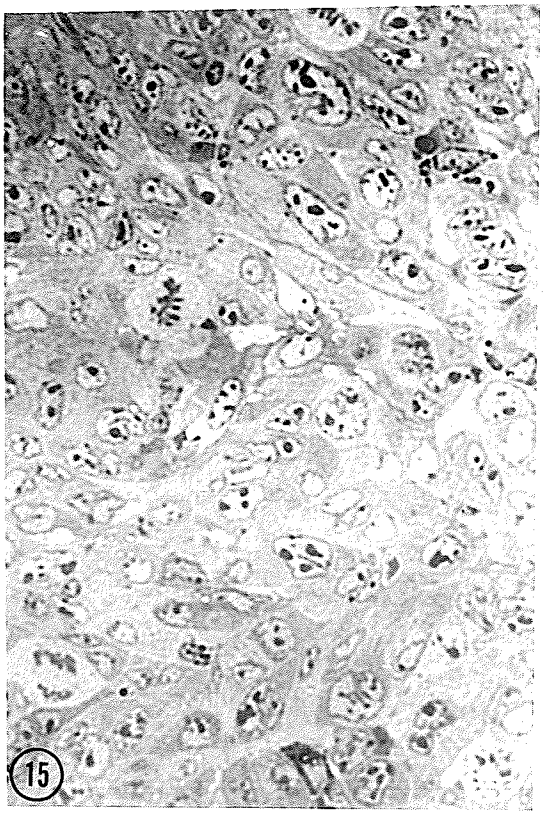
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IV

OBSERVATIONS

ELECTRON MICROSCOPY

UNTREATED TUMOR

ELECTRON MICROSCOPY OF UNTREATED TUMOR

Electron microscopic observations confirmed the optical microscopic findings that the tumor was highly cellular with negligable amounts of collagen. Morphologically the cells of the non-treated tumors were pleomorphic in nature. The presence of melanin within the cells afforded a positive criterion for identification of these cells as melanocytes.

The Interphase Cell

Basically two types of cells were encountered; light and dark, dependent upon the amount of free and membrane attached ribosomes within the cytoplasm (Fig. 16). The light cells made up the greater proportion of the tumor cells. The nuclei of the tumor cells varied from an oval to a highly irregular contour. A distinct perinuclear space characterized the tumor nuclei with a considerable number of ribosomes being attached to the outer nuclear membrane (Fig. 17). Moderate to heavy accumulation of chromatin was found along the nuclear margin. Large and multiple nucleoli were found in many tumor cells.

The cytoplasm was rich in free and granular endoplasmic reticulum which accounted for the basophilia observed with the light microscope. It appeared that the free ribosomal content in the dark cell was considerably greater than in the light cell (Fig. 16). Mitochondria of the tubulo-vesicular type were found in the tumor cells. It is interesting to note that tubulo-vesicular mitochondria were not observed in other melanomas. Well developed Golgi complexes were found in these cells (Fig. 17).

Virus like particles resembling Type A particles of Bernhard (1958)

measuring 700-800Å in diameter were seen in clusters in the tumor cells, principally in relation to the endoplasmic reticulum (Fig. 18, 19, & 20). These particles were dougnhut shaped enclosing a 250Å translucent core. It is believed that these particles represent a virus. The evolution of the virus particles is considered to occur as follows: the earliest indication of formation of these particles was a thickening of the membrane of the endoplasmic reticulum which gradually invaginated into the lumen of the endoplasmic reticulum. This budding or invagination process continued until a nearly complete double membrane sphere, connected by a narrow stalk adherent to the endoplasmic reticulum, was formed. Subsequently the connection is lost and the doughnut shaped particle lay free in the cistern of the granular endoplasmic reticulum.

Melanin granules were observed in all the cells of the tumor and formed the basis of identification of the cell as a melanocyte. The younger or immature forms of the melanin granules were usually located well within the cytoplasm of the tumor cells, whereas the mature granules had a peripheral location. Since the aim of this dissertation was not to elucidate the origin of the melanin granule, no attempt will be made to describe or discuss the probable origin of the granule. Suffice it is to say that both the granular endoplasmic reticulum and the Golgi complex play a part in its elaboration. Formation of melanin progressed through a biochemical series of pigmentation starting from tyrosinase laden premelanosomes, the precursor form: to fully pigmented, virtually tyrosinase free melanin granules (Seiji et al., 1960). Figures 21, 22, 23 & 24 depict several forms of melanin observed within the melanoma; from early premelanosomes

to melanin granules, with some described as autophagic vacuoles by Seiji and Otaki (1972).

The Mitotic Cell

Several instances of mitosis were observed in the tumor. Mitotic cells were seen in all phases of the mitotic cycle. Figure 25 illustrates a portion of a mitotic melanocyte with numerous chromosomal masses lying in a cytoplasmic matrix. It may be seen that organelles such as mitochondria and segments of endoplasmic reticulum are generally excluded from amongst the chromosomes and are situated in the peripheral portion of the cell. Late telophase may be observed in Figure 26 where the two daughter cells are connected by the last stringent means of tether known as the 'midbody' which is composed of some microtubules and dense material (Fig. 27).

Melanin granules were observed in tumor cells in all phases of the mitotic cycle.

ELECTRON MICROGRAPHS

UNTREATED TUMOR

FIGURES 16-27

Figure 16. NON-TREATED TUMOR

This electron micrograph demonstrates the typical features of a non-treated tumor tissue. In this figure are a number of "light" cells with their nuclei, and a portion of a "dark" cell and its nucleus. Cytoplasmic inclusions include tubulo-vesicular mitochondria, Golgi complex, endoplasmic reticulum and melanin in different stages of melanization. The dark cell cytoplasm is much more dense due to an increased ribosomal content.

X 15,048

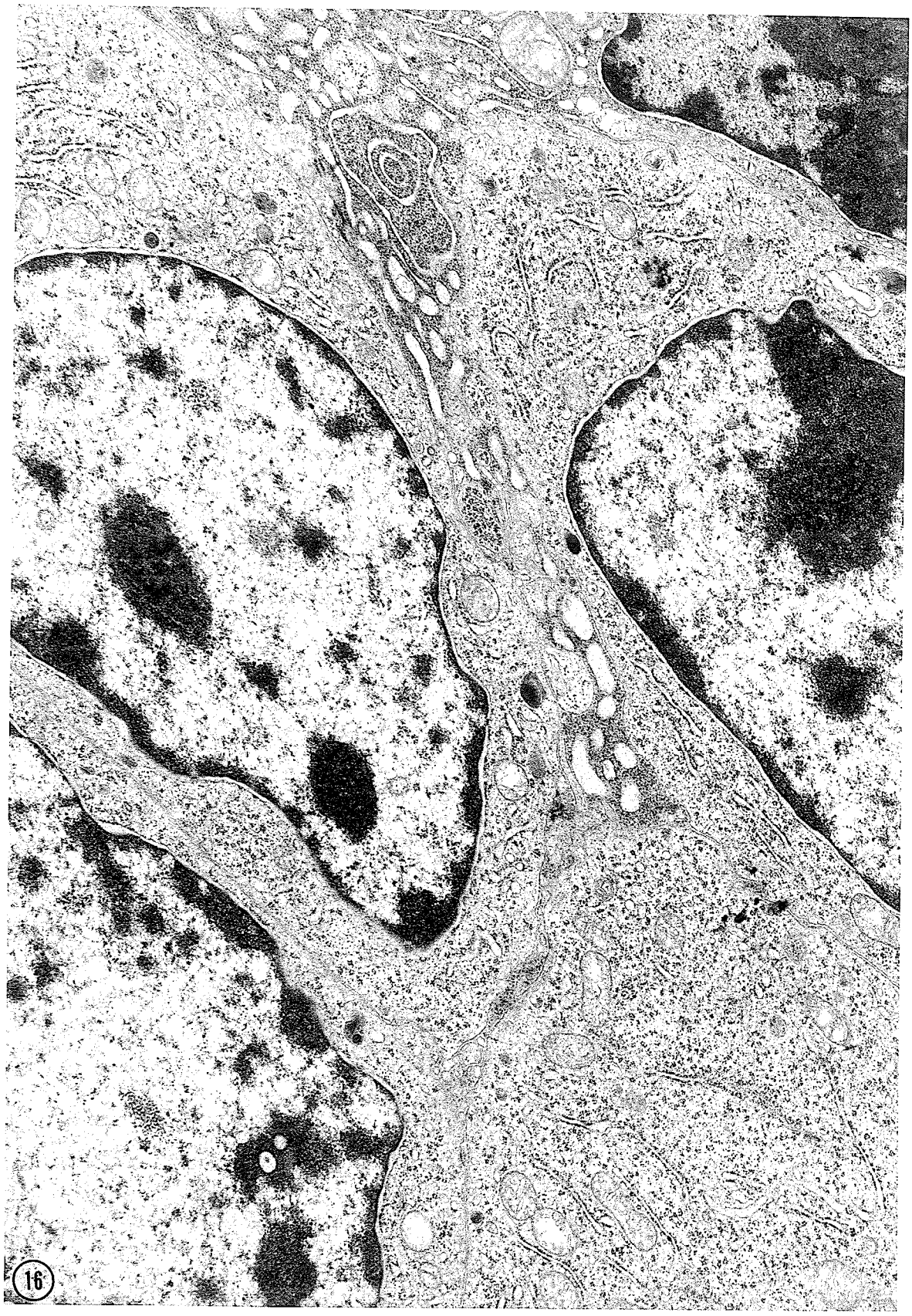
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Figure 17. NON-TREATED TUMOR

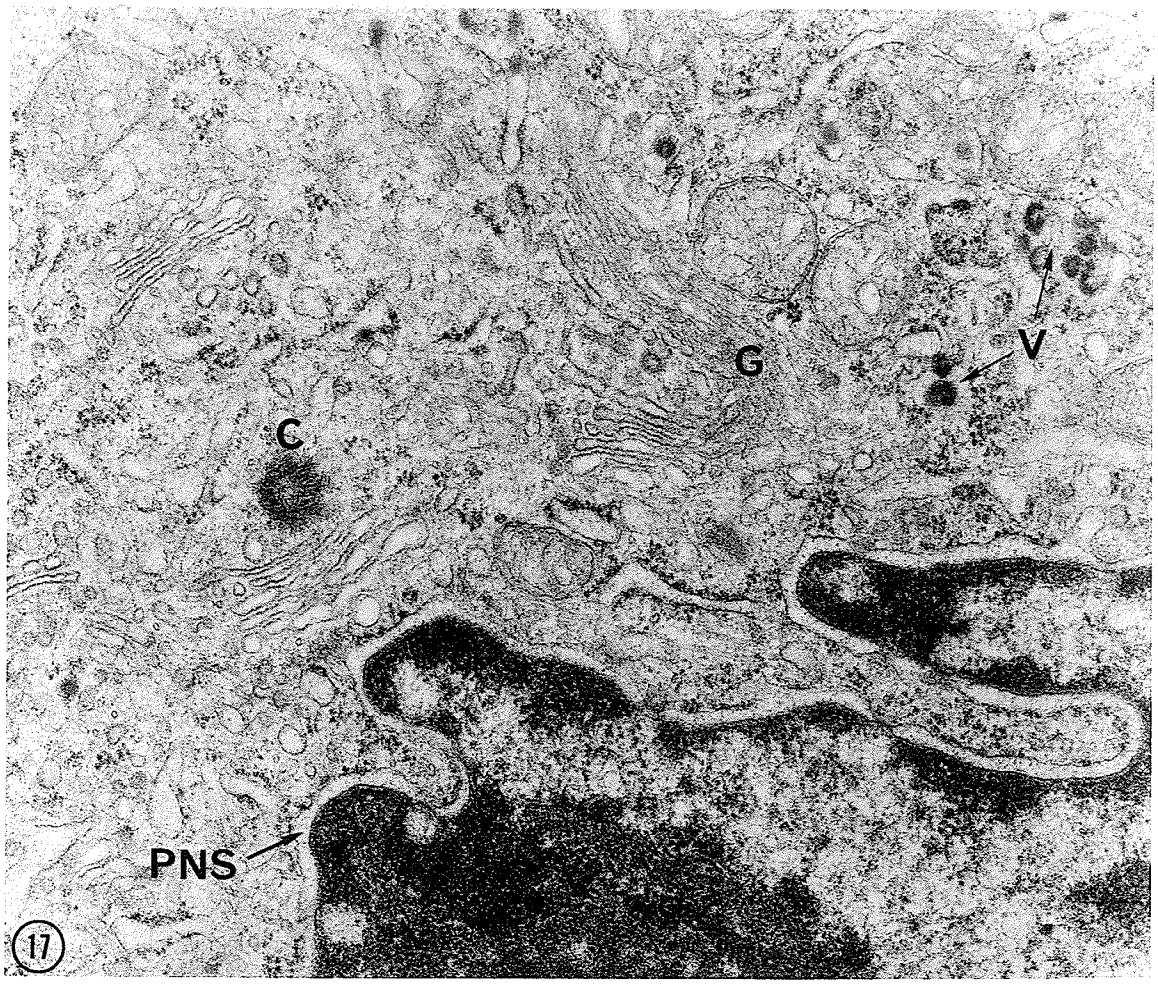
Micrograph showing the presence of a highly irregular nucleus, with peripheral accumulation of chromatin. A distinct perinuclear space (PNS) is evident with the outer limiting nuclear membrane ribosome studded. Multiple well-developed Golgi complexes (G) are evident as are tubulo-vesicular mitochondria. A centriole (C) is also seen, as are a number of Type A virus particles (V) which are budding from the endoplasmic reticulum.

X 47,242

Figure 18.
Figure 19. NON-TREATED TUMOR
Figure 20.

Electron micrographs showing the formation of virus particles by budding from the endoplasmic reticulum. Arrows point to virus particles which have not completely severed themselves from the endoplasmic reticulum. Complete double-membraned virus particles approximately 700A^{o} in diameter enclosing an electron translucent 250A^{o} space are evident within the cisternae of the endoplasmic reticulum in all three micrographs.

Fig. 18. - X 39,026
Fig. 19. - X 41,496
Fig. 20. - X 37,072



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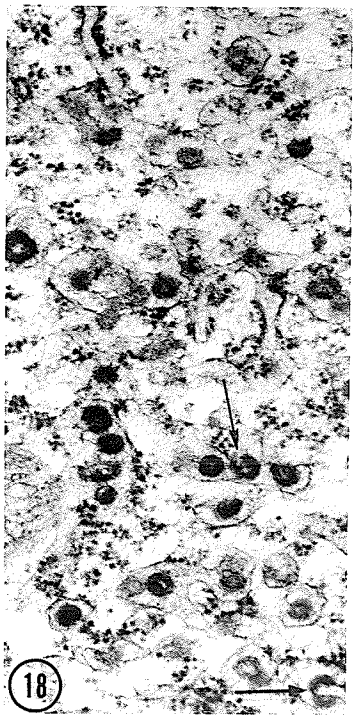

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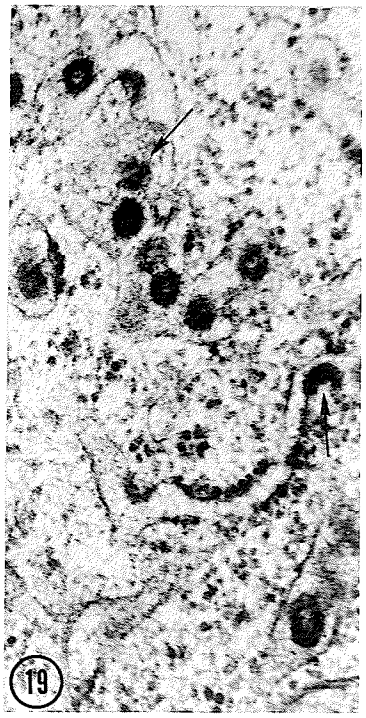

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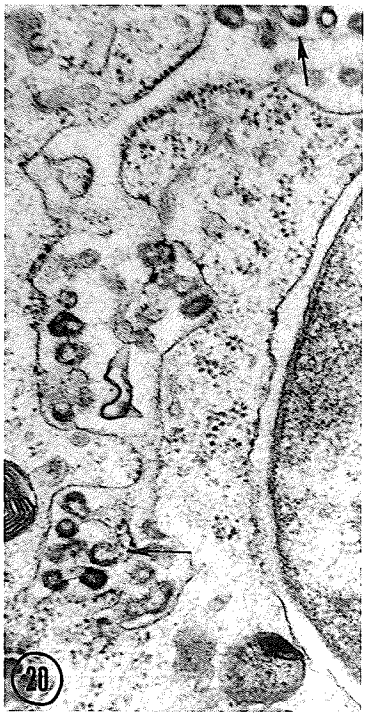

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Figure 21. MELANIZATION IN AN UNTREATED TUMOR

This low power electron micrograph shows the presence of melanin within the cytoplasm of an interphase cell. Many fully pigmented melanin granules (MG) are observed along with a few melanosomes (MS).

X 17,442

Figure 22. MELANIZATION IN AN UNTREATED TUMOR

This high power micrograph shows a lamellar structure during melanosome formation. Also seen in this figure are a Golgi complex, tubulo-vesicular mitochondria, rough endoplasmic reticulum and virus particles associated with the endoplasmic reticulum.

X 32,832

Figure 23. MELANIZATION IN AN UNTREATED TUMOR

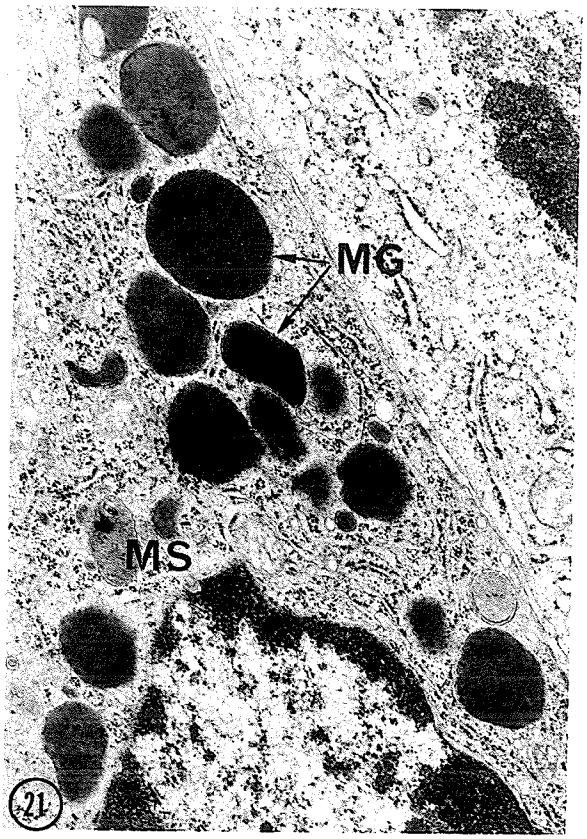
This micrograph demonstrates numerous melanin granules, lamellar melanosomes, and some smaller pre-melanosomes within the cytoplasm. Mitochondria and Golgi complexes are also seen within the cell.

X 15,390

Figure 24. MELANIZATION IN AN UNTREATED TUMOR

This micrograph again illustrates the composite lamellar structure of a melanosome (MS), a precursor form to the completely tyrosine free melanin granule as are shown by the darkly pigmented cellular inclusions.

X 30,780



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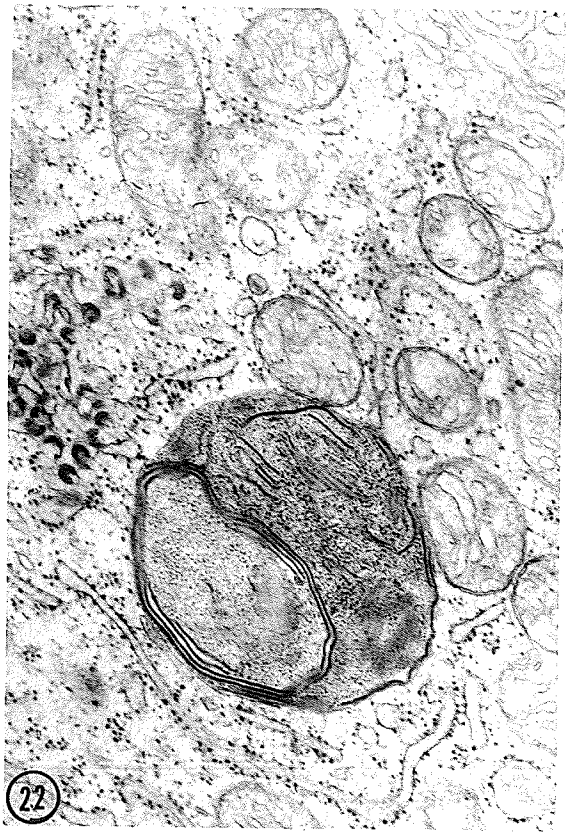

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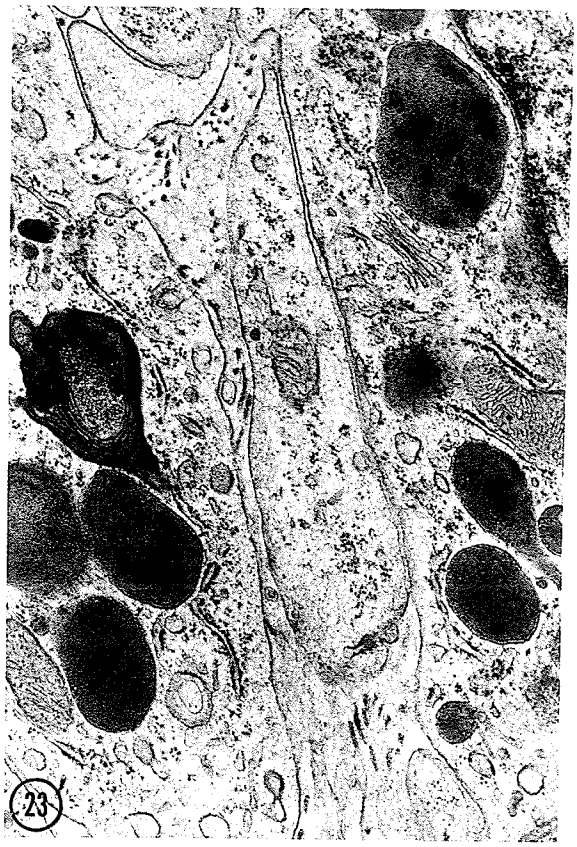

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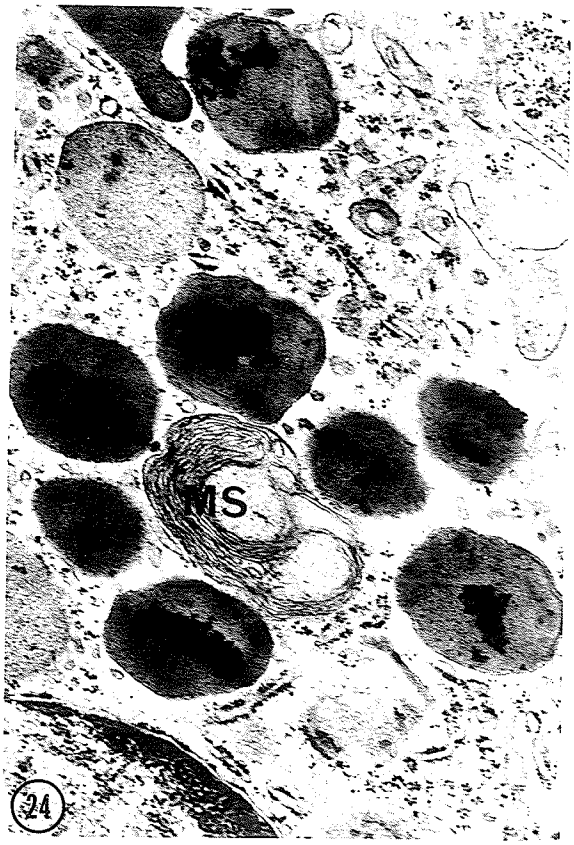

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Figure 25. NON-TREATED TUMOR

This electron micrograph shows the greater part of a non-treated mitotic cell. Note the presence of chromosomes, tubulo-vesicular mitochondria, short segments of rough endoplasmic reticulum, and melanin. Some microtubules are also seen in association with the chromosomes.

X 11,628

Figure 26. NON-TREATED TUMOR

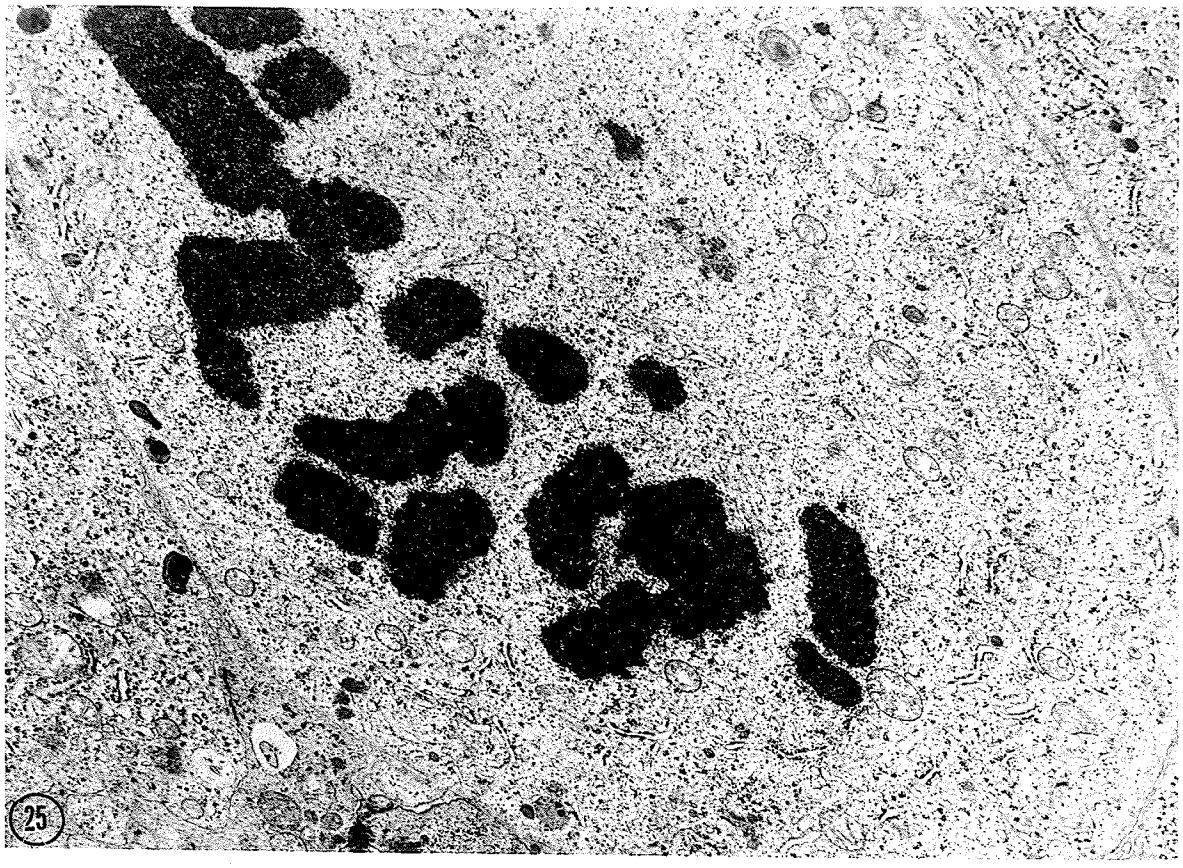
This figure shows a mitotic cell in a late stage of division i.e., telophase. Note the presence of the "midbody" (MB) which is the last remnant of attachment between the two prospective daughter cells before complete separation.

X 6,783

Figure 27. NON-TREATED TUMOR

Micrograph of a tumor cell in late telophase of Figure showing two daughter cells connected by a constricted portion of cytoplasm termed "midbody" containing some microtubules and dense substance.

X 11,172



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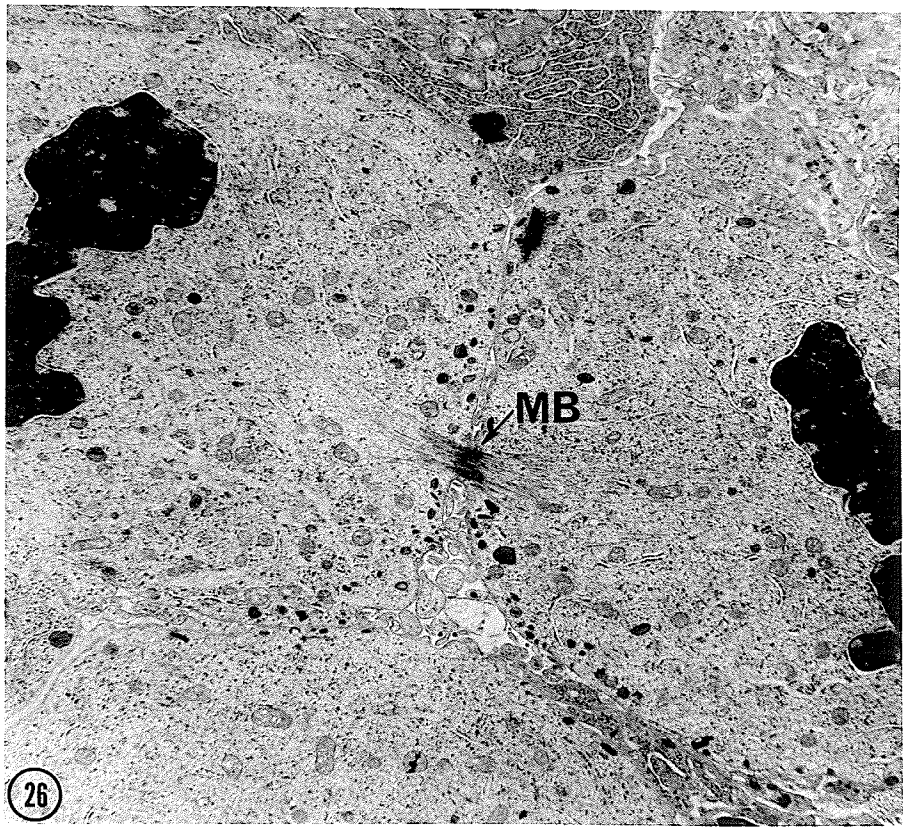

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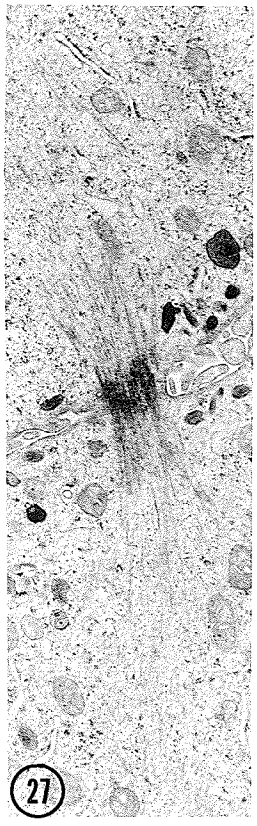

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IV

OBSERVATIONS

ELECTRON MICROSCOPY

EXPERIMENT A - MAXIMUM DOSAGE

EXPERIMENT A - MAXIMAL DOSAGE

TREATED TUMOR

Electron microscopic observations of Treated tumor revealed the presence of numerous colchicine mitotic cells. The colchicine mitotic cell was enlarged and was comprised of a central portion consisting of chromosomes which were located in a dense cytoplasmic matrix (Fig. 28). Quite often a centriole with spindle tubules radiating from it was observed in this area. The peripheral portion of the cell exhibited dilated cisternae of endoplasmic reticulum. The most significant feature of the C-mitotic cell was the presence of bundles of microfilaments, 35-50Å in diameter which were generally located in the peripheral aspect of the cell. Cellular organelles such as mitochondria were often excluded from the midst of the filament bundles. Ribosomes were often found bordering the filaments (Figs. 28 & 29).

Interphase cells of Treated-tumor presented many of the features of non-treated cells such as tubulo-vesicular mitochondria, multiple well-developed Golgi complexes, smooth and rough endoplasmic reticulum, distinct perinuclear space, and peripheral accumulation of chromatin. Distinguishing the interphase cells of Treated-tumor from untreated-tumor were the presence of bundles of cytoplasmic filaments which were often perinuclear in position (Fig. 30). These filaments had a close spatial arrangement with ribosomes, although few ribosomes appeared amongst the filaments.

Margination of melanin granules was also observed in some cells following colchicine chemotherapy (Fig. 31). The pattern of melanin formation continued as before treatment with colchicine (Fig. 33). Complex patterns of endoplasmic reticulum resembling "finger-prints"

were encountered in a few cells (Fig. 32). Melanization and amount of melanin tended to increase after colchicine treatment as was observed by Friedman and Drutz (1958) and Nathaniel et al., (1968).

GENERATION I - TWO WEEKS AFTER CESSATION OF COLCHICINE TREATMENT

Cells of Generation I were not unlike those observed in Treated-tumor. Interphase cells containing bundles of filaments in the cytoplasm were readily observed (Figs. 35 & 36). The filaments, 35-50Å in diameter were in close proximity to ribosomes (Figs. 35 & 36) as seen in Treated-tumor. Type A virus particles, seen in both non-treated and Treated-tumor, were also observed in Generation I cells (Fig. 34). Blood vessels were seen to permeate the tumor and often contained remanants of red blood cells (Fig. 35).

Mitotic cells also continue to contain bundles of filaments peripheral to the centrally located chromosomes (Fig. 37). Another feature seen in several cells and not observed in untreated tumors were desmosome-like thickenings of plasma membranes of adjacent cells (Figs. 38 & 39).

GENERATION II - FOUR WEEKS AFTER CESSATION OF COLCHICINE TREATMENT

The presence of fine filaments continued to be observed in both interphase and mitotic cells. Figure 40 illustrates an interphase cell with a large bundle of filaments, some appearing in longitudinal section, some in cross-section. Figure 41 shows a mitotic cell containing a bundle of filaments with chromosomes surrounded by dilated cisternae of endoplasmic reticulum. These filaments can be easily distinguishable from spindle tubules which arise from the centriole.

Large amounts of melanin in various stages of formation were

observed to accumulate in the periphery of an interphase cell (Fig. 40), as was seen in Treated and Generation I tumor cells.

Virus particles continue to be present and are seen to arise from the endoplasmic reticulum. The double-membraned 700Å virus particles are seen in Figure 42 and continue to persist in cells through all the generations.

GENERATION III - SIX WEEKS AFTER CESSATION OF COLCHICINE TREATMENT

By this stage of transplantation very few cells in mitosis were observed to contain filaments. The majority of cells containing filaments were seen in interphase (Figs. 43, 44 & 45). Cells seen to contain filaments also exhibited many of those features as described previously for other generations. Multiple well-developed Golgi complexes (Fig. 45), accumulation of melanin in varying stages of melanization (Figs. 43 & 44), tubulo-vesicular mitochondria (Fig. 45) and virus particles could be found in most cells. Filaments were seen in a perinuclear position in some cells (Fig. 43), but more often they appeared in the rest of the cytoplasm (Figs. 44 & 45).

GENERATION IV - EIGHT WEEKS AFTER CESSATION OF COLCHICINE TREATMENT

By this stage of transplantation the tumor cells containing filaments have become fewer in numbers, and only by means of scanning large numbers of cells, could some cells containing distinct bundles of filaments be found. Those filaments that were present still maintained the features as exhibited in cells of previous generations. In general the filamentous bundles have become smaller in content and in number (Fig. 46), and were now most frequently observed to permeate the cytoplasm (Fig. 47) rather than form distinct bundles as seen in

Treated-tumor and Generations I, II. Both light and dark cells were visualized, but only light cells throughout all the generations were seen to contain filaments. Virus particles continue to persist and remain associated with the endoplasmic reticulum (Figs. 47 & 48). Melanin granules appear less abundantly at this stage than were seen in the first few generations following treatment.

GENERATION V - TEN WEEKS AFTER CESSATION OF COLCHICINE TREATMENT

By Generation V very few cells were observed to contain well defined bundles of filaments. Figure 49 and 50 illustrate cells containing conspicuous bundles of filaments both perinuclear in position, and permeating within the cytoplasm. These cells also show well-developed Golgi complexes, tubulo-vesicular mitochondria, well organized granular endoplasmic reticulum, developing melanin, and virus particles. Desmosomal connections between adjacent cells were rarely observed in the cells of this generation.

It is to be emphasized that the tumor cells of this generation contained microfilaments which permeated the cytoplasm. Only in few cells such as those shown in Figures 49 and 50 did one observe distinct bundles of microfilaments.

ELECTRON MICROGRAPHS

EXPERIMENT A - MAXIMUM DOSAGE

FIGURES 28-50

Figure 28. EXPERIMENT A - MAXIMUM DOSAGE - TREATED TUMOR

This figure shows a mitotic cell in a colchicine treated tumor. A centriole is present, as are microtubules radiating towards some of the chromosomes. Numerous dilated cisternae of endoplasmic reticulium are also evident. In the periphery of the cell are three bundles of filaments (fil), 35-50A^{o} in diameter, with few ribosomes found amongst them.

X 19,426

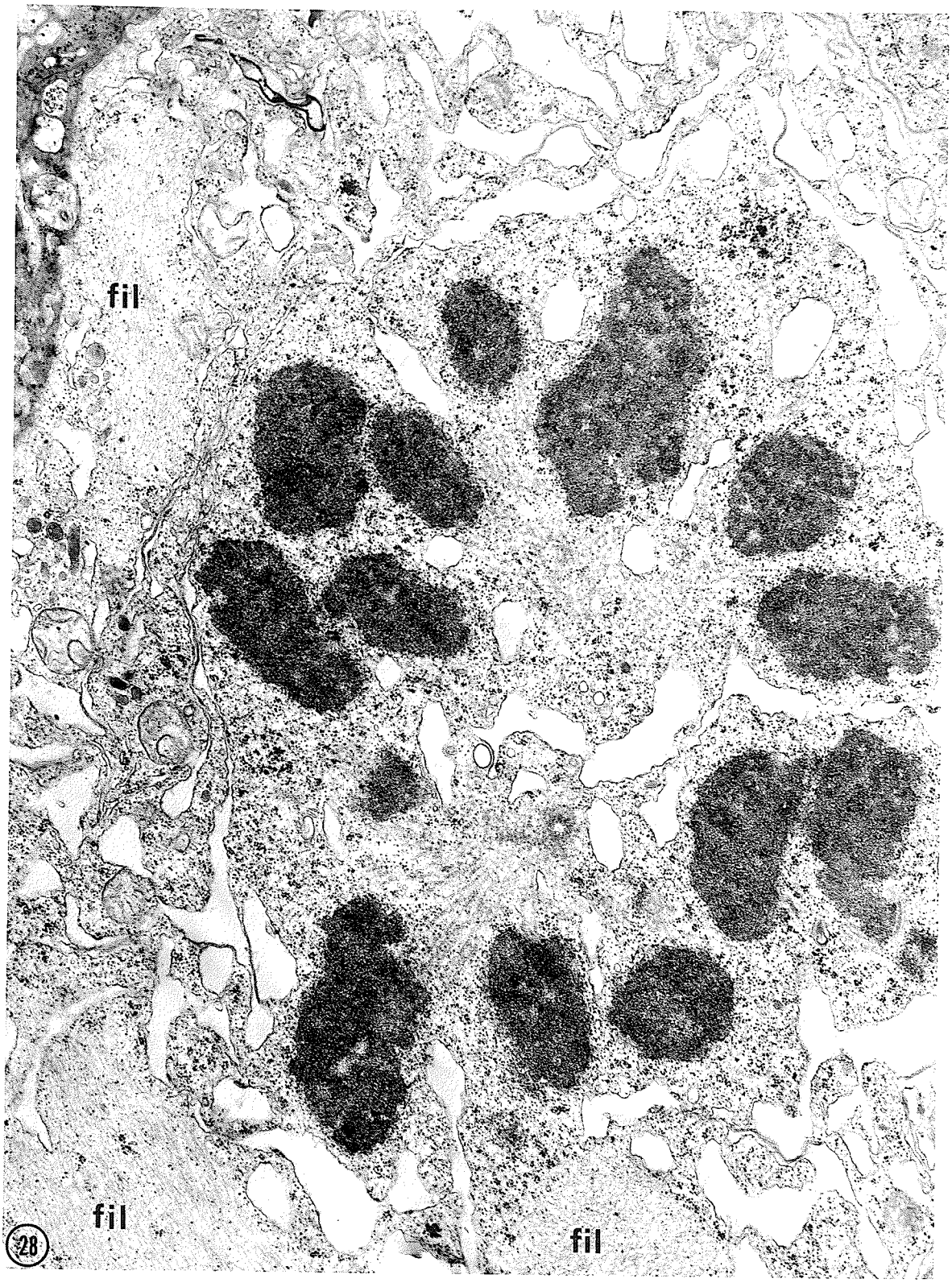
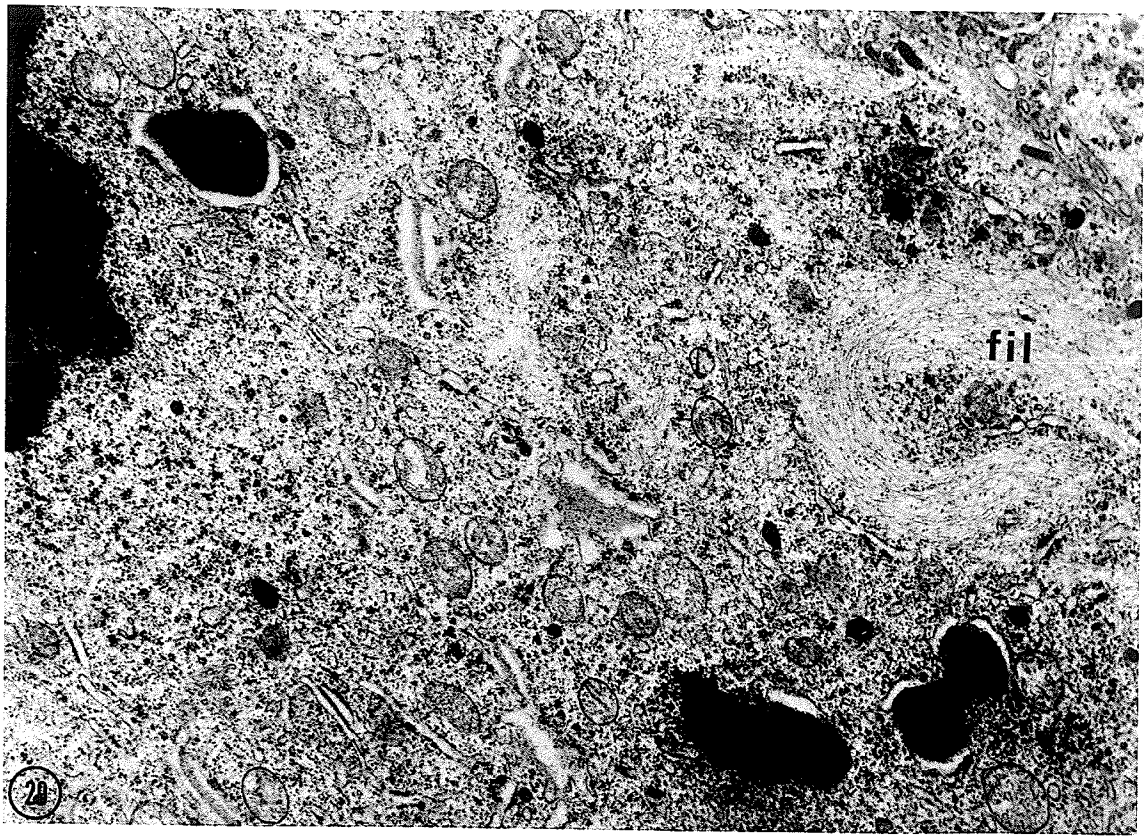

fil
fil
fil
28

Figure 29. EXPERIMENT A - MAXIMUM DOSAGE - TREATED TUMOR
This micrograph shows the presence of bundles of filaments (fil) which measure 35-50A^{o} in diameter within the cytoplasm of a melanocyte. Filaments can also be seen permeating the cytoplasm of the cell. The presence of nuclear masses, some of which show perinuclear space while others do not, suggest that this cell is in early prophase or late telophase. Melanin in various stages of development are found in the cytoplasm.

X 14,165

Figure 30. EXPERIMENT A - MAXIMUM DOSAGE - TREATED TUMOR
A highly irregular nucleus is depicted containing a large, relatively ribosome free matrix of filaments (fil). Within the micrograph can also be seen, tubulo-vesicular mitochondria, Golgi complex, segments of endoplasmic reticulum and melanin.

X 21,854


fil
28

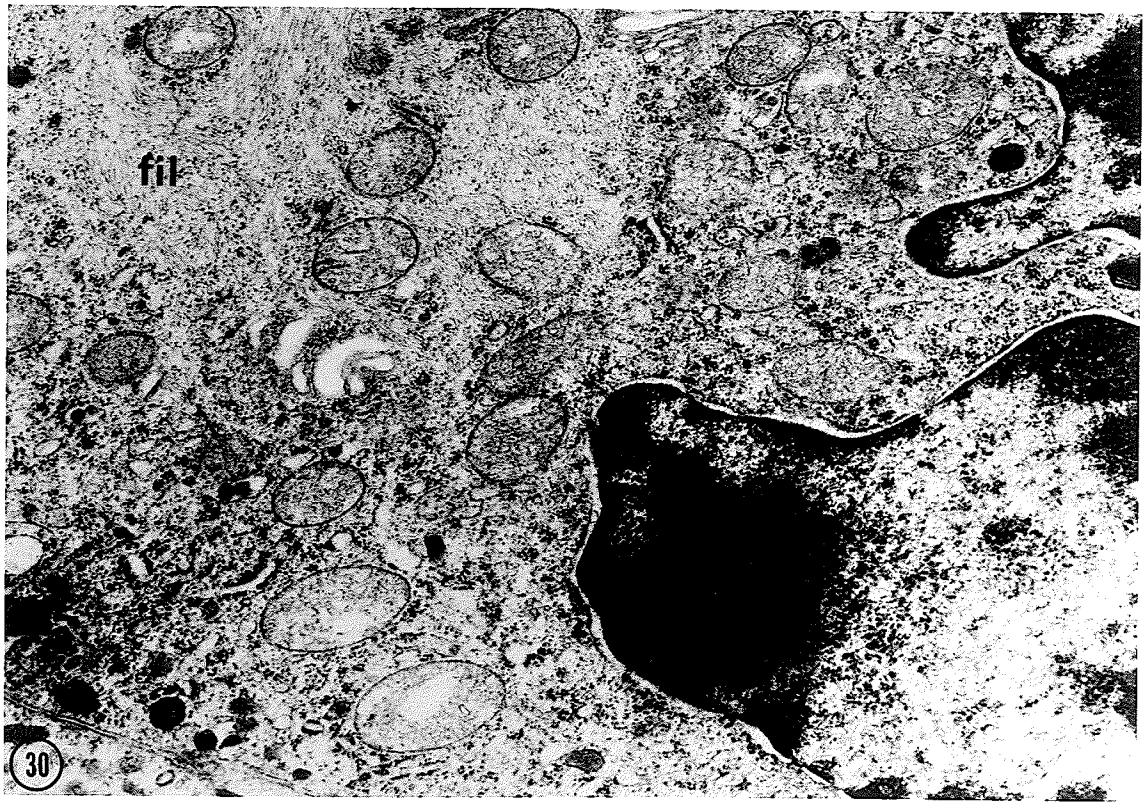

fil
30

Figure 31. EXPERIMENT A - MAXIMUM DOSAGE - TREATED TUMOR
The greater part of a sectionally-cut nucleus is shown in the figure. The cytoplasm of this cell shows "margination" by numerous premelanosomes (PM), melanosomes (M), melanin granules (MG). Type A virus particles (arrow) may be observed in close association with the endoplasmic reticulum in the lower part of the figure.

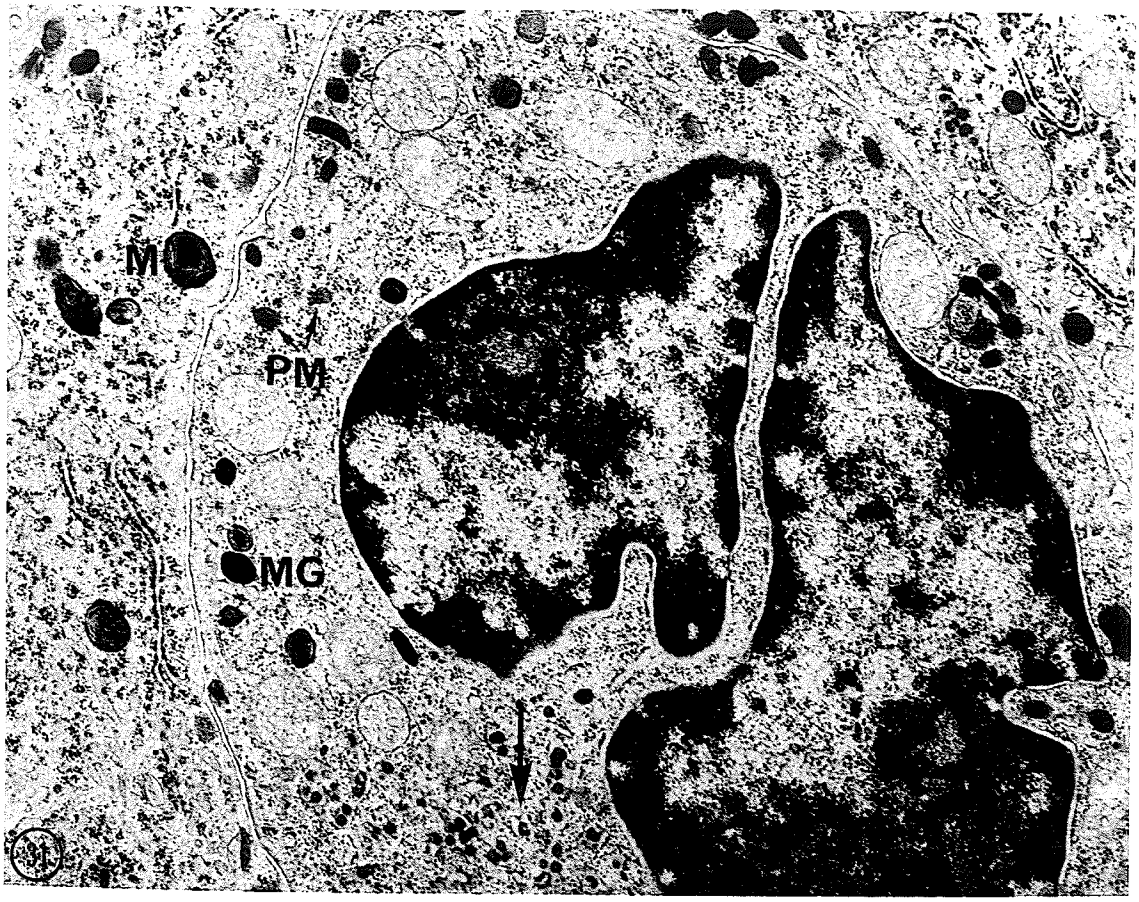
X 20,064

Figure 32. EXPERIMENT A - MAXIMUM DOSAGE - TREATED TUMOR
Shows an atypical endoplasmic reticulum rarely seen in melanocytes. Such a configuration is generally considered as a "fingerprint" endoplasmic reticulum.

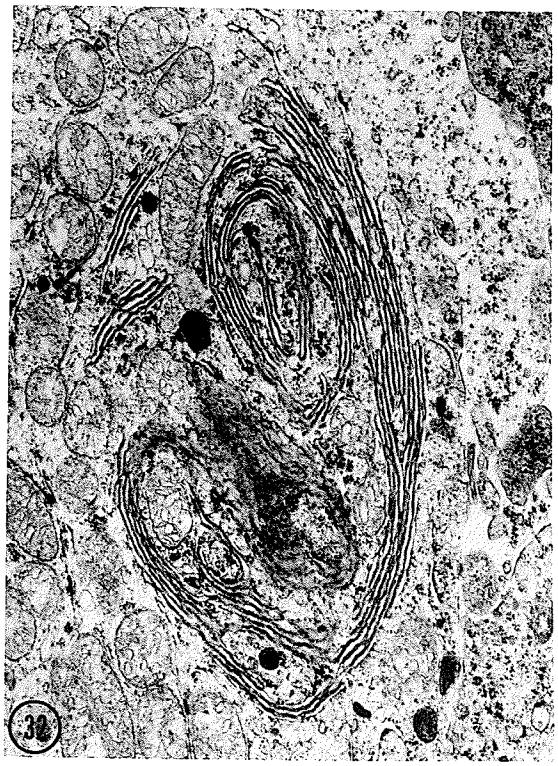
X 20,748

Figure 33. EXPERIMENT A - MAXIMUM DOSAGE - TREATED TUMOR
An electron micrograph of a melanosome complex showing various stages of the melanization process. Less densely pigmented granules showing substructure represent precursor forms to the densely pigmented mature melanin granules.

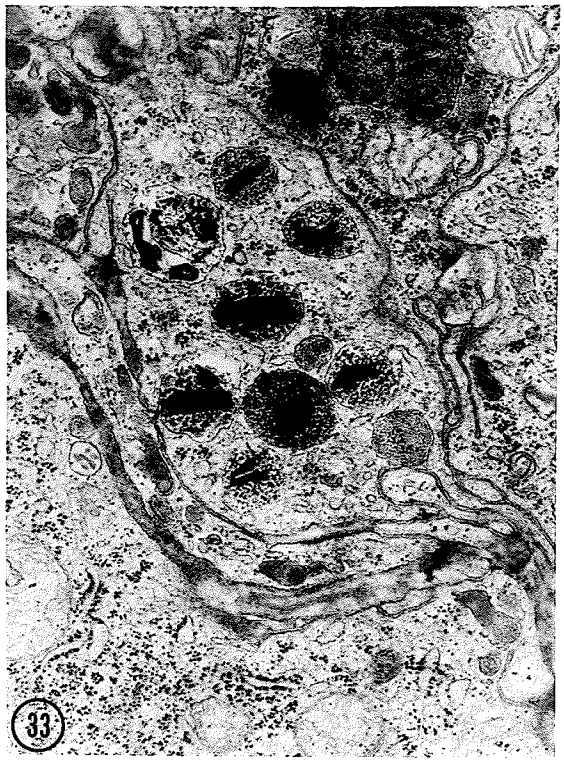
X 25,650



31



32



33

Figure 34. EXPERIMENT A - MAXIMUM DOSAGE - GENERATION I - 2 weeks after cessation of colchicine treatment.

Micrograph of portion of an interphase cell showing the presence of a large bundle of filaments (fil), 35-50A^{o} in diameter, within the cytoplasm. Large numbers of virus particles are seen associated with the endoplasmic reticulum.

X 30,096

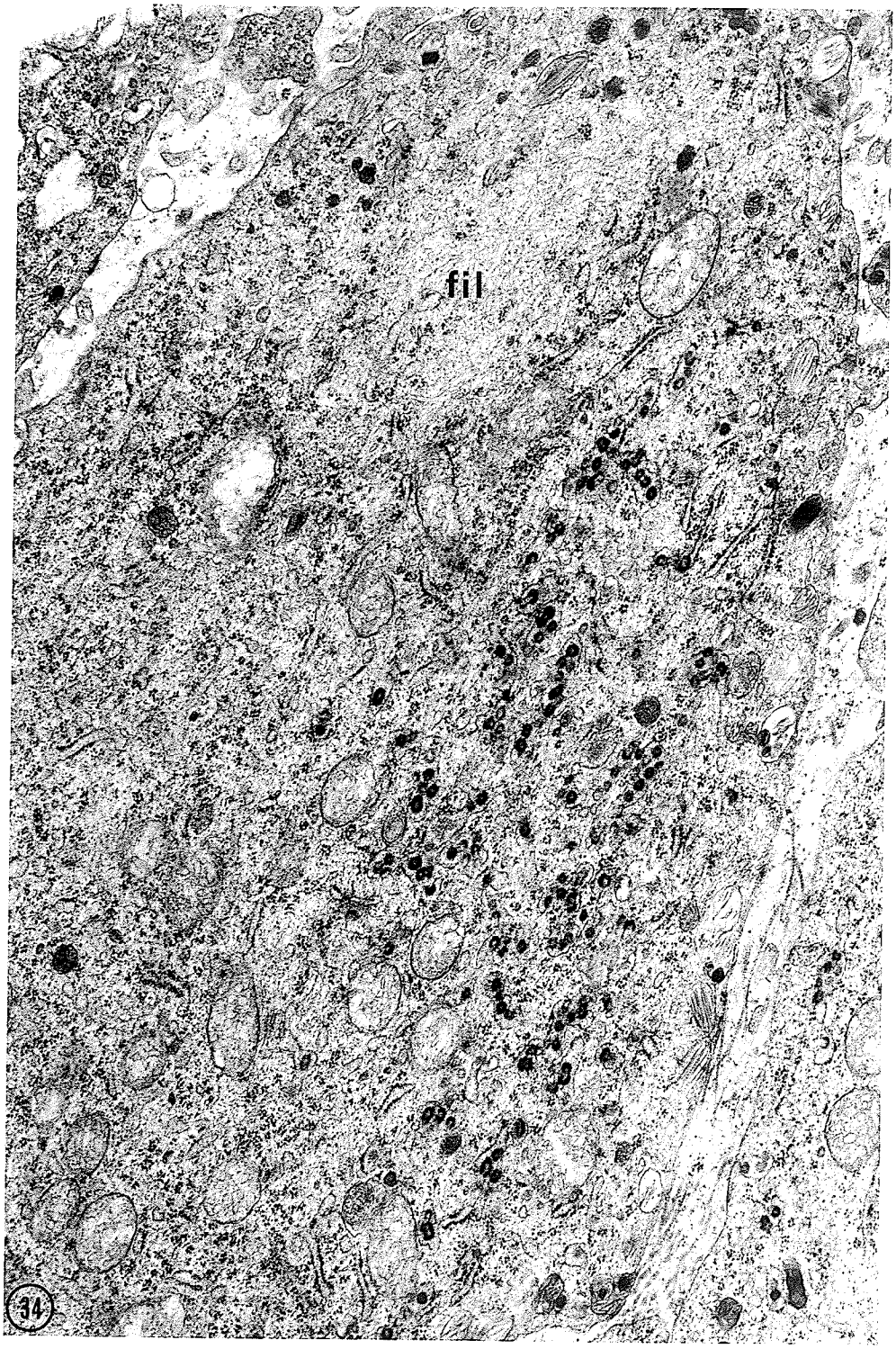

fil
34

Figure 35. EXPERIMENT A - MAXIMUM DOSAGE - GENERATION I - 2 weeks after cessation of colchicine treatment

Low power micrograph showing a blood vessel containing red blood cells. Also evident are a number of interphase cells containing cytoplasmic organelles. A portion of a tumor cell in mitosis may be seen in the top left portion of the figure. A bundle of filaments is seen within a cell adjacent to the blood vessel.

X 6,977

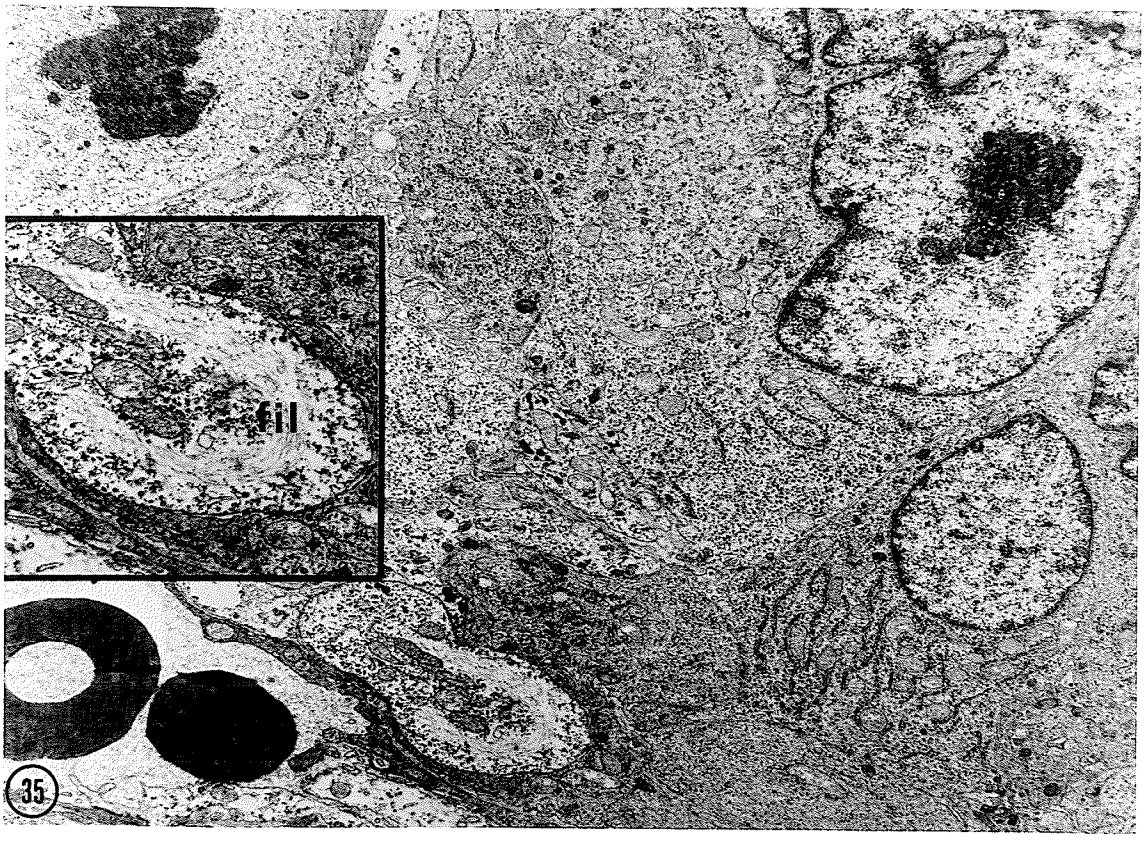
Inset shows at a higher magnification filaments within the cell shown in figure

X 12,403

Figure 36. EXPERIMENT A - MAXIMUM DOSAGE - GENERATION I - 2 weeks after cessation of colchicine treatment

High power micrograph of a tumor cell showing a large bundle of filaments (fil) surrounded peripherally by ribosomes.

X 30,096


fil
35

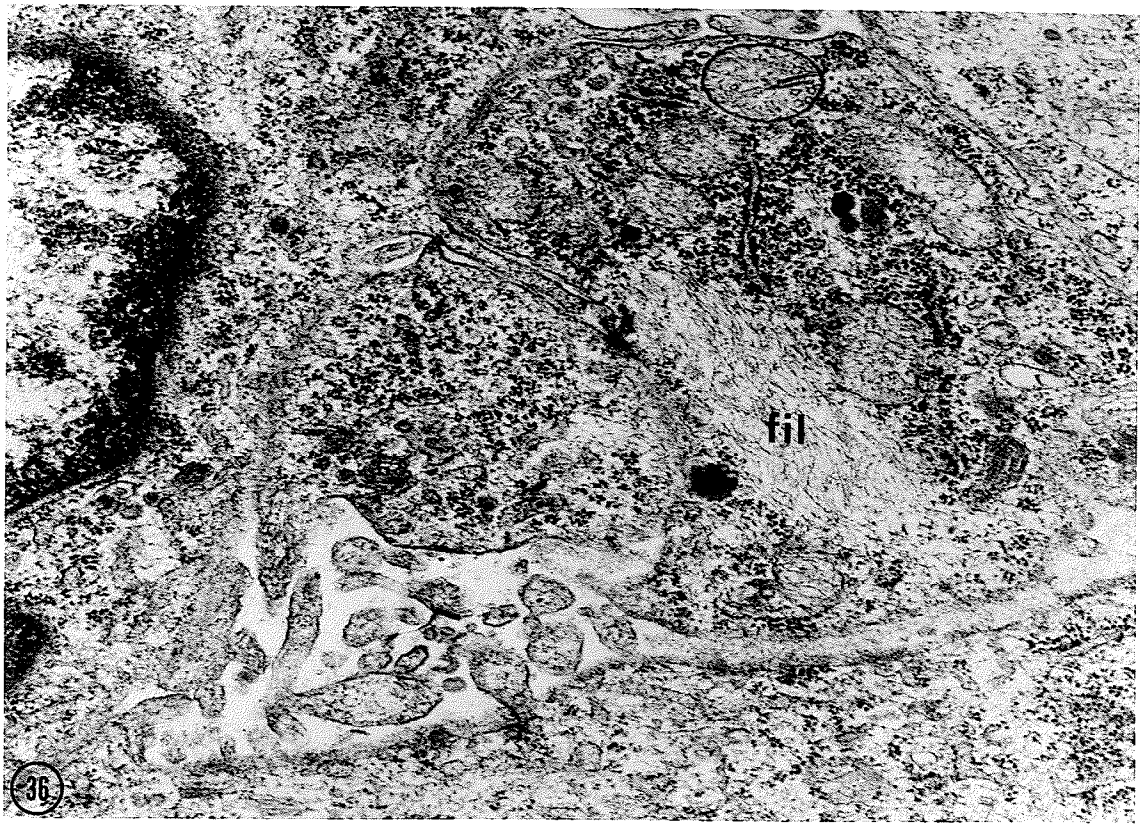

fil
36

Figure 37. EXPERIMENT A - MAXIMUM DOSAGE - GENERATION I - 2 weeks after cessation of colchicine treatment

This micrograph demonstrates a mitotic cell with centrally located chromosomes, and an elongated bundle of filaments (fil) peripheral to them. Observe an unusual pattern of granular endoplasmic reticulum (arrows).

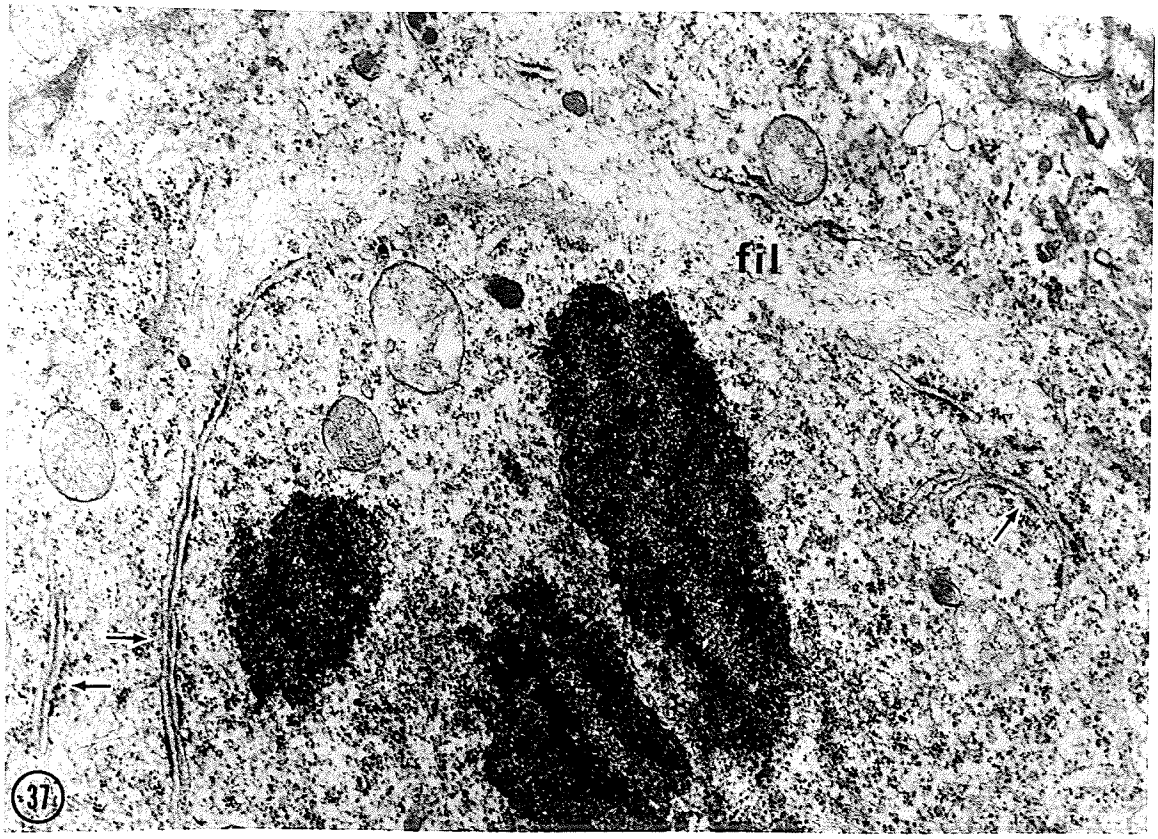
X 27,132

Figs. 38 & 39. EXPERIMENT A - MAXIMUM DOSAGE - GENERATION I - 2 weeks after cessation of colchicine treatment

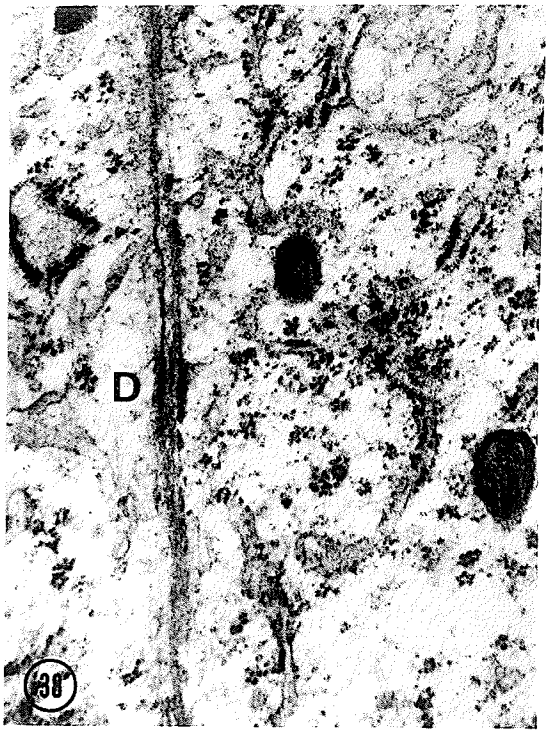
These high power micrographs demonstrate intercellular attachments (D) between the cell membranes of two cells. Such membrane specilizations were not observed in non-treated tumor cells.

Fig. 38 - X 35,112

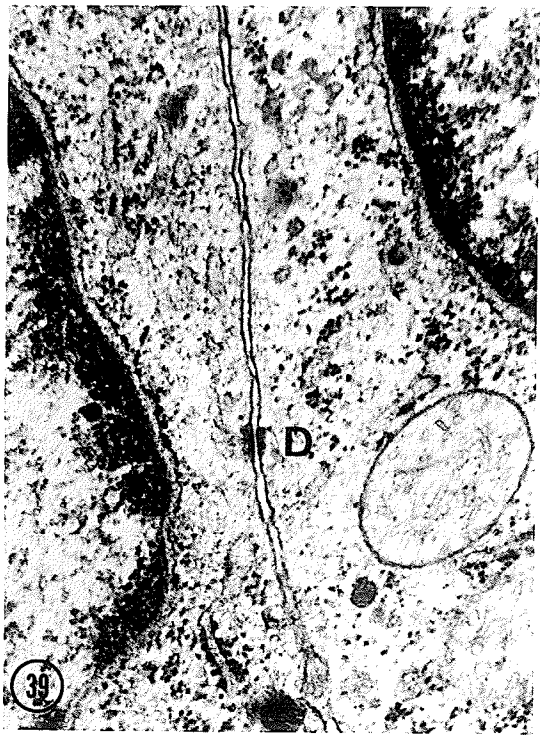
Fig. 39 - X 35,112



37



38



39

Figure 40. EXPERIMENT A - MAXIMUM DOSAGE - GENERATION II - 4 weeks after cessation of colchicine treatment

This figure illustrates a large bundle of filaments (fil) within a cell. Numerous ribosomes, both free and membrane bound, border the filaments. Considerable amounts of melanin is found in the periphery of the cell. Substantial numbers of mitochondria are also visualized in the cell.

X 54,264

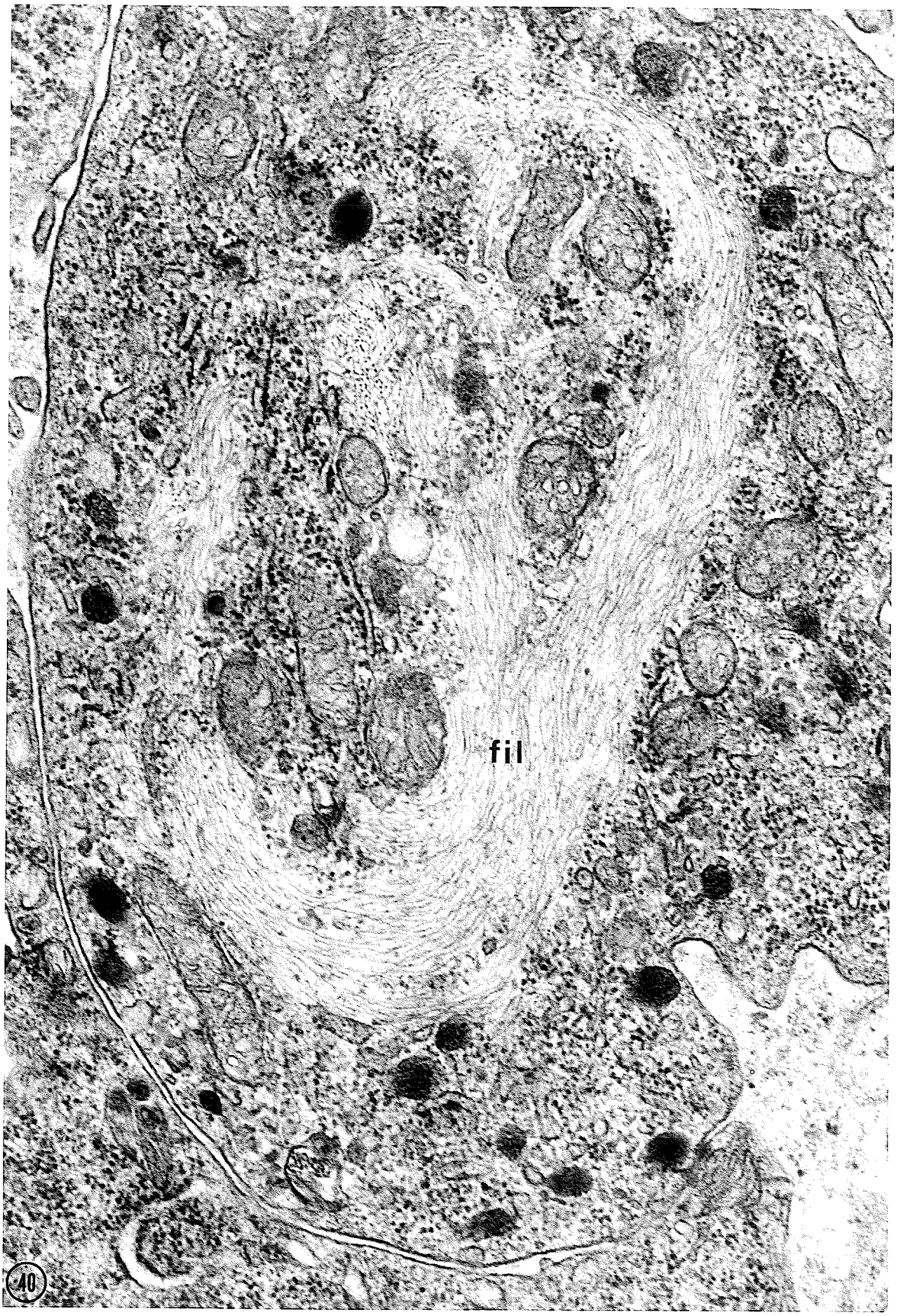
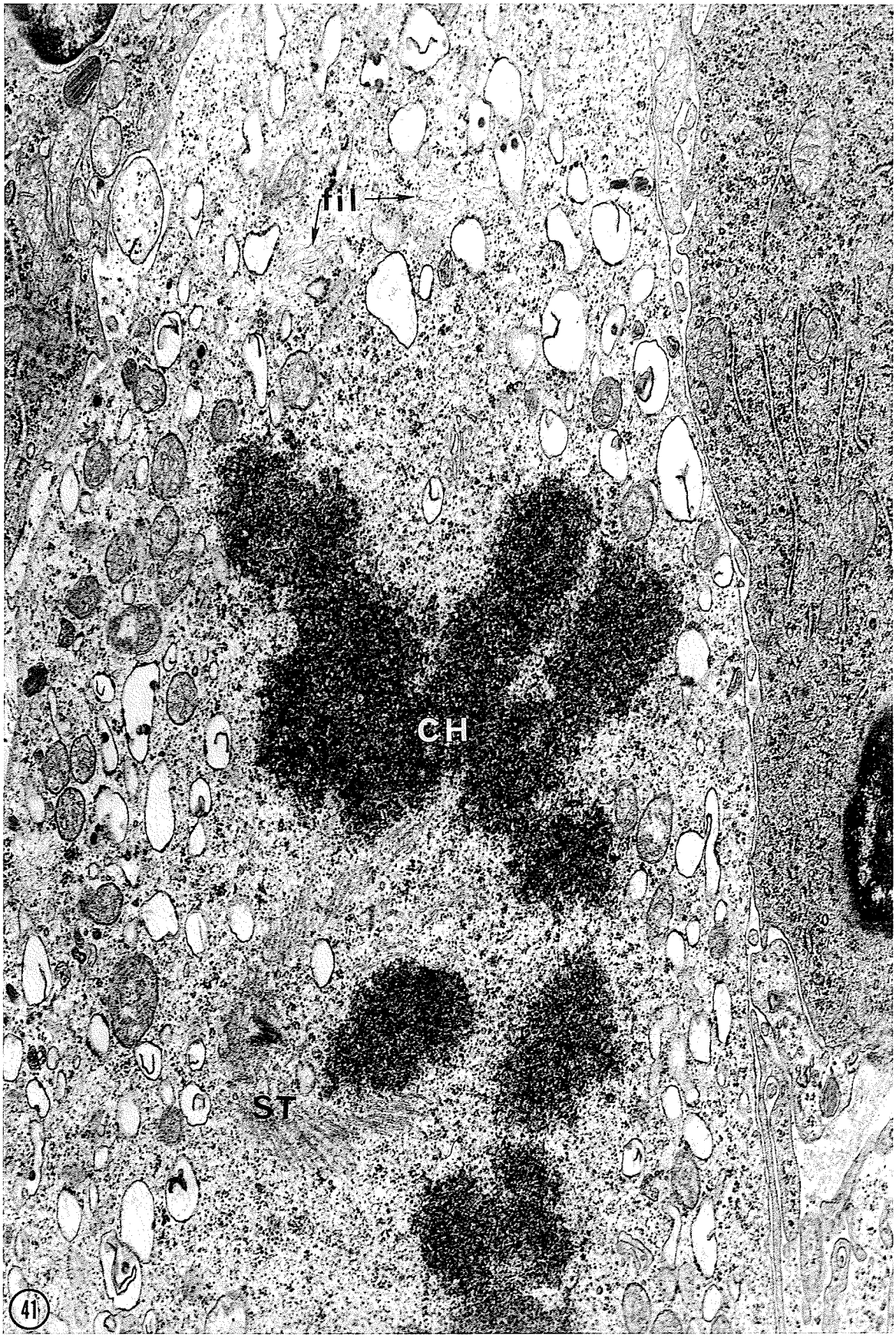

fil
40

Figure 41. EXPERIMENT A - MAXIMUM DOSAGE - GENERATION II - 4 weeks after cessation of colchicine treatment

This micrograph shows a mitotic cell with centrally located chromosomes (CH), spindle tubules (ST), and a centriole. In the periphery of the cell are dilated cisternae of endoplasmic reticulum and tubulo-vesicular mitochondria. Two small bundles of filaments (fil), readily distinguishable from spindle tubules, are seen in the cytoplasm of the cell.

X 24,624

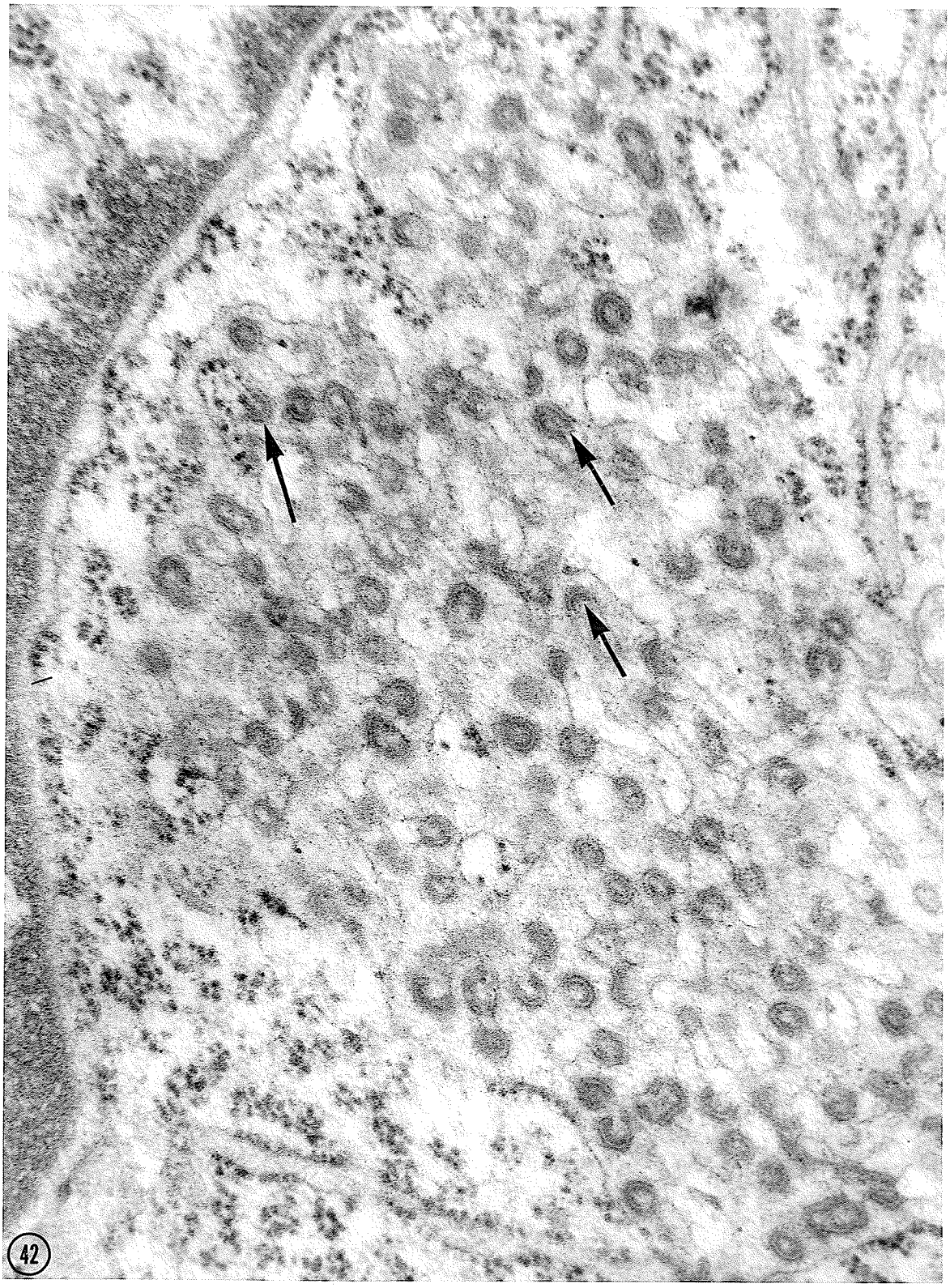


41

Figure 42. EXPERIMENT A - MAXIMUM DOSAGE - GENERATION II - 4 weeks after cessation of colchicine treatment

High power micrograph showing evolution of Type A double membrane virus particles from the endoplasmic reticulum. These virus particles are 700A^{o} in diameter and enclose a 250A^{o} translucent space. Arrows indicate particles which are presumably in the process of budding.

X 72,732

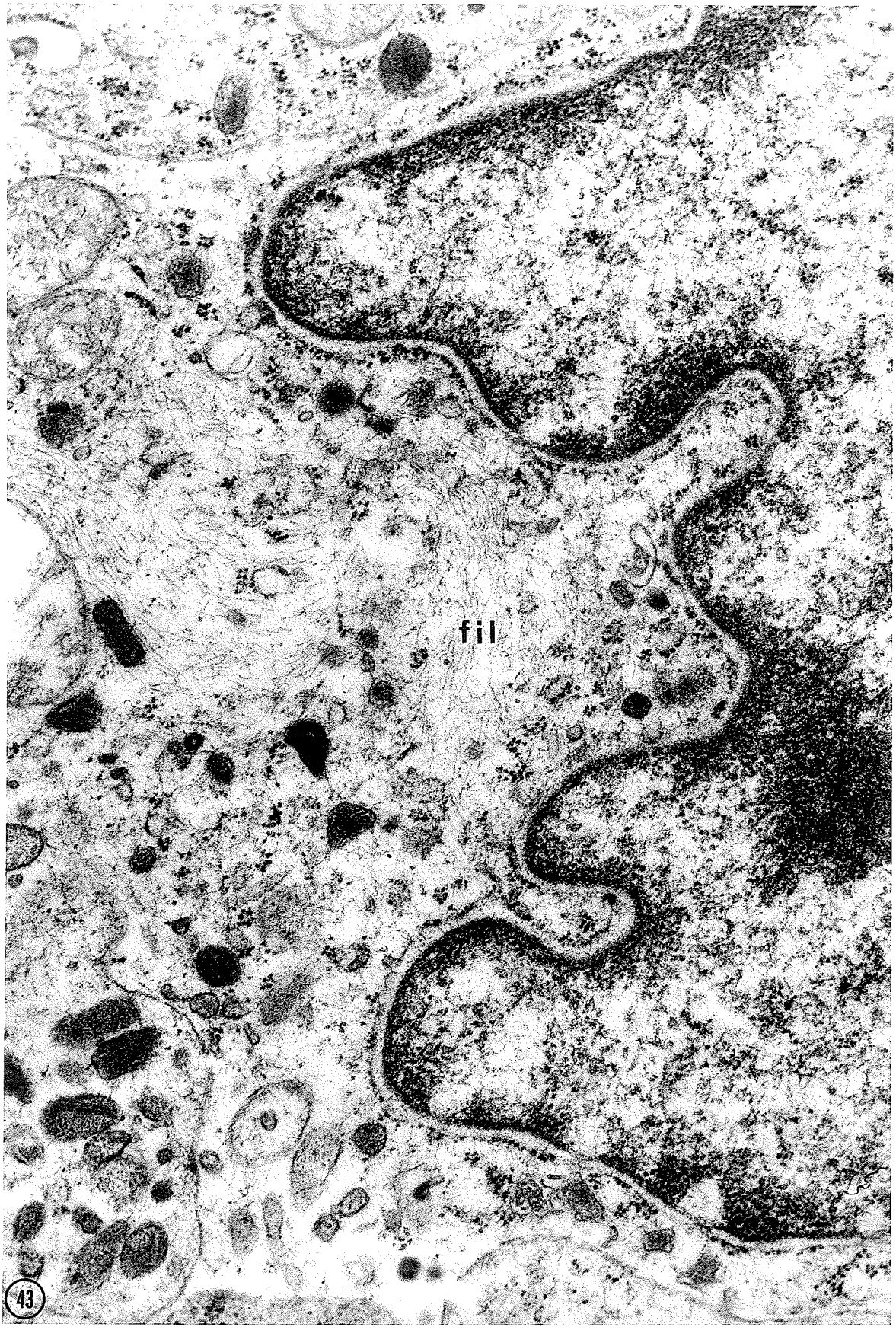


42

Figure 43. EXPERIMENT A - MAXIMUM DOSAGE - GENERATION III - 6 weeks after cessation of colchicine treatment

This micrograph illustrates an interphase cell possessing numerous microfilaments (fil), essentially perinuclear in position. Observe the attachment of ribosomes to the outer nuclear membrane. Melanin granules can also be seen in the cytoplasm.

X 47,196



43

Figure 44. EXPERIMENT A - MAXIMUM DOSAGE - GENERATION III - 6 weeks after cessation of colchicine treatment

An electron micrograph showing part of a cell containing a bundle of filaments (fil). Many melanosomes and melanin granules are seen adjacent to the filaments.

X 43,092

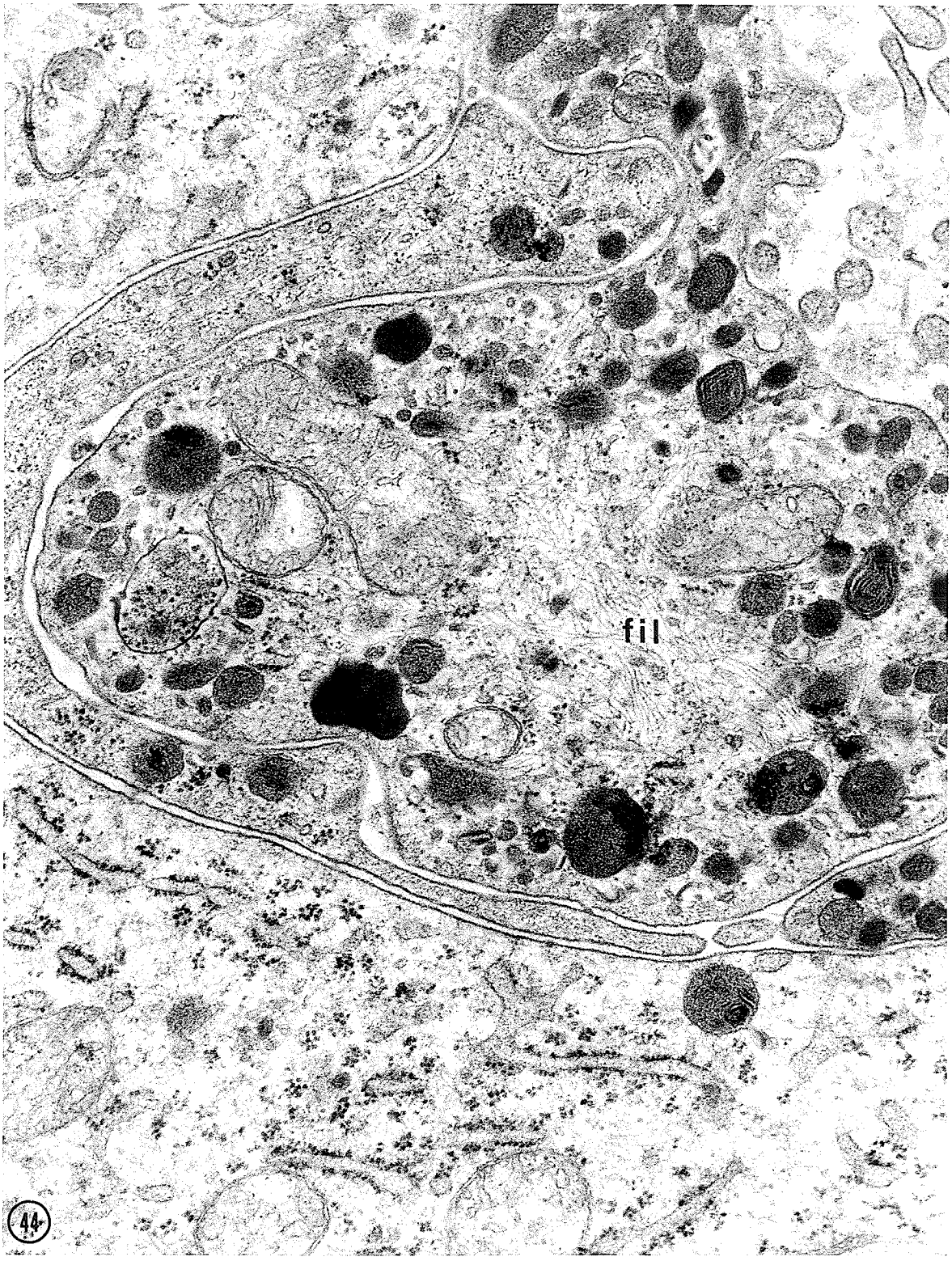
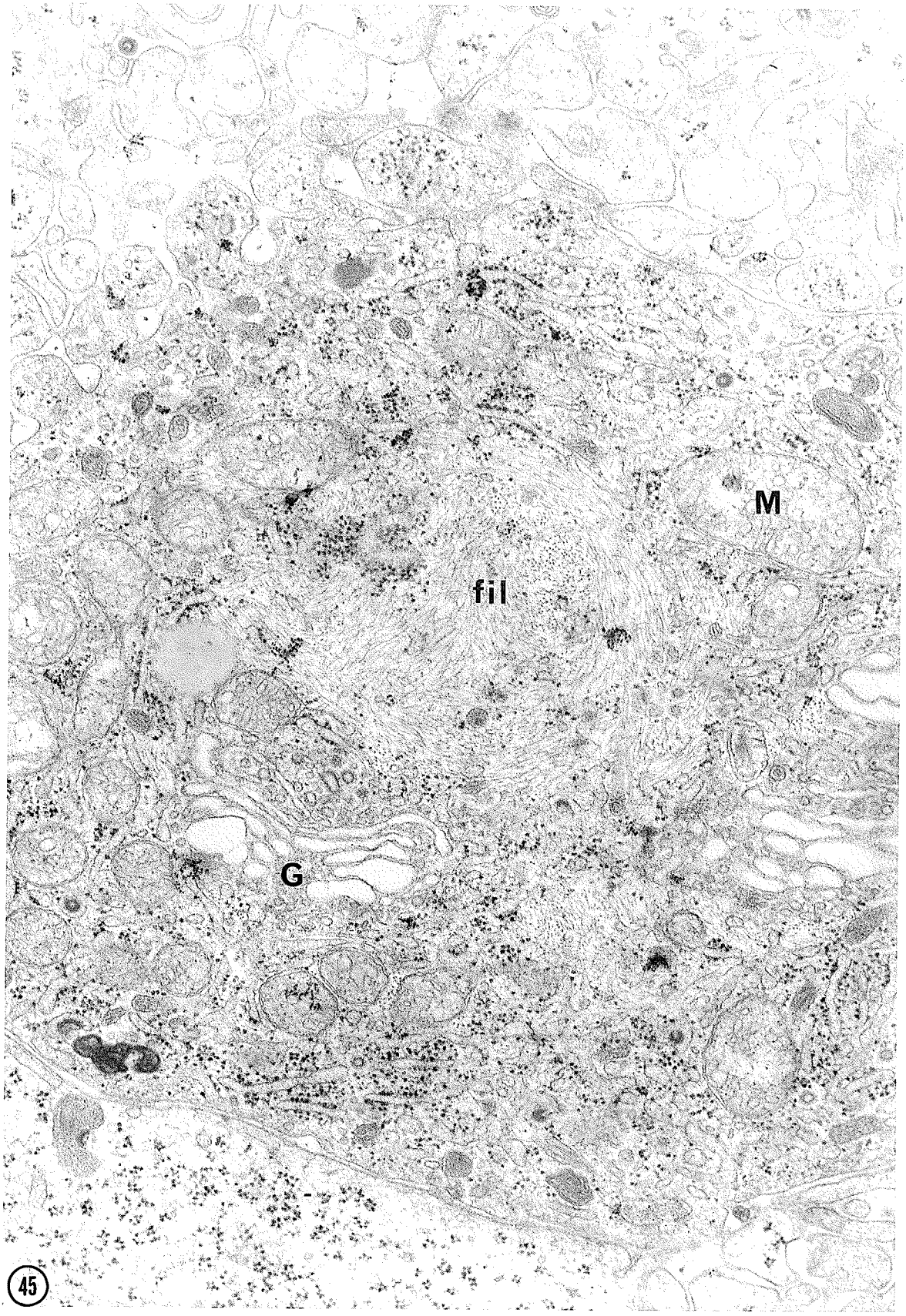

fil

Figure 45. EXPERIMENT A - MAXIMUM DOSAGE - GENERATION III - 6 weeks after cessation of colchicine treatment

This micrograph depicts a portion of an interphase cell containing a large bundle of filaments (fil) within the cytoplasm. Numerous well-developed Golgi complexes (G) and tubulo-vesicular mitochondria (M) are evident. Melanin in various stages of development can be seen scattered throughout the cytoplasm. A few virus particles may also be observed.

X 35,112



45

Figure 46. EXPERIMENT A - MAXIMUM DOSAGE - GENERATION IV - 8 weeks after cessation of colchicine treatment

Electron micrograph showing a cell containing a bundle of filaments (fil) with a few ribosomes interspersed amongst them, but mostly located at the periphery of the filaments. Other cellular inclusions evident are melanin and a lipid-like (L) vacuole.

X 35,112

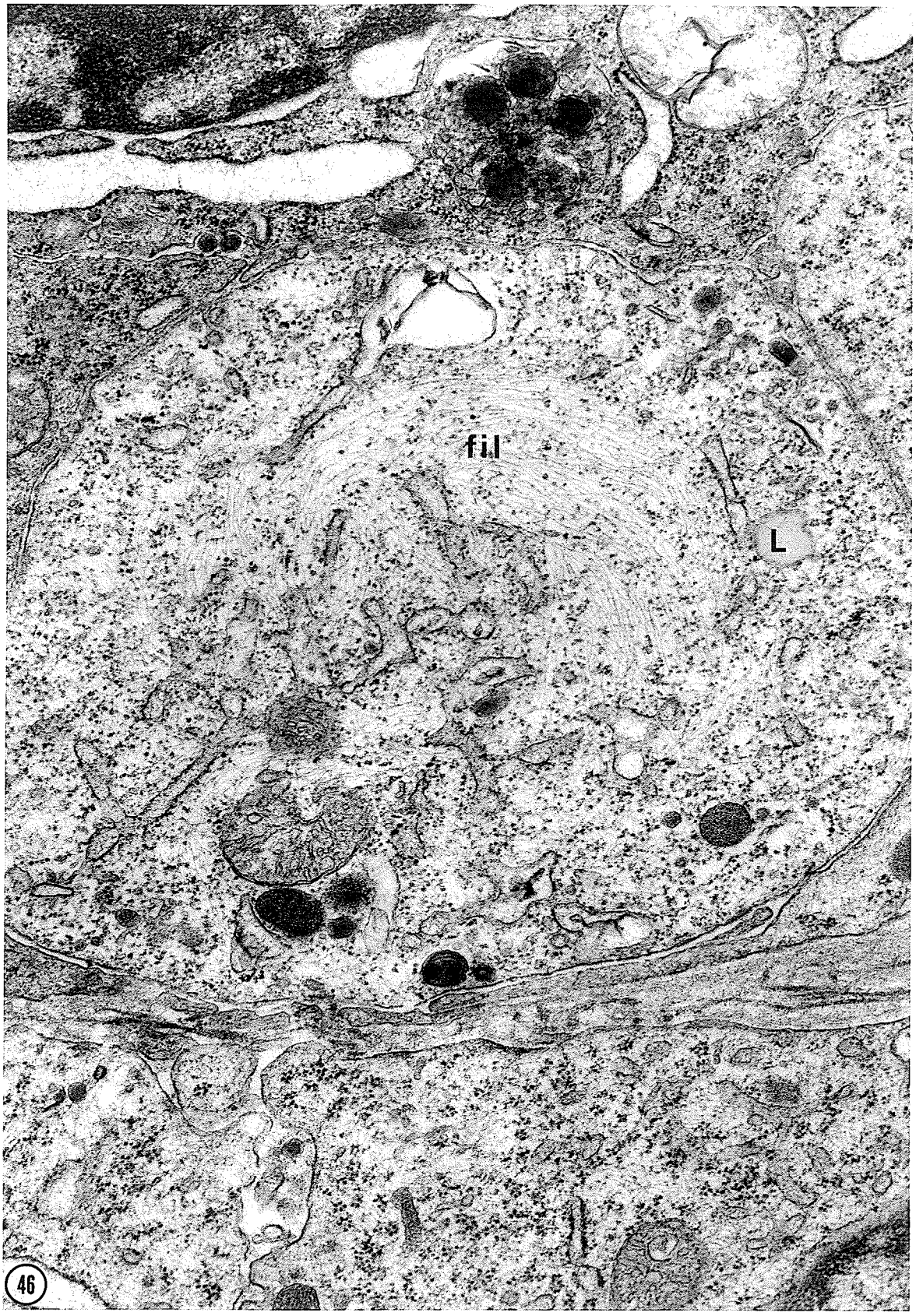

fil
L
46

Figure 47. EXPERIMENT A - MAXIMUM DOSAGE - GENERATION IV - 8 weeks after cessation of colchicine treatment

Micrograph of an interphase cell showing many filaments permeating the cytoplasm of the cell. Mitochondria and melanin are readily seen. Observe virus particles (V) which are associated with the endoplasmic reticulum.

X 35,112

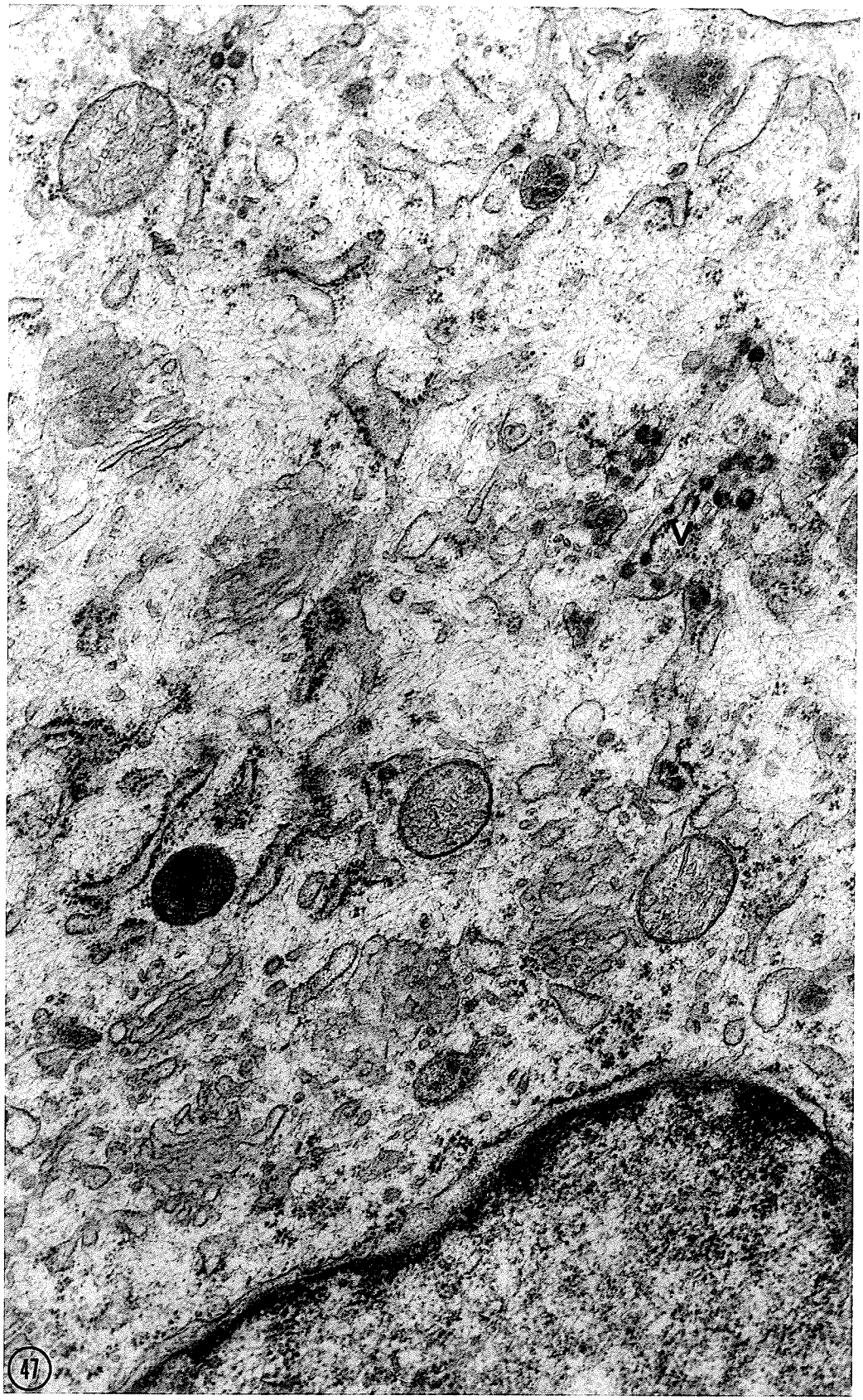

V
47

Figure 48. EXPERIMENT A - MAXIMUM DOSAGE - GENERATION IV - 8 weeks after cessation of colchicine treatment

Low power micrograph showing a moderately basophilic "light cell" (LC) and several heavily basophilic "dark cells" (DC). Filaments (fil) are seen in the cytoplasm of the light cell. Note the presence of virus particles in the dark cell seen in the lower, right portion of figure.

X 12,289

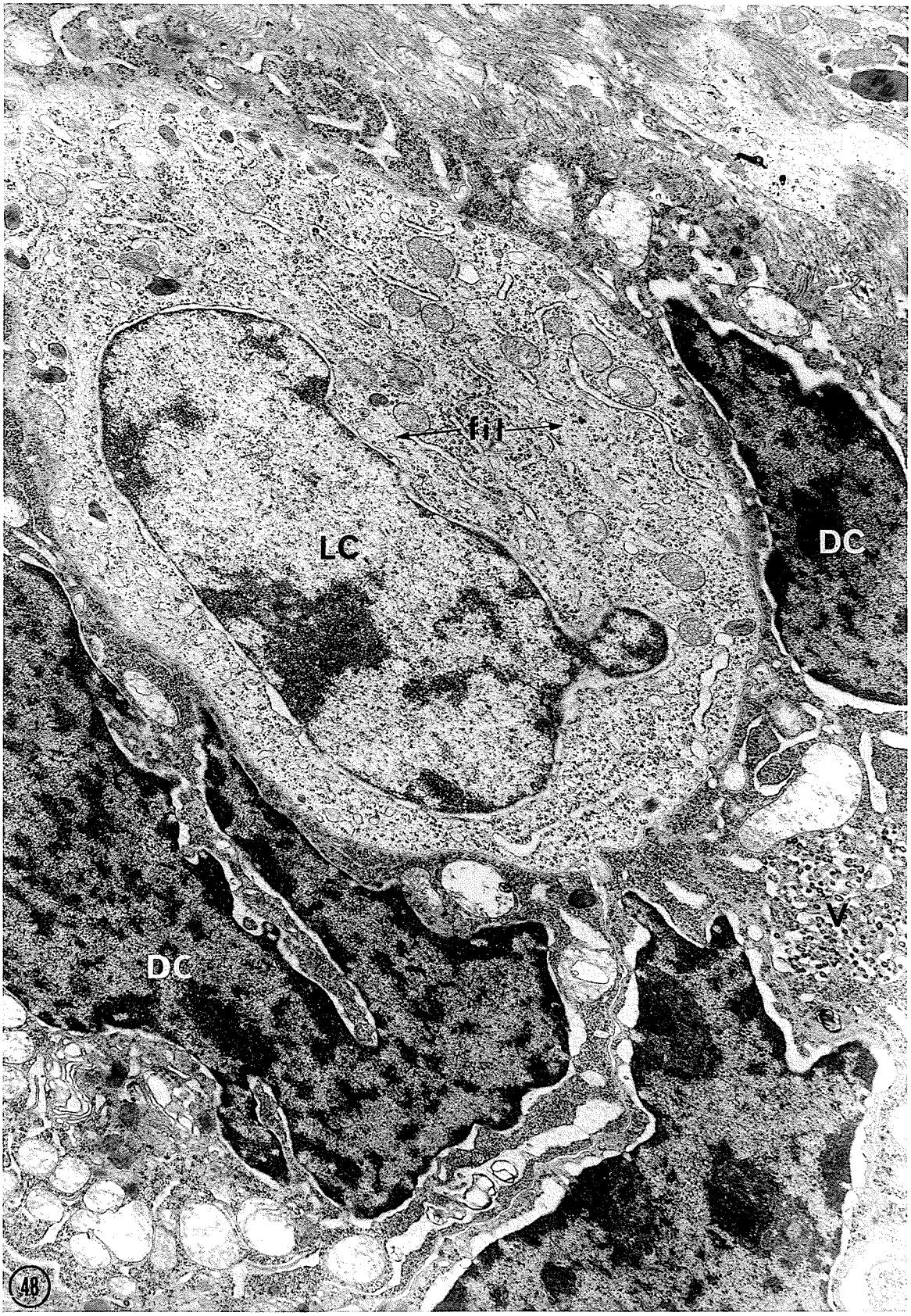

fil
LC
DC
DC
V
48

Figure 49. EXPERIMENT A - MAXIMUM DOSAGE - GENERATION V - 10 weeks after cessation of colchicine treatment

Electron micrograph of an interphase cell with an irregular nucleus and perinuclear space. Present also are a well-developed Golgi complex, tubulo-vesicular mitochondria and granular endoplasmic reticulum. A conspicious bundle of filaments, peri-nuclear in position, may be observed in this figure.

X 22,572

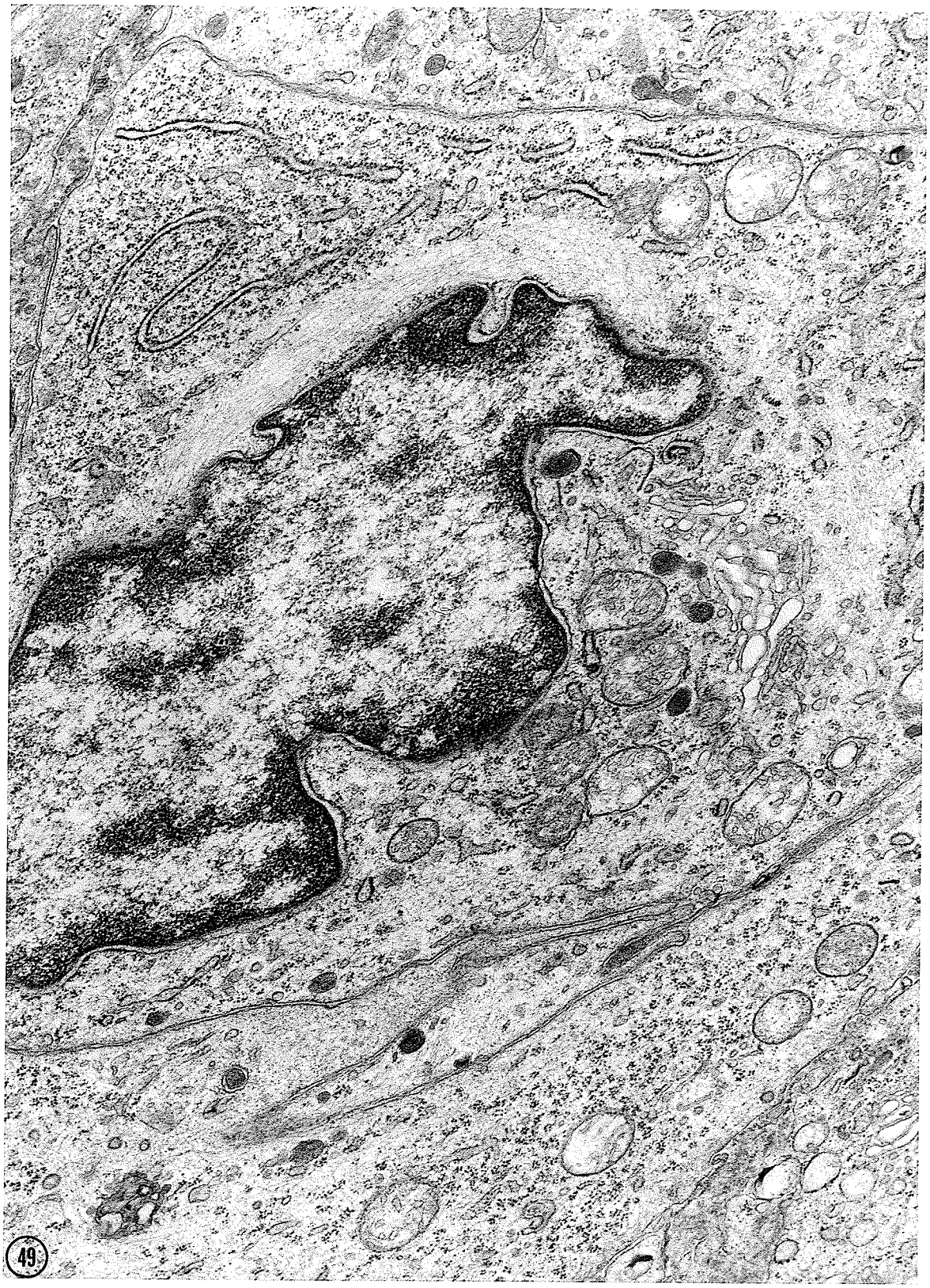
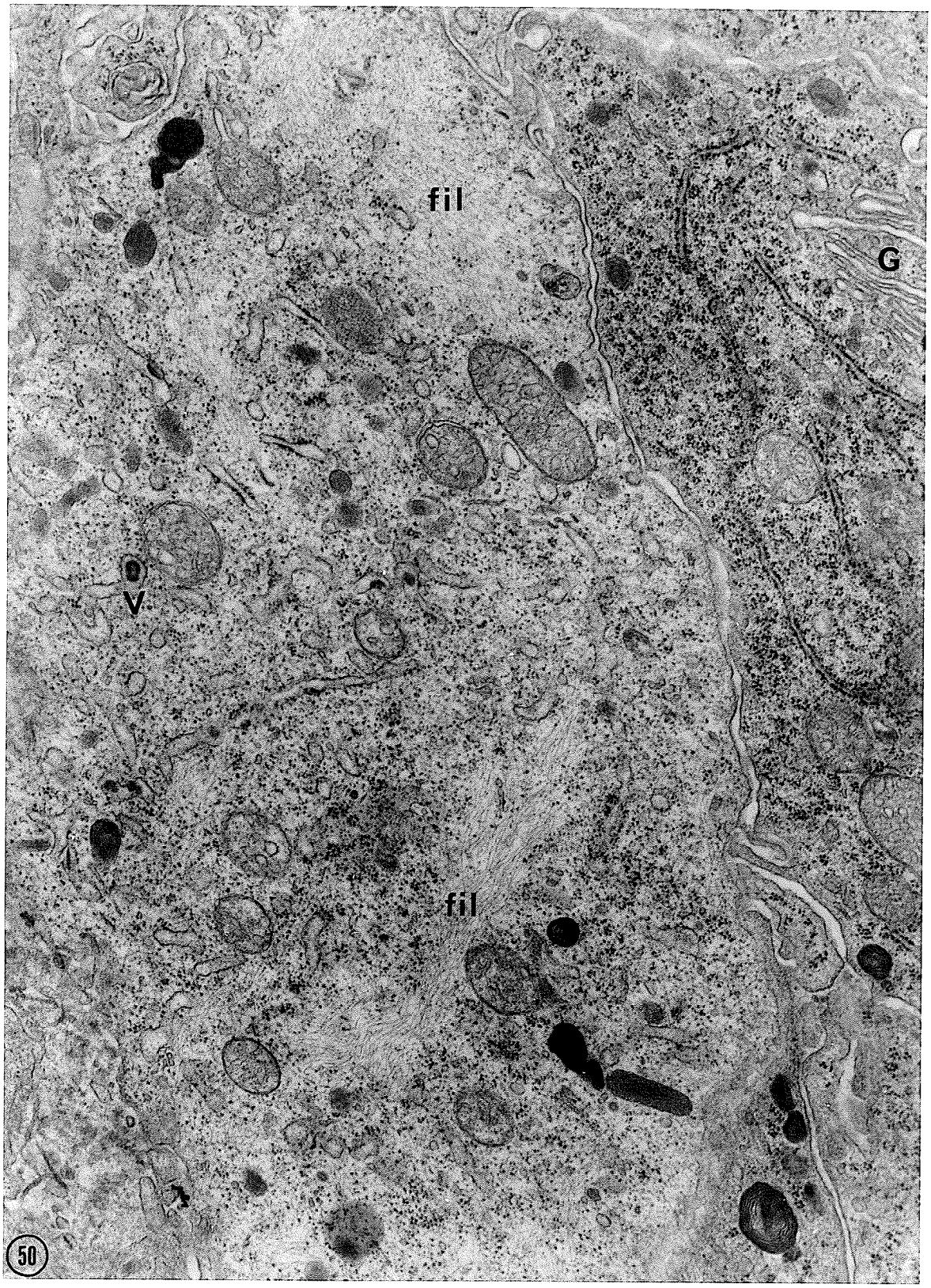


Figure 50. EXPERIMENT A - MAXIMUM DOSAGE - GENERATION V - 10 weeks after cessation of colchicine treatment

Micrograph of portions of two cells, one containing bundles of filaments (fil) 35-50A^{o} in diameter. Also seen in this cell are tubulo-vesicular mitochondria, melanin granules, precursor forms of melanin, and a lone virus (V) particle. A well-developed Golgi complex (G) and rough endoplasmic reticulum may be seen in the adjacent cell.

X 27,588


fil
G
V
fil
50

IV

OBSERVATIONS

ELECTRON MICROSCOPY

EXPERIMENT B - MINIMUM DOSAGE

EXPERIMENT B - MINIMUM DOSAGE

TREATED TUMOR

Cells of this experimental tumor were also generally pleomorphic in nature, with nuclei containing a peripheral accumulation of chromatin and distinct perinuclear space (Figs. 51 & 52). Melanin in various stages of development tended to increase in amount immediately following this colchicine treatment, but to a lesser extent than observed after maximal dosage treatment. Tubulo-vesicular mitochondria, well-develop Golgi complexes, and smooth and rough endoplasmic reticulum were observed in many cells (Figs. 51 & 52). Virus particles developing from the granular endoplasmic reticulum of the cell were often seen (Figs. 51 & 53). Centrioles were found adjacent to the nucleus of the cell (Figs. 51, 52 & 53). The most significant feature, was the presence of fine filaments in close proximity to the nucleus (Fig. 53) as well as in the rest of the cytoplasmic (Fig. 52). Emphasis must be placed on the fact that the number of cells containing filaments was much less than seen in experiment A, presumably due to the decreased dosage of colchicine.

GENERATION I - TWO WEEKS AFTER CESSATION OF COLCHICINE TREATMENT

The majority of cells seen in this generation which contained filaments were in the resting stage, or interphase (Fig. 54). Very few mitotic cells were observed to contain filaments in contrast to that seen in tumor cells of a comparable time of transplantation after maximal colchicine chemotherapy. Tubulo-vesicular mitochondria, smooth and rough endoplasmic reticulum and melanin granules could be observed to occur in most cells. Filaments were more often seen permeating the cytoplasm rather than in distinct bundles (Fig. 54).

GENERATION II - FOUR WEEKS AFTER CESSATION OF COLCHICINE TREATMENT

Figures 55 and 56 demonstrate the features of tumor cells in this generation of transplantation. The tumor nuclei are highly irregular in their outline and exhibit considerable condensation of chromatin along their margin. Some of them have hypertrophied nucleoli (Fig. 55). The nuclei possess a distinct perinuclear space with ribosomes attached to the outer nuclear membrane. In Figure 55 is a cell which apparently appears to be binucleated. It is felt that this nuclear configuration is due to the highly irregular nature of the tumor nucleus, which in a thin section results in two distinct nuclear masses giving it a fallacious binuclear condition. Well defined bundles of filaments may be seen between the nuclear masses. The cell also exhibits tubulo-vesicular mitochondria, a centriole lipid and melanin granules. Virus particles in relation to granular endoplasmic reticulum may be seen as well (Fig. 56).

GENERATION III - SIX WEEKS AFTER CESSATION OF COLCHICINE TREATMENT

Tumor cells of Generation III presented features similar to those seen in cells of other generations of this experiment. The melanin granules observed in this tumor were fewer in number than were seen in tumor cells of generation III after maximal dosage of colchicine. Mitochondria, ribosomes, free and attached to endoplasmic reticulum, and Golgi complexes all resembled those previously seen with no unusual features being encountered. Filaments were principally observed in interphase cells. Figure 57 depicts a multilobed nucleus with considerable margination of chromatin. Thy cytoplasm enclosed between the confines of the horse-shoe shaped nucleus exhibited

microfilaments. In some cells filaments were located in the peripheral portions of the cytoplasm (Fig. 58). Most of these interphase cells displayed well organized granular endoplasmic reticulum and numerous mitochondria. Fine bundles of microfilaments were observed in mitotic cells (Fig. 59).

GENERATION IV - EIGHT WEEKS AFTER CESSATION OF COLCHICINE TREATMENT

Fewer cells in this generation than in the previous generations presented filaments within the cytoplasm. Some cells had bundles, but the more often observed feature was filaments permeating throughout the cytoplasm (Fig. 60). Virus particles appeared at random in many cells (Fig. 60) and continue to be derived from the cisternal walls of endoplasmic reticulum. The usual cytoplasmic organelles appeared in the cytoplasm and were generally excluded from the midst of the filaments (Fig. 60).

GENERATION V - TEN WEEKS AFTER CESSATION OF COLCHICINE TREATMENT

In the last generation observed in this experiment, tumor tissue which had gone through five transplantations of host animals following cessation of colchicine treatment showed several cells containing bundles of filaments within the cytoplasm (Figs. 61, 62 & 63). Very few junctional specilizations could be seen between cells (Fig. 62), and extracellular space was seen to contain collagen (Figs. 61, 62 & 63). Filaments were identical to those observed in previous generations. They were seen in cross-section as well as longitudinal section; those in cross-section distinguishable from ribosomes by their much smaller size. Well defined granular endoplasmic reticulum (Fig. 61), free ribosomes, Golgi complexes, mitochondria and virus particles were all observed in the tumor cells. Emphasis must be

placed on the fact that large numbers of cells were observed before cells with filaments could be found at this stage of transplantation. The micrographs used showing filaments were only found after much scanning.

ELECTRON MICROGRAPHS

EXPERIMENT B - MINIMUM DOSAGE

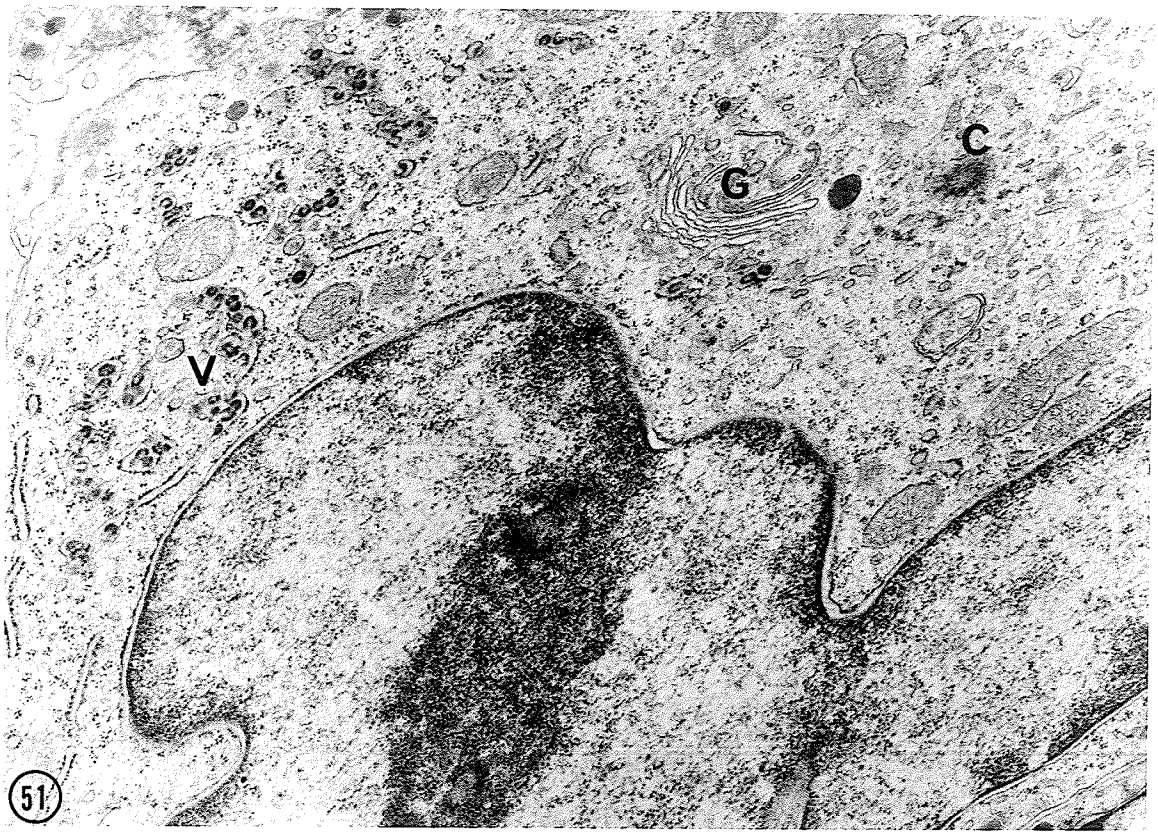
FIGURES 51-63

Figure 51. EXPERIMENT B - MINIMUM DOSAGE - TREATED TUMOR
Electron micrograph of an interphase cell showing a well-developed Golgi complex (G), centriole (C), and mitochondria. Note the presence of virus particles (V) deriving from the endoplasmic reticulum.

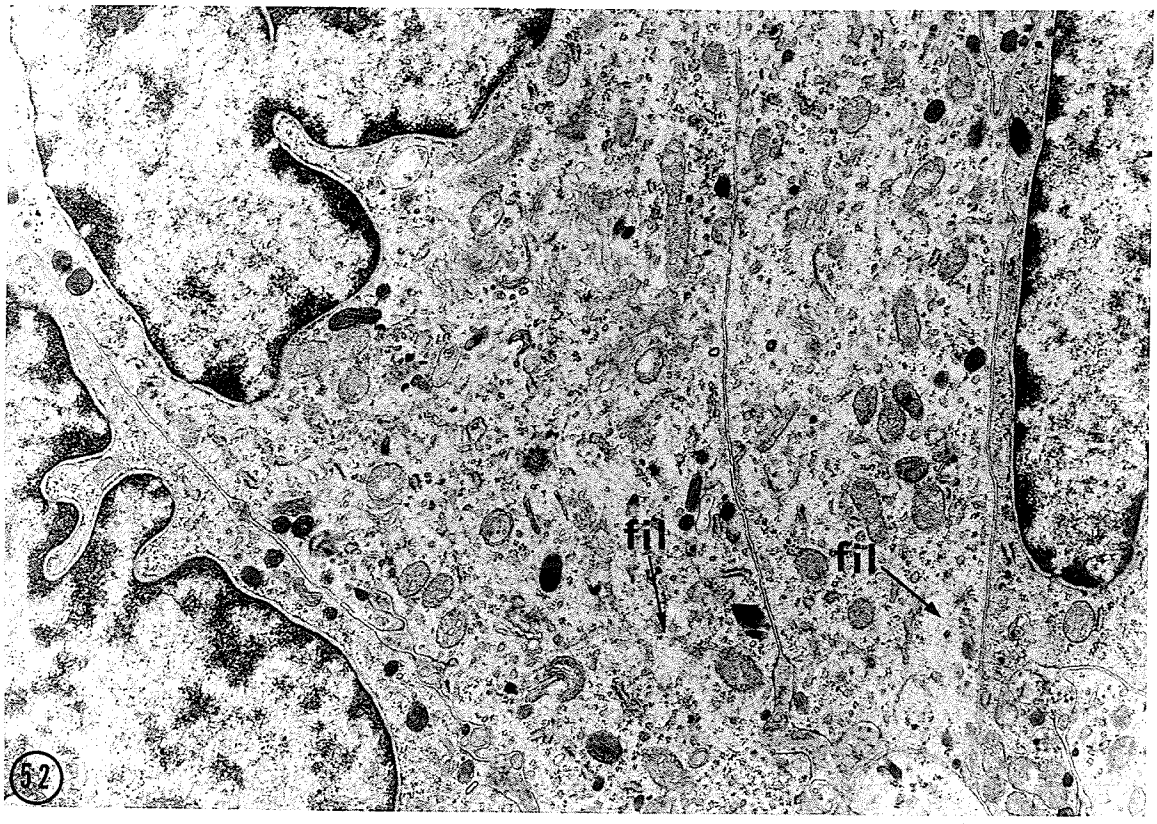
X 21,318

Figure 52. EXPERIMENT B - MINIMUM DOSAGE - TREATED TUMOR
Interphase cells illustrating presence of a Golgi complex, endoplasmic reticulum, centriole, mitochondria and some fine filaments (fil) permeating the cytoplasm are seen.

X 13,760



51



52

Figure 53. EXPERIMENT B - MINIMUM DOSAGE - TREATED TUMOR
Portion on an interphase cell showing a centriole (C) and fine filaments (fil) permeating the cytoplasm. Virus particles (V) are shown in association with the endoplasmic reticulum.

X 47,196

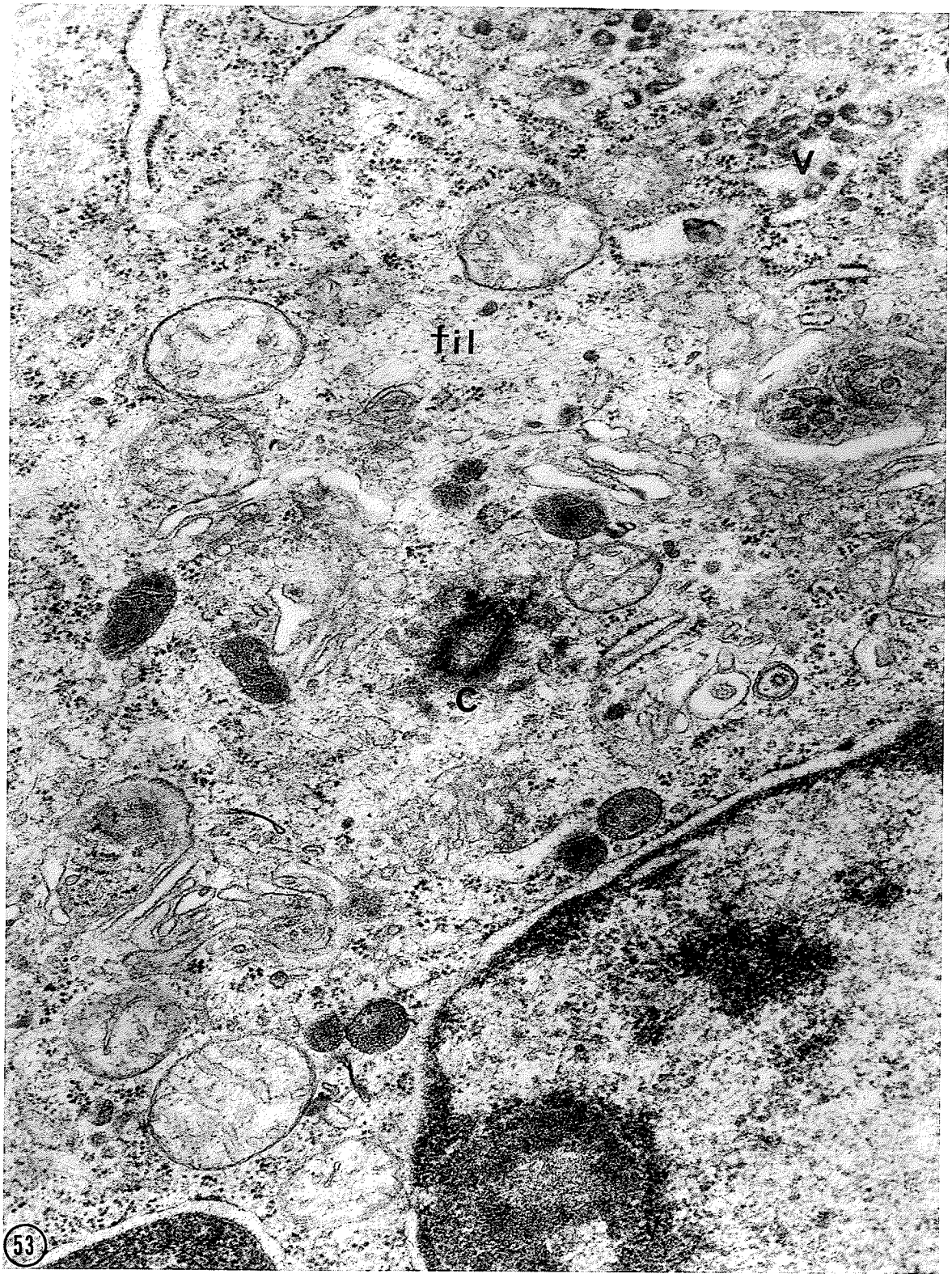

V
fil
C
53

Figure 54. EXPERIMENT B - MINIMUM DOSAGE - GENERATION I - 2 weeks after cessation of colchicine treatment

An interphase cell showing filaments (fil) permeating the cytoplasm rather than in distinct bundles. The cell demonstrates the usual cytoplasmic organelles viz., centriole, tubulo-vesicular mitochondria, Golgi complex, rough endoplasmic reticulum and melanin granules.

X 25,650


fil
fil
54

Figure 55. EXPERIMENT B - MINIMUM DOSAGE - GENERATION II - 4 weeks after cessation of colchicine treatment

This figure shows a cell containing a substantial amount of microfilaments (fil), with few ribosomes amongst them. Also present is a centriole, tubulo-vesicular mitochondria and melanin in various stages of melanization. Large numbers of free ribosomes and multiple vacuoles, which presumably contained neutral lipid (L) in the living state, can be seen in the cell.

X 28,842

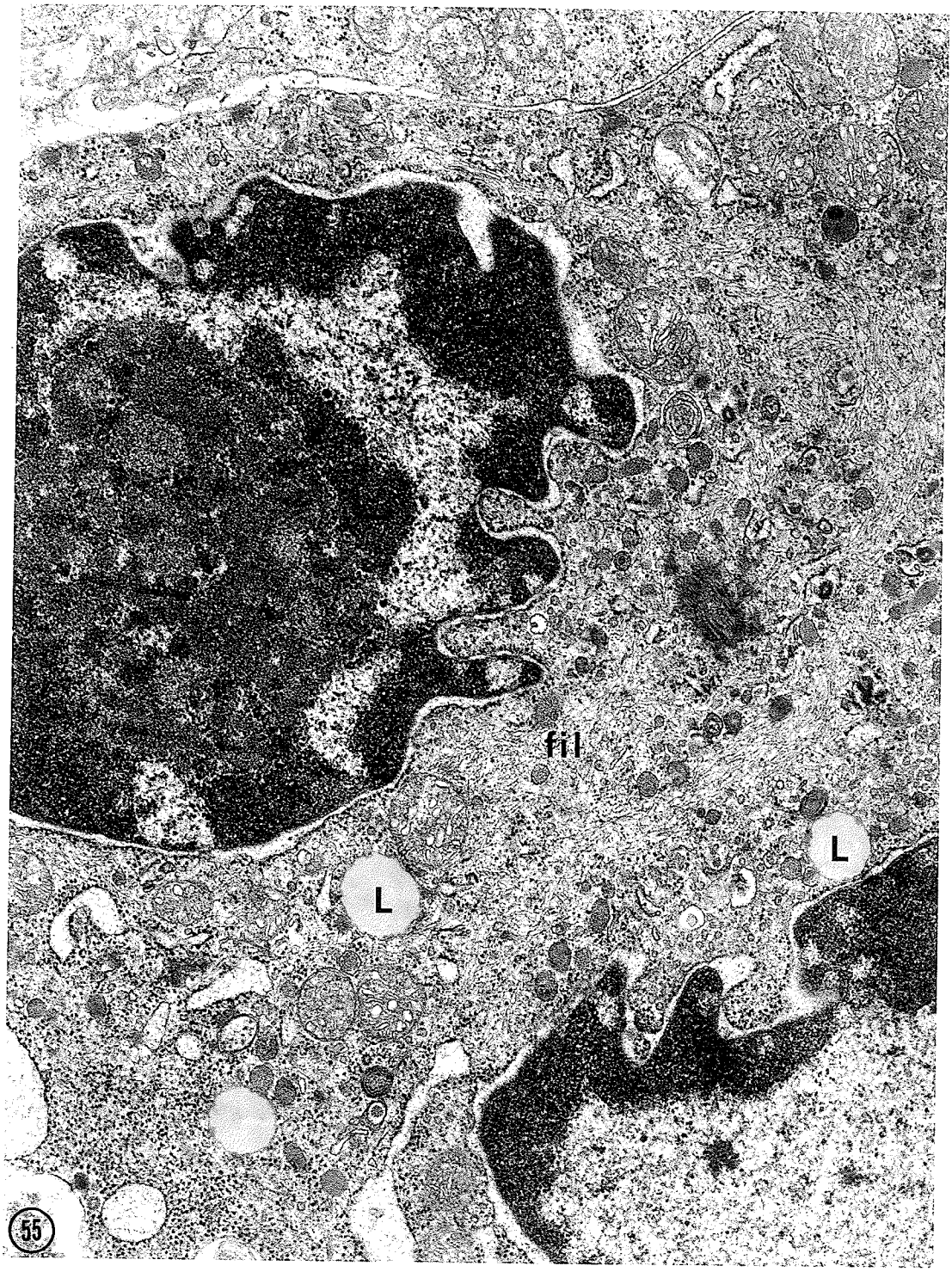

fil
L
L
55

Figure 56. EXPERIMENT B - MINIMUM DOSAGE - GENERATION II - 4 weeks after cessation of colchicine treatment

This figure depicts the more usual pattern of filaments (fil) observed in the cytoplasm of tumor cells treated with minimum dosage. Melanin, Golgi complexes and mitochondria are frequently observed in the cell cytoplasm. Virus particles (V) enclosed within rough endoplasmic reticulum are visualized.

X 23,598

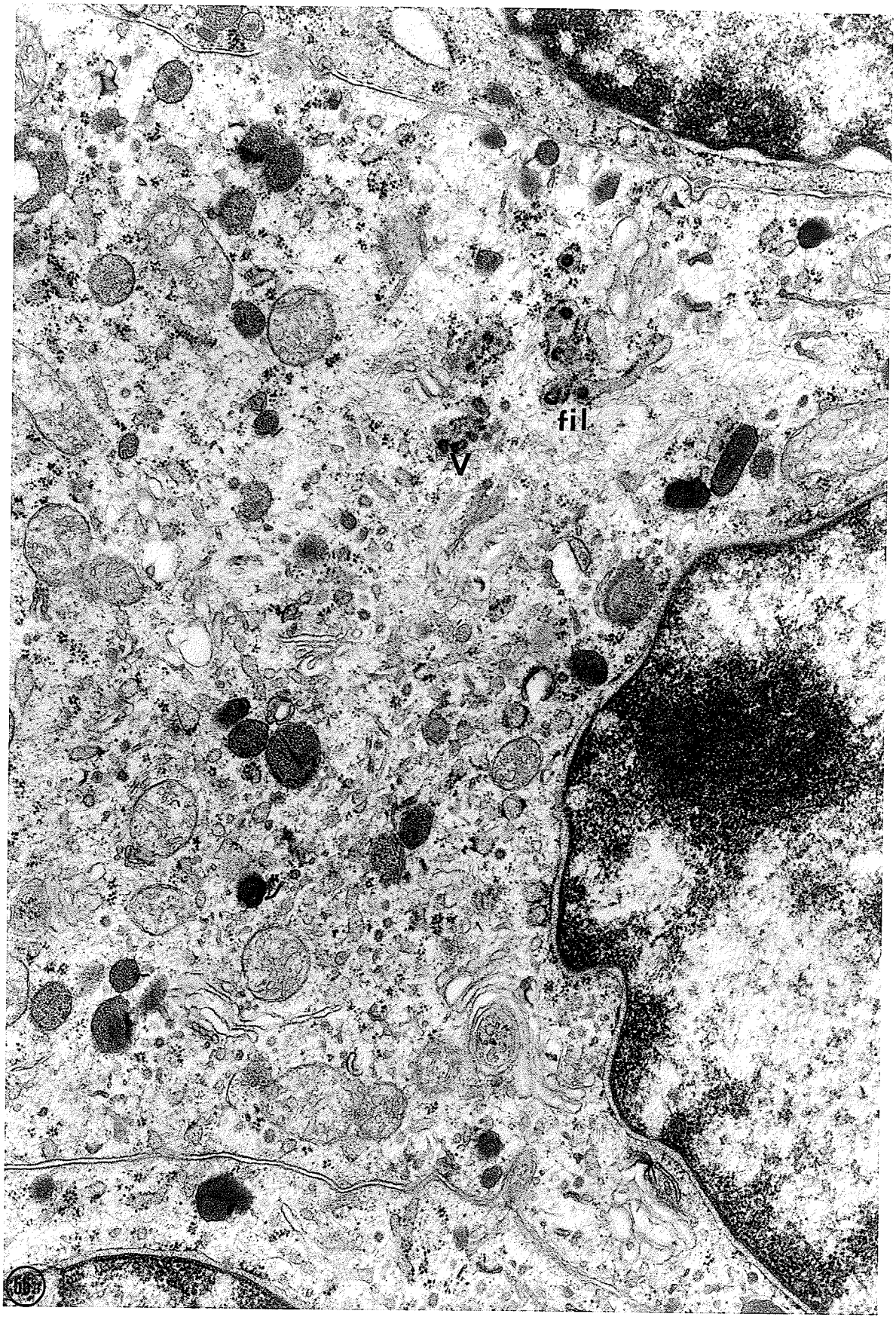

fil
V

Figure 57. EXPERIMENT B - MINIMUM DOSAGE - GENERATION III - 6 weeks after cessation of colchicine treatment

This low power micrograph shows an interphase cell containing a large bundle of filaments (fil) adjacent to the very irregular nucleus. Melanin in various stages of melanization are seen throughout the cytoplasm. Distinct endoplasmic reticulum, tubulo-vesicular mitochondria and well-developed Golgi complexes are also seen in this micrograph. The continuity between rough surfaced endoplasmic reticulum and smooth endoplasmic reticulum is indicated (arrows).

X 15,732

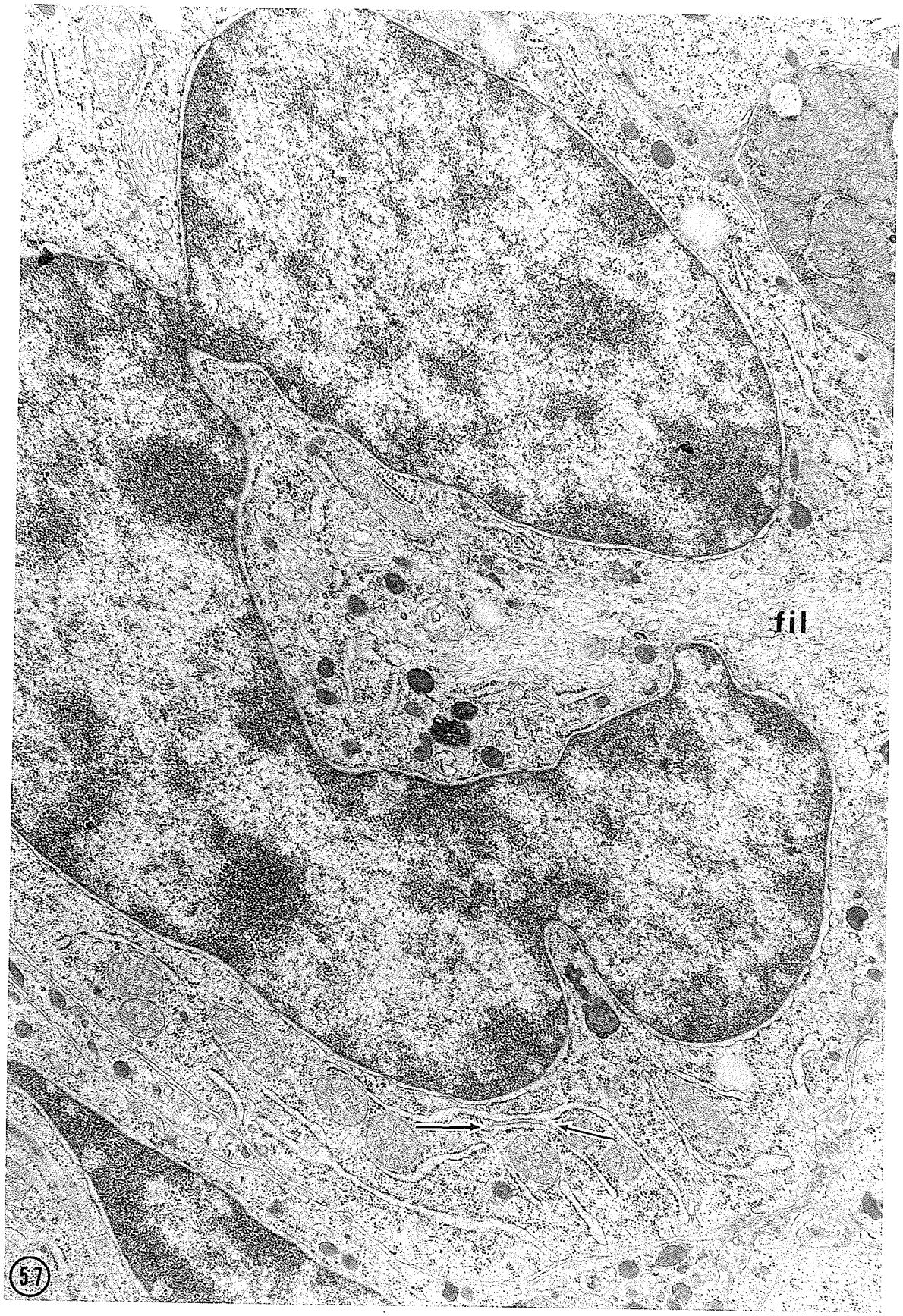

fil
5.7

Figure 58. EXPERIMENT B - MINIMUM DOSAGE - GENERATION III - 6 weeks after cessation of colchicine treatment

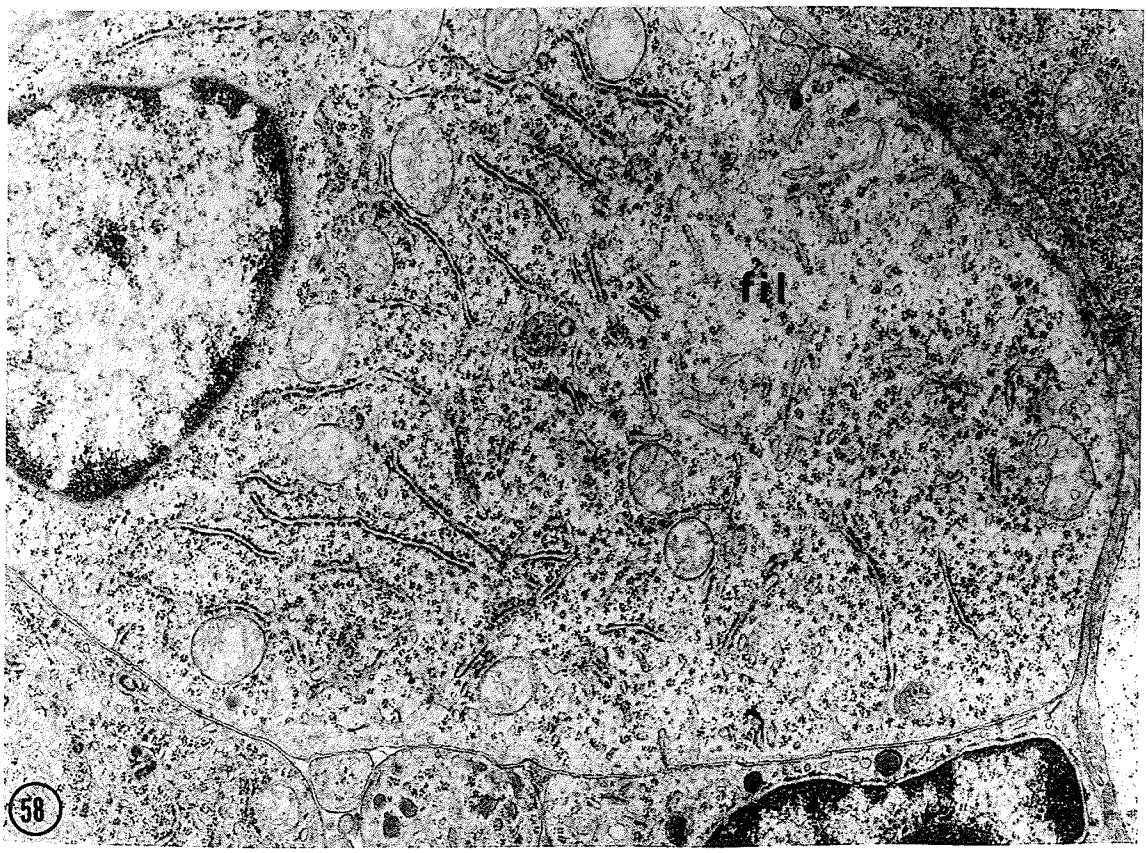
This micrograph shows the greater part of an interphase cell containing microfilaments within the cytoplasm (fil). Numerous mitochondria and endoplasmic reticulum are also present within the cell.

X 18,468

Figure 59. EXPERIMENT B - MINIMUM DOSAGE - GENERATION III - 6 weeks after cessation of colchicine treatment

This figure shows a mitotic cell containing chromosomes, endoplasmic reticulum, tubulo-vesicular mitochondria and melanin. Fine filaments are seen in the form of a small bundle (fil) as well as permeating the cytoplasm (arrows).

X 15,732


fil
58

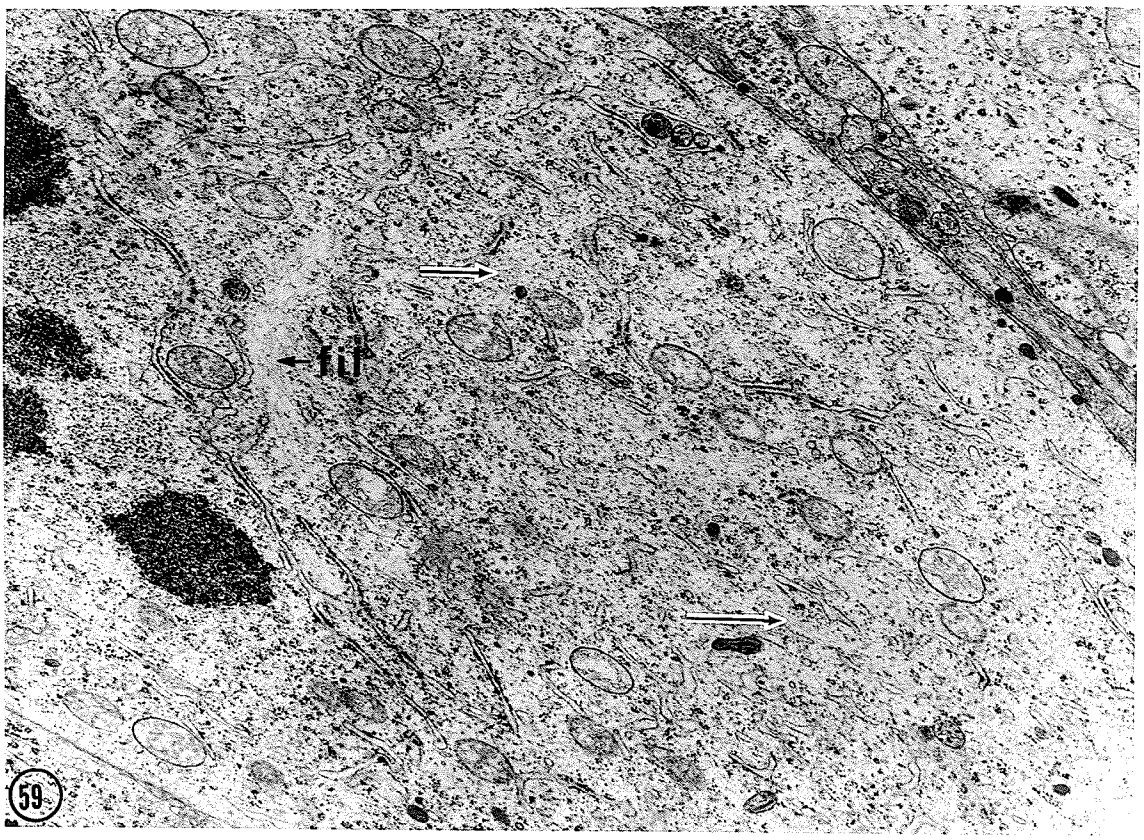

fil
59

Figure 60. EXPERIMENT B - MINIMUM DOSAGE - GENERATION IV - 8 weeks after cessation of colchicine treatment

Micrograph illustrating microfilaments (fil) within the tumor cell. Note that the ribosomes are generally excluded from the midst of the filaments, but are located bordering them. Mitochondria and melanin granules are found in the cell. Virus particles (V) showing several stages in their evolution from the rough surfaced endoplasmic reticulum are also visualized in this micrograph.

X 57,456

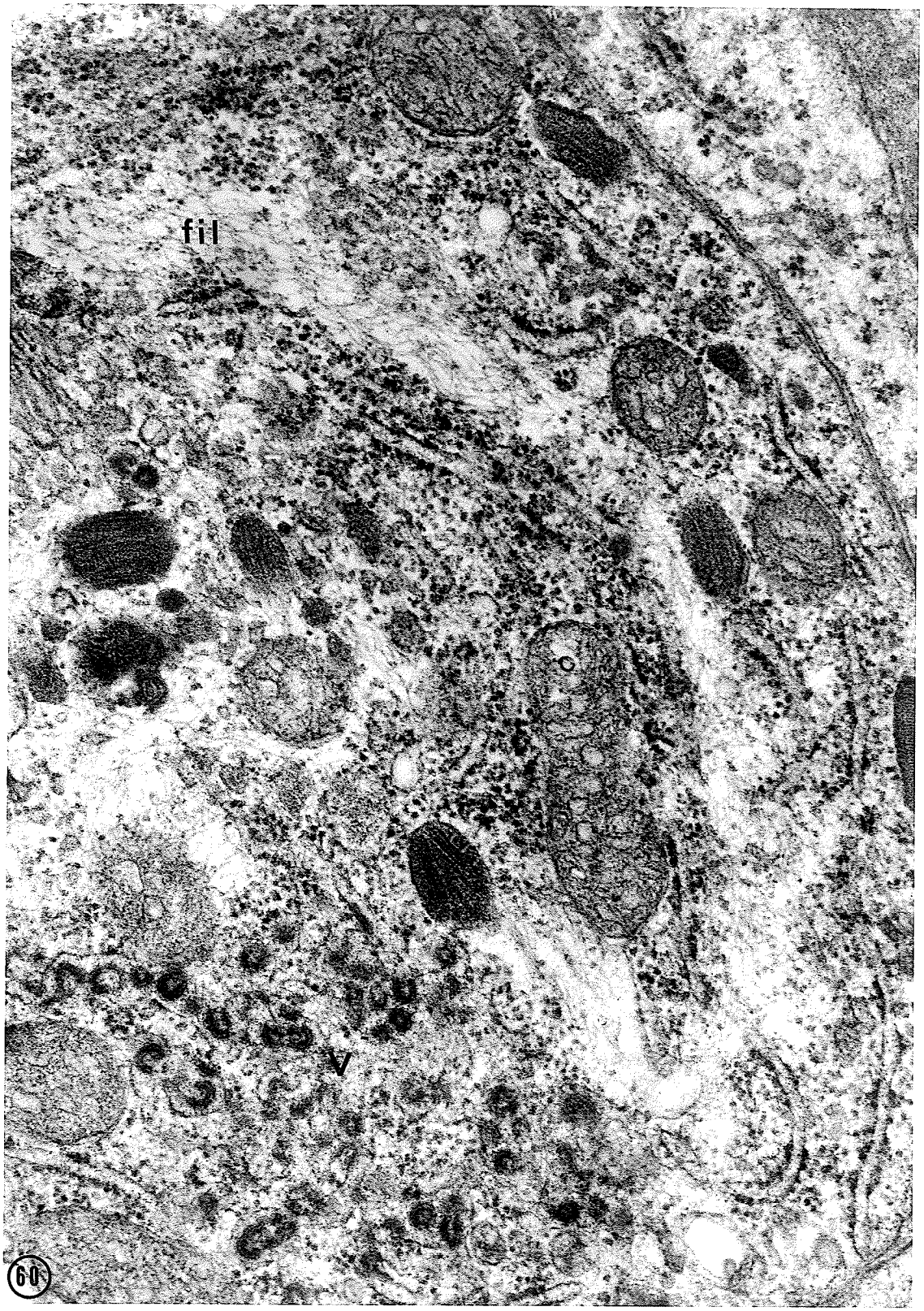
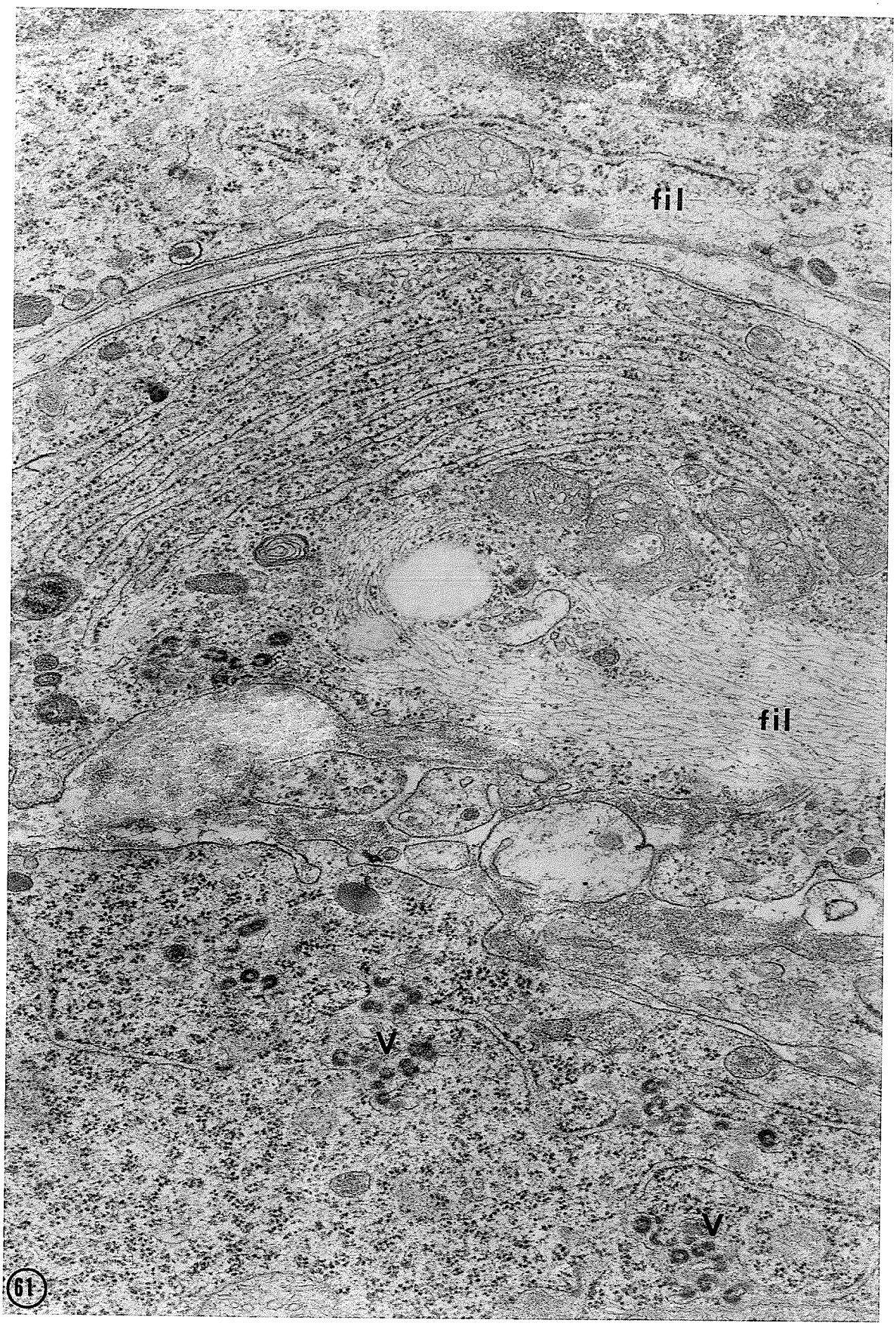

fil
V
60

Figure 61. EXPERIMENT B - MINIMUM DOSAGE - GENERATION V - 10 weeks after cessation of colchicine treatment

A conspicuous bundle of filaments (fil) is vizualized within the cell located in the center of the micrograph. A dramatic parallel array of rough endoplasmic reticulum is also seen in this cell. The cell in the lower part of the figure shows a considerable amount of polyribosomes. Numerous virus particles (V) in relation to rough endoplasmic reticulum are seen in this cell also. A portion of the cell in the top of the figure displays a fine bundle of filaments (fil).

X 36,908



61

Figure 62. EXPERIMENT B - MINIMUM DOSAGE - GENERATION V - 10 weeks after cessation of colchicine treatment

Portions of interphase cells are seen, some containing filaments (fil) in large bundles, or small bundles (*) within the cytoplasm. In the lower portion of the figure, plasma membranes of adjacent cells exhibit slight thickening (arrow) which resembles junctional attachments. Collagen (coll) can be observed in the intercellular spaces.

X 17,100

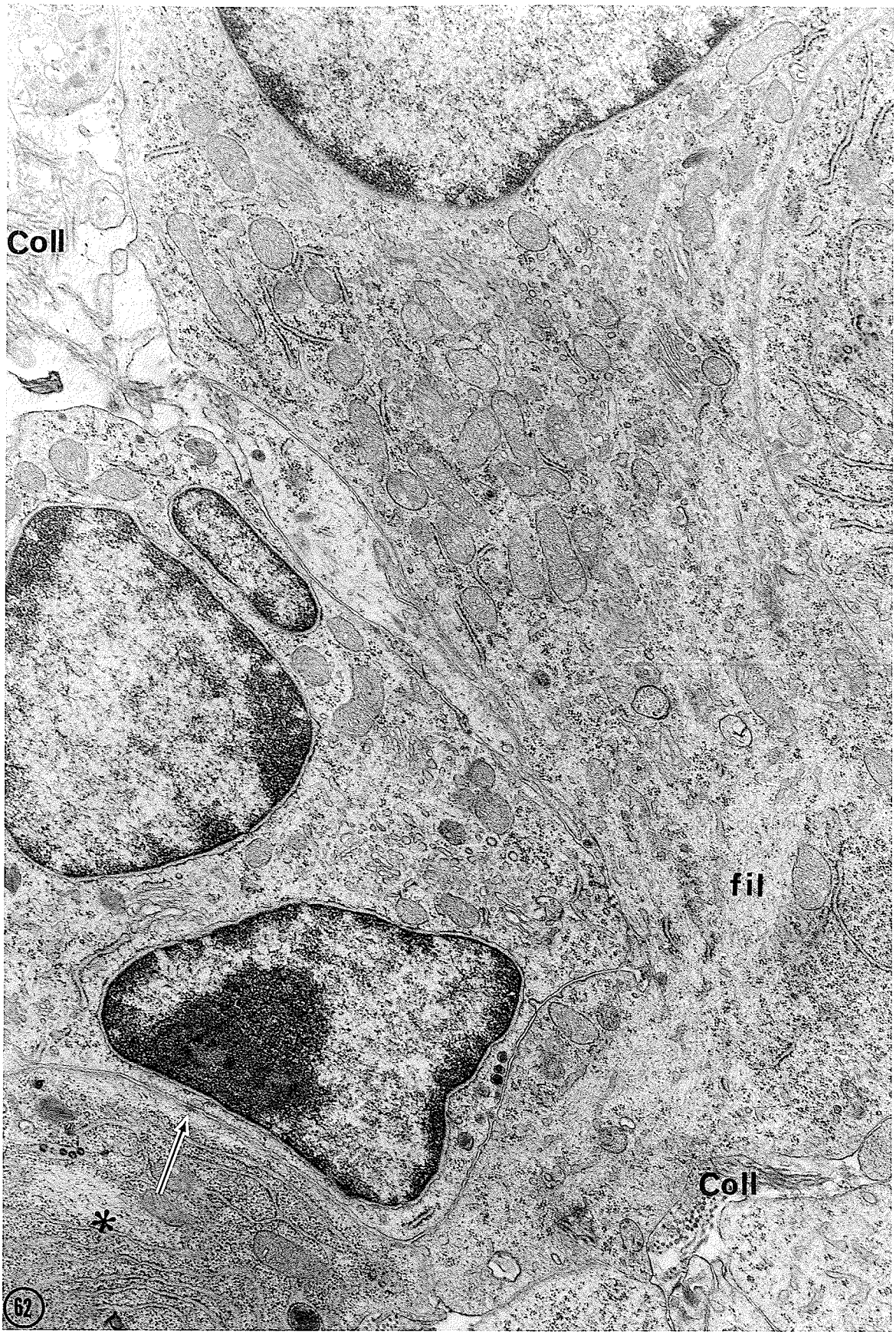
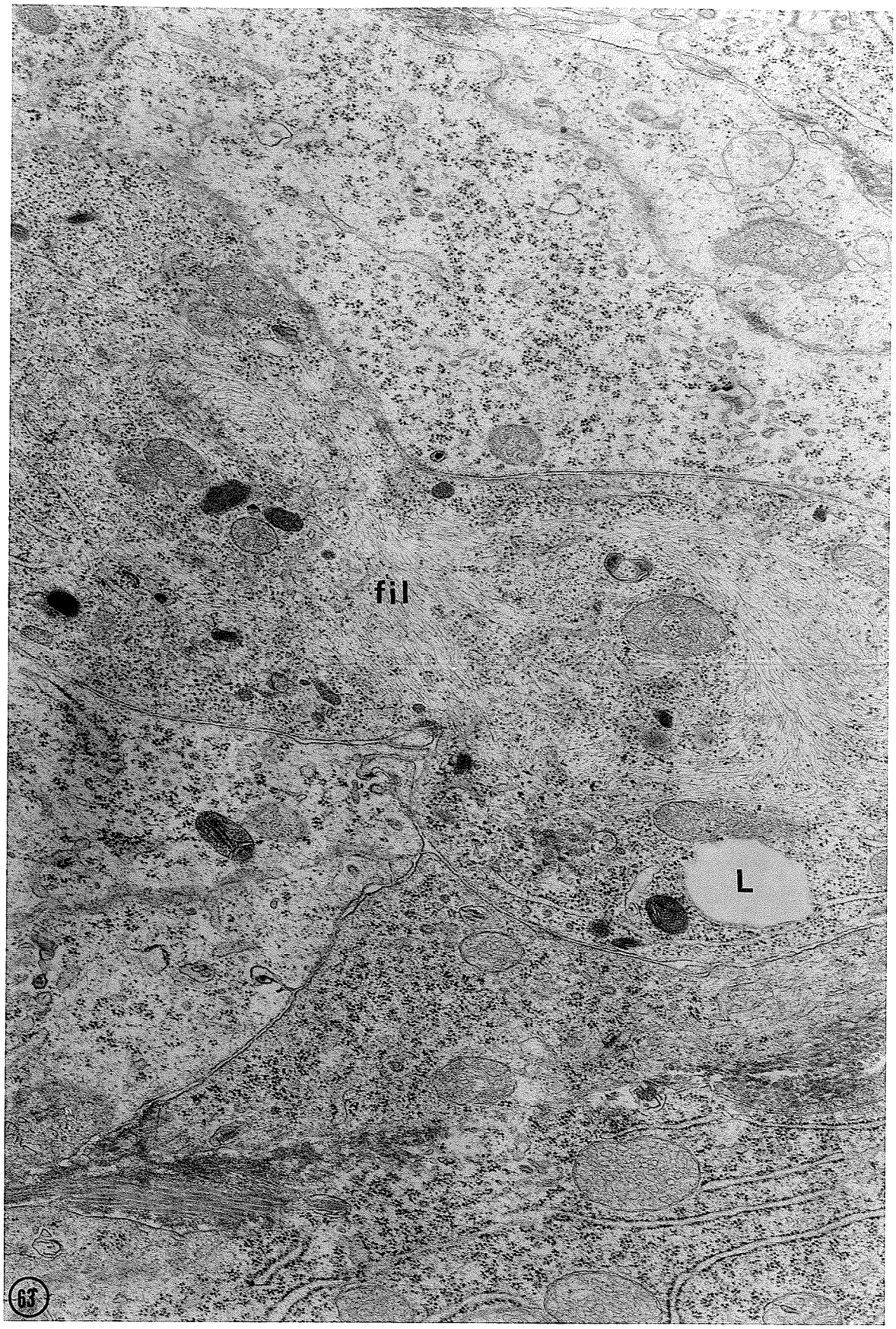

Coll
fil
Coll
62

Figure 63. EXPERIMENT B - MINIMUM DOSAGE - GENERATION V - 10 weeks after cessation of colchicine treatment

Shows a portion of a cell containing a large bundle of filaments (fil). As in previous generations, the filaments are 35-50A^{O} in diameter spatially close to ribosomes. A large vacuole presumably lipid (L) material is also seen in the figure.

X 24,624


fil
L
63

IV

OBSERVATIONS

ELECTRON MICROSCOPY

EXPERIMENT C - INTERMITTENT DOSAGE

EXPERIMENT C - INTERMITTENT CHEMOTHERAPY

1^{o} TREATED TUMOR

Cells treated with a smaller dose of colchicine in this phase of the experiment showed features which were identical to those seen in Treated tumor of Experiment B. This observation is accredited to the fact that animals in both experiments at this phase received the same dosage of the drug, colchicine. Filaments 35-50Å in diameter were most easily seen to be permeating the cytoplasm adjacent to the nucleus (Fig. 64). These filaments were easily distinguishable from microtubules which were also observed in the cytoplasm (Fig. 64). Mitochondria were tubulo-vesicular in nature and found in large numbers within the cytoplasm (Figs. 64, 65 & 66). Type A virus particle formation was observed in many cells (Fig. 66). The presence of melanin within the cells afforded a criterion for establishing these cells as melanocytes, with features such as melanosome complexes being present (Fig. 64 & 65). Blood vessels appeared with as much frequency as was observed in the other two experiments. The endothelial cell cytoplasm exhibited dilated cisternae (Fig. 65).

GENERATION I - TWO WEEKS AFTER FIRST COLCHICINE TREATMENT

The features of this generation, again, resembled those seen in Generation I of Experiment B with minimum dosage. Filaments in bundles and permeating the cytoplasm were seen in some cells (Figs. 67, 68 & 69). Tubulo-vesicular mitochondria, rough and smooth endoplasmic reticulum, free ribosomes, melanin granules, premelanosomes, and multiple well-developed Golgi complexes were found in tumor cells (Figs. 67, 68 & 69). The pronounced nuclear irregularity was present in most cells (Fig. 69). Formation of virus particles followed the usual

pattern (Fig. 69). Mitotic cells containing filaments were rarely seen in this generation.

2^{o} TREATED TUMOR

A striking change took place in the morphology of the Harding-Passey melanoma following the second administration of colchicine. Mitotic cells which in the previous generations were rarely seen to contain filaments were strikingly evident in this phase. 2^{o} Treated tumor tissue contained many colchicine induced mitotic cells containing bundles of filaments. Mitotic cells contained centrally located chromosomes surrounded by dilated cisternae of endoplasmic reticulum (Figs. 70 & 71) as was seen in treated tumor cells of Experiment A given maximum dosage of colchicine. The presence of numerous bundles of filaments of various dimensions was a consistent pattern in these mitotic cells. A significant observation at this stage is that filaments were seen more often in mitotic cells than in interphase cells.

Interphase cells showed the typical margination effect of melanin as was seen in Treated tumor of Experiment A (Fig. 72). In addition to the presence of filaments, interphase cells contained all the organelles as described in tumor cells of the previous generations (Figs. 73, 74 & 75).

GENERATION III - TWO WEEKS AFTER SECOND COLCHICINE TREATMENT

Cells in this generation closely resemble those observed in 2^{o} Treated tumor. There were numerous mitotic cells containing one or more bundles of filaments (Fig. 76). Very little cell searching was required in order to visualize the presence of filaments. In

contrast to the more common pattern of observing filaments in mitotic cells as seen in 2^{o} Treated cells, interphase cells seem to predominate in containing filaments (Figs. 77 & 78). The interphase cells contained filaments in the form of distinct bundles (Fig. 77), or as large amounts of filaments permeating the cytoplasm of the cell (Fig. 78). Presence of junctional complexes was observed between some tumor cells. Typical cell organelles and inclusions such as mitochondria, endoplasmic reticulum, melanin and the ever present cytoplasmic organelle, virus particles;were readily seen (Figs. 76, 77 & 78).

3^{o} TREATED TUMOR

After the third intermittent regime of colchicine, the cellular morphology tends to revert back to having the same characteristic features as in 2^{o} Treated tumor. Numerous mitotic figures are evident, but an even greater number of interphase cells now contain filaments than were seen in interphase cells of 2^{o} Treated tumor. Mitotic cells contained filaments in large amounts (Fig. 79) surrounded by dilated cisternae of endoplasmic reticulum. Portions of mitotic cells with chromosomes and spindle tubules could also be observed which served to morphologically distinguish the filaments from spindle fibers (Fig. 80). Interphase cells contained, in some cases, large bundle of filaments with relatively few ribosomes amongst them (Figs. 81 & 82). Smaller bundles of filaments were also observed in different portions of the cytoplasm (Figs. 82 & 83).

In Figure 83 is seen an unusual pattern of cytomembranes. This consisted of a group of four membranes, the outer two possessing ribosomes attached to them while the inner two were smooth and devoid of ribosomes. In addition to two inner smooth membranes were closely

approximated, the space between them being in the order of about 150Å, continuous with the cytoplasmic matrix. The cisternal space located between the granular and agranular membrane was of the dimension normally encountered in granular endoplasmic reticulum.

The cells of this tumor possessed the usual organelles such as tubulo-vesicular mitochondria, Golgi complexes, melanin granules and virus particles.

GENERATION V - TWO WEEKS AFTER THIRD COLCHICINE TREATMENT

Numerous cells in interphase could be observed to contain bundles of filaments. There were fewer mitotic cells which contained filaments, a characteristic feature in Generation III. Filaments could be identified as dispersed bundles within the cytoplasm (Figs. 84, 85, 86 & 87), or adjacent to the nucleus (Fig. 85).

In Figure 86 a melanocyte reveals a concentric configuration of granular endoplasmic reticulum with melanin granules located in the peripheral portion of the cell exemplifying marginal distribution of melanin. Lamellar rough endoplasmic reticulum, multivesiculated Golgi complexes, tubulo-vesicular mitochondria, virus particles and melanin could be identified in most cells.

ELECTRON MICROGRAPHS

EXPERIMENT C - INTERMITTENT DOSAGE

FIGURES 64-87

Figure 64. EXPERIMENT C - INTERMITTENT DOSAGE - 1^{O} TREATED TUMOR
This micrograph illustrates a highly irregular nucleus with a distinct perinuclear space. Also shown are fine filaments (fil) 35-50A^{O} in diameter permeating the cytoplasm which are easily distinguishable from microtubules (MT) seen adjacent to the nucleus. Type A virus particles (V) are seen in relation to granular endoplasmic reticulum.

X 35,112

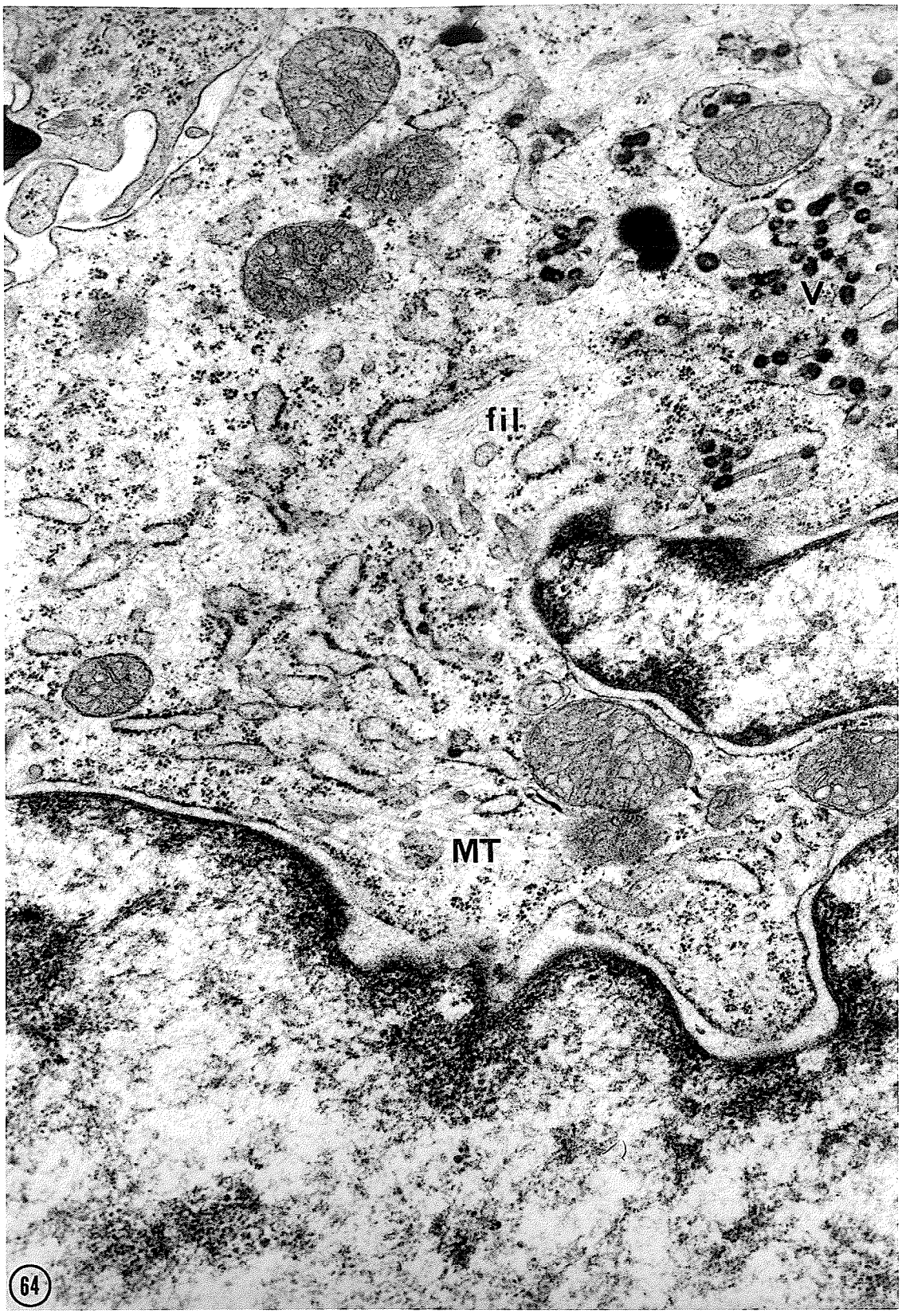

V
fil
MT
64

Figure 65. EXPERIMENT C - INTERMITTENT DOSAGE - 1^{o} TREATED TUMOR
This electron micrograph illustrates a portion of a small blood vessel. The nucleus of the endothelial cell may be seen in the top of the figure. Adjacent to the blood vessel is a portion of a cell containing filaments (fil). In the top, right hand portion of the figure is a cell showing numerous virus particles and marginated melanin granules.

X 10,032

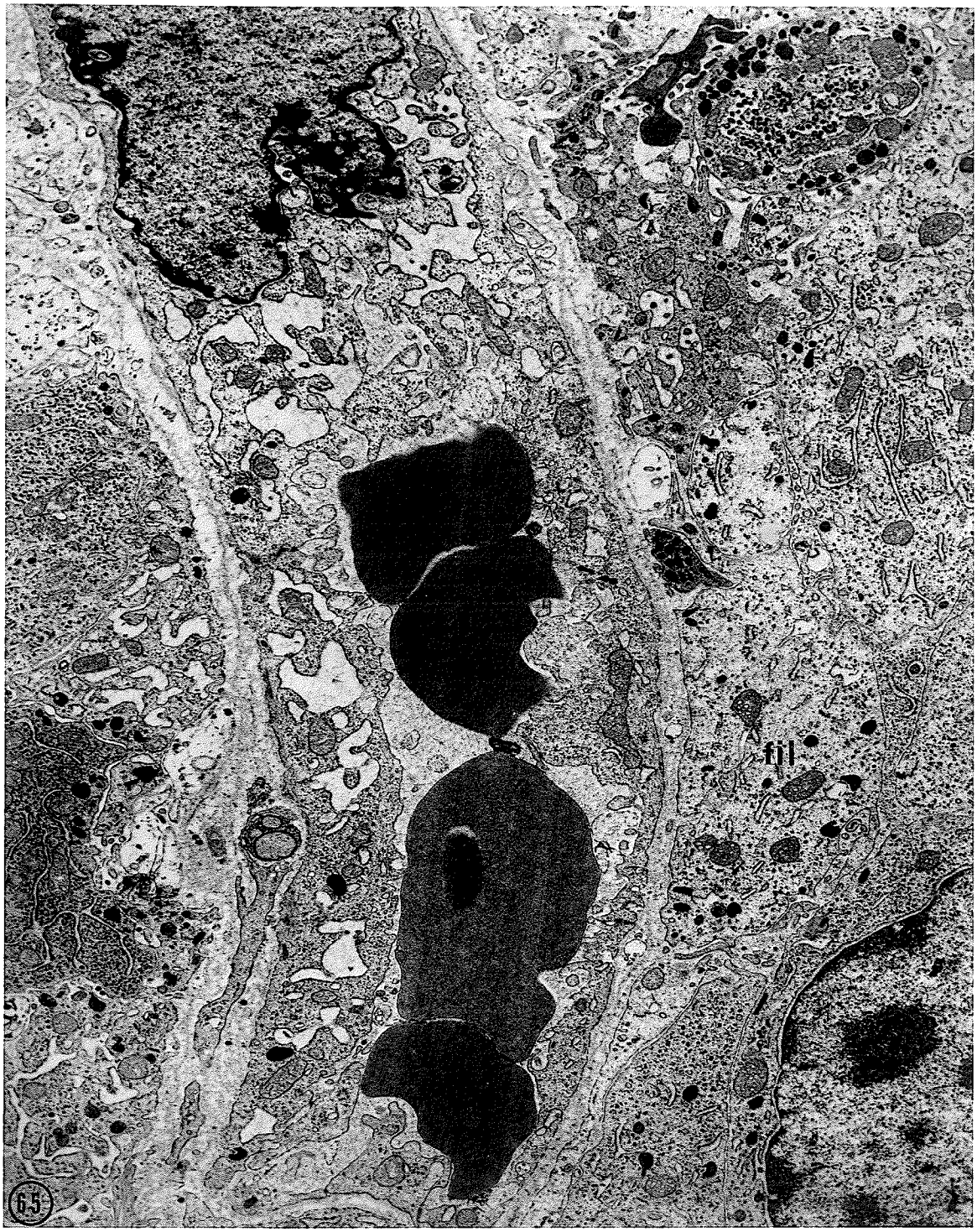

fil
6.5

Figure 66. EXPERIMENT C - INTERMITTENT DOSAGE - 1^{o} TREATED TUMOR
This figure demonstrates a number of virus particles located in relation to the cisternae of granular endoplasmic reticulum. In this micrograph the various stages in the evolution of the virus particles, from its beginning as a mere thickening of the membrane of the granular endoplasmic reticulum, to fully formed doughnut shaped virus particles lying within the lumen of the granular endoplasmic reticulum, are visualized. The arrows point to virus particles which are nearly fully formed but still demonstrate an attachment to the endoplasmic reticulum by means of a stalk. Such particles are presumed to be in the process of budding.

X 45,144

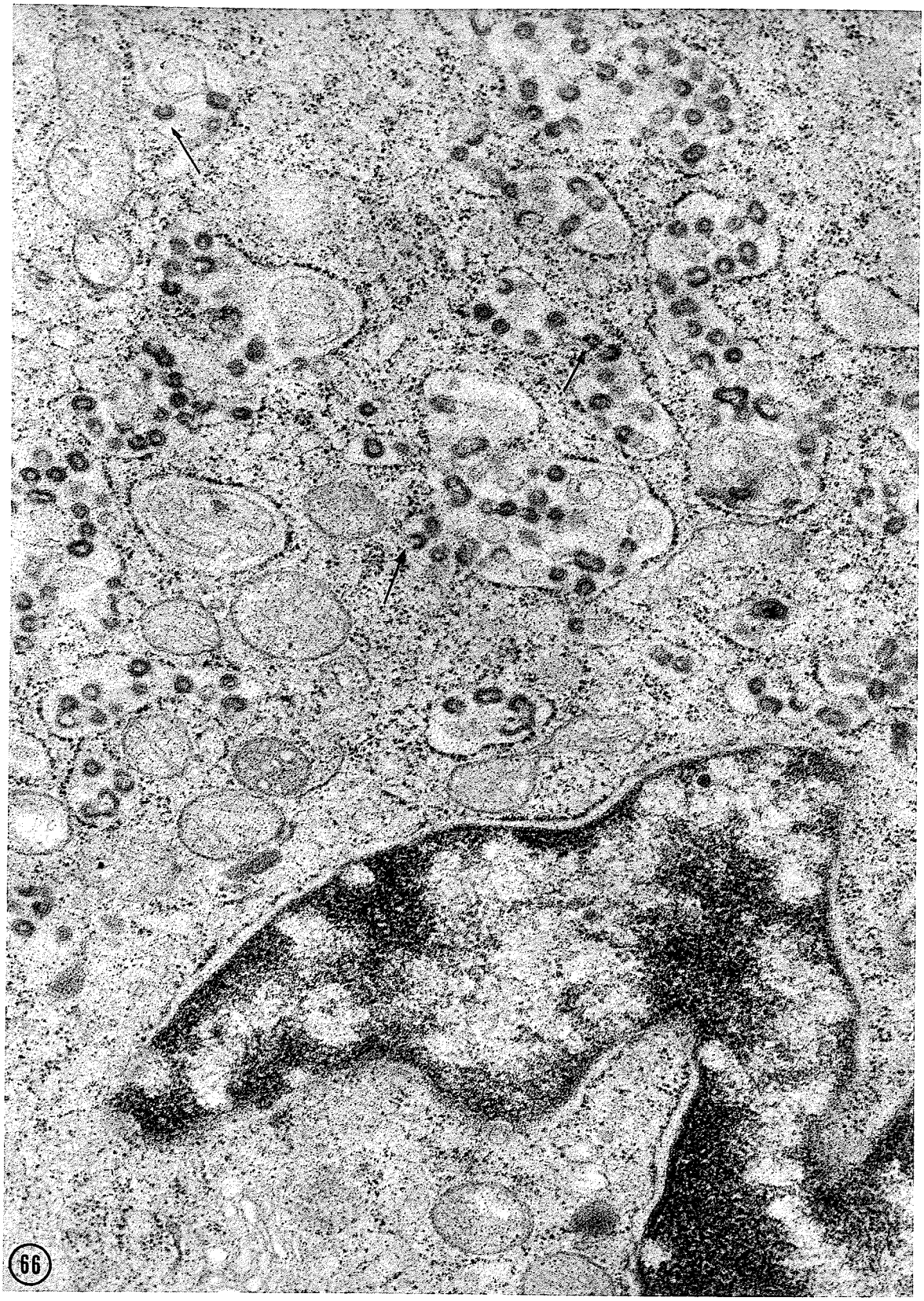

66

Figure 67. EXPERIMENT C - INTERMITTENT DOSAGE - GENERATION I - 2 weeks after first colchicine treatment

This micrograph demonstrates a long bundle of filaments (fil) adjacent to the cytoplasmic membrane of a cell. Centrioles, tubulo-vesicular mitochondria and melanin among other cytoplasmic organelles are also evident in this figure.

X 15,732

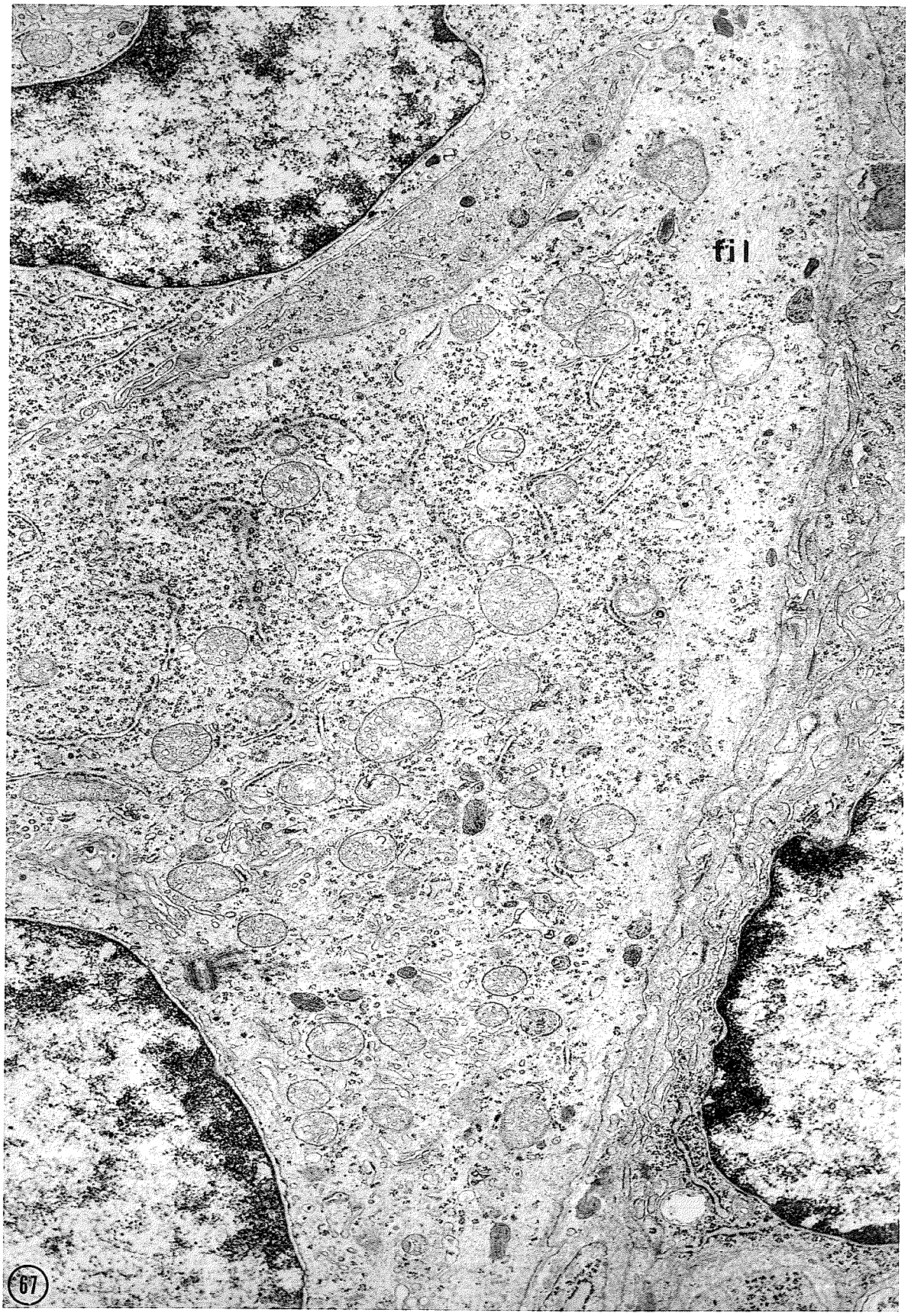
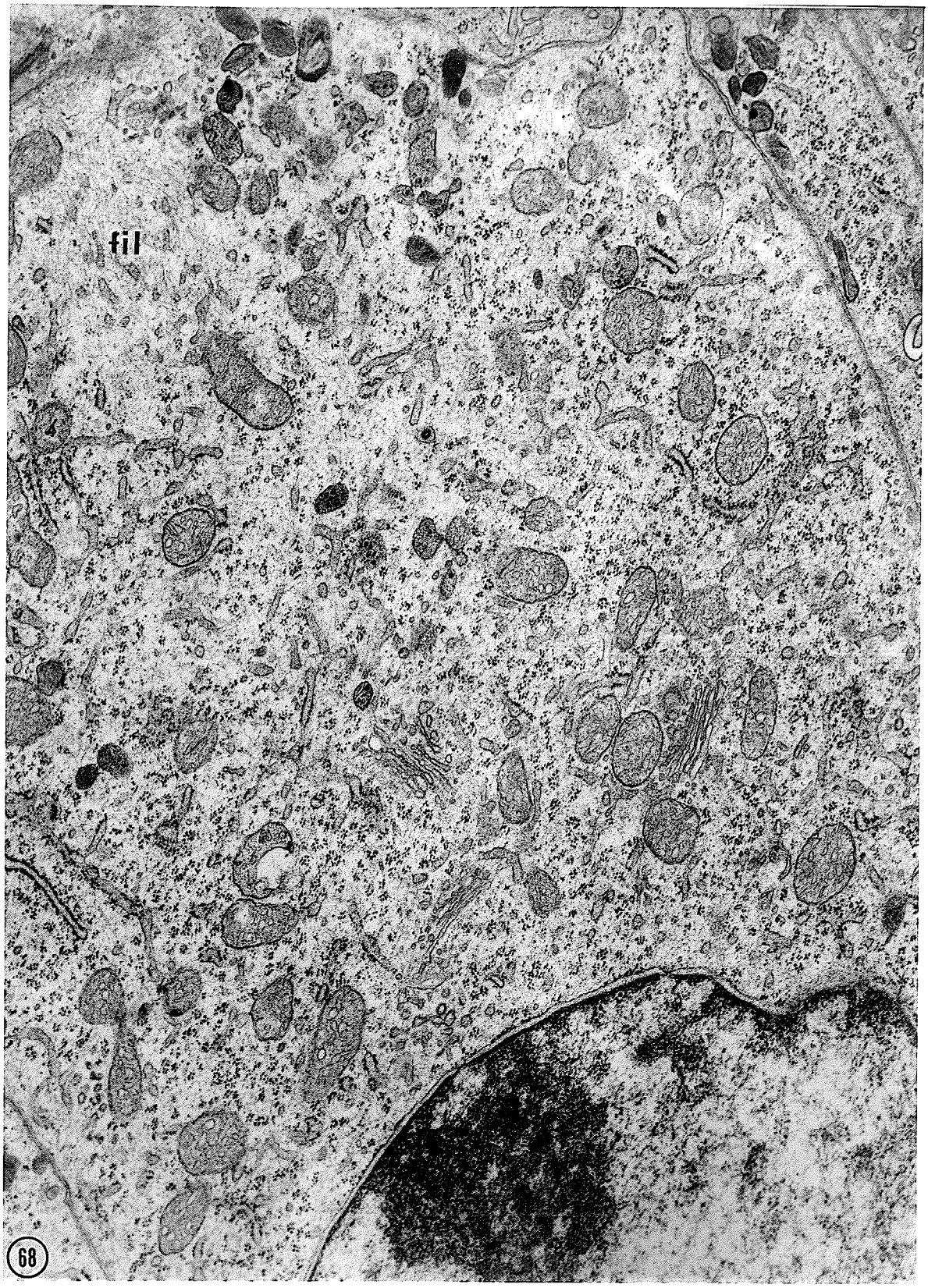

fil
67

Figure 68. EXPERIMENT C - INTERMITTENT DOSAGE - GENERATION I - 2 weeks after first colchicine treatment

Filaments (fil) are seen permeating the cytoplasm following the low dosage of colchicine. The general pattern is one of dispersed filaments rather than discrete bundles. All previously described cytoplasmic organelles are still seen, as well as melanin inclusions.

X 23,598



68

Figure 69. EXPERIMENT C - INTERMITTENT DOSAGE - GENERATION I - 2 weeks after first colchicine treatment

Cytoplasmic filaments (fil) are seen in this figure. Numerous well-developed Golgi complex (G) are also seen, as are developing virus (V) particles associated with the endoplasmic reticulum. Numerous melanosomes (MS) are also evident in the figure.

X 26,842

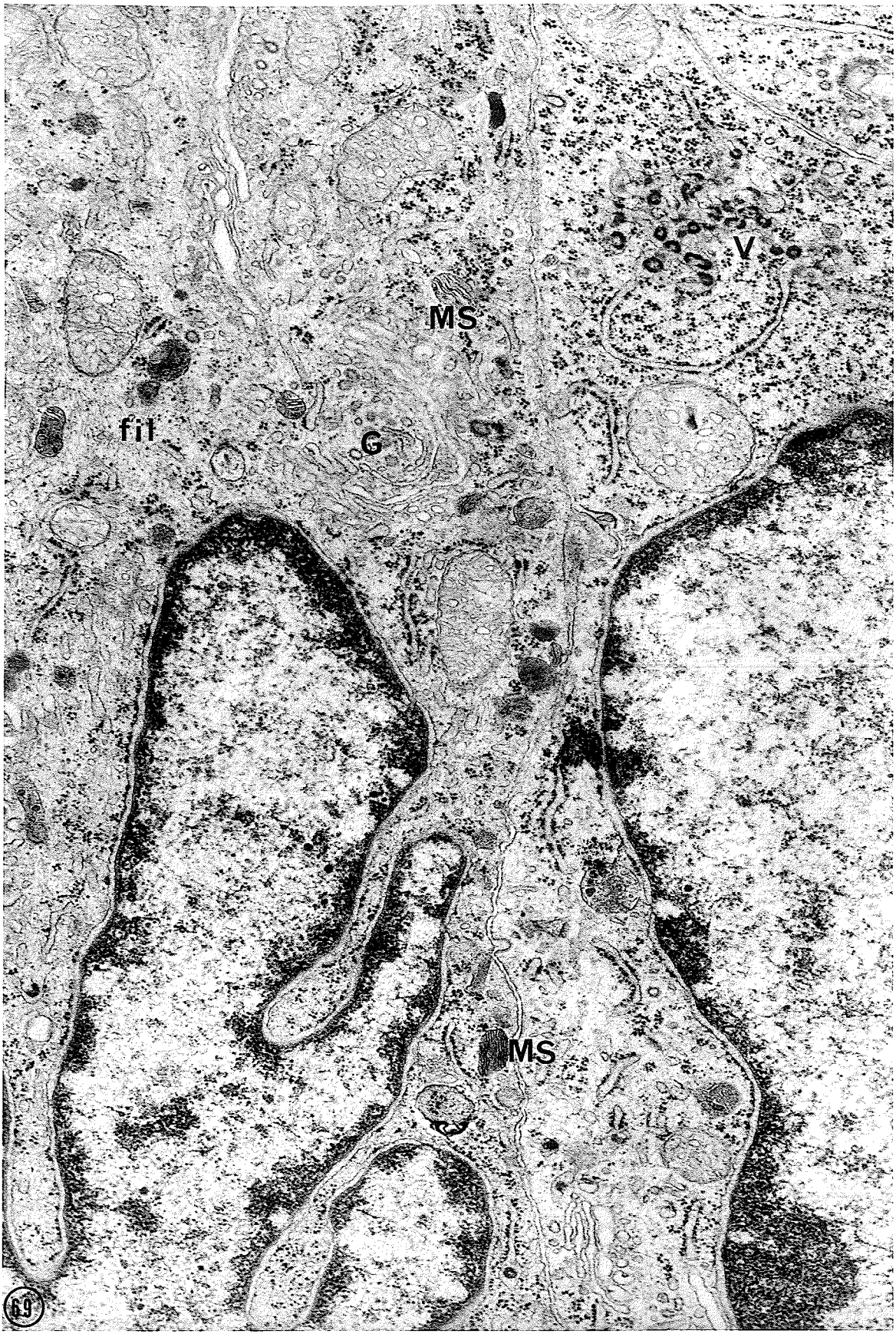
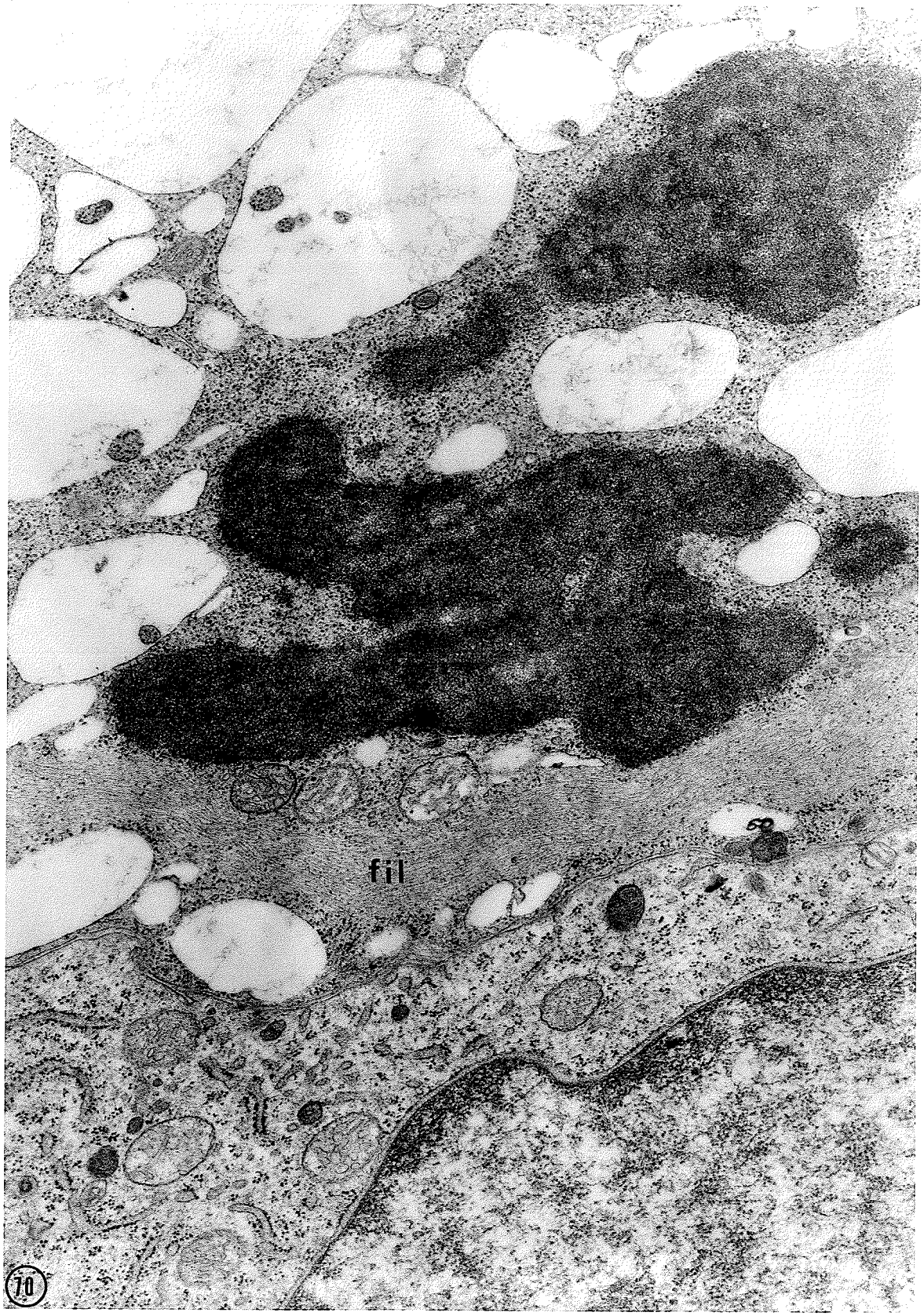

V
MS
fil
G
MS
69

Figure 70. EXPERIMENT C - INTERMITTENT DOSAGE - 2^{o} TREATED TUMOR
This electron micrograph demonstrates a mitotic cell with a dense matrix containing chromosomes. A large bundle of filaments (fil) are seen in the periphery of the mitotic cell adjacent to the chromosomes. Few ribosomes are found among the filaments. Note the presence also of the dilated cisternae of endoplasmic reticulum, a discernable feature always present in the mitotic cell.

X 27,588



70

Figure 71. EXPERIMENT C - INTERMITTENT DOSAGE - 2^{o} TREATED TUMOR

An electron micrograph showing two mitotic cells. One of the mitotic cells has several bundles of filaments (fil) within the cytoplasm with ribosomes adjacent to them. Dilated cisternae of endoplasmic reticulum are also seen, as are tubulo-vesicular mitochondria and melanin.

X 15,732

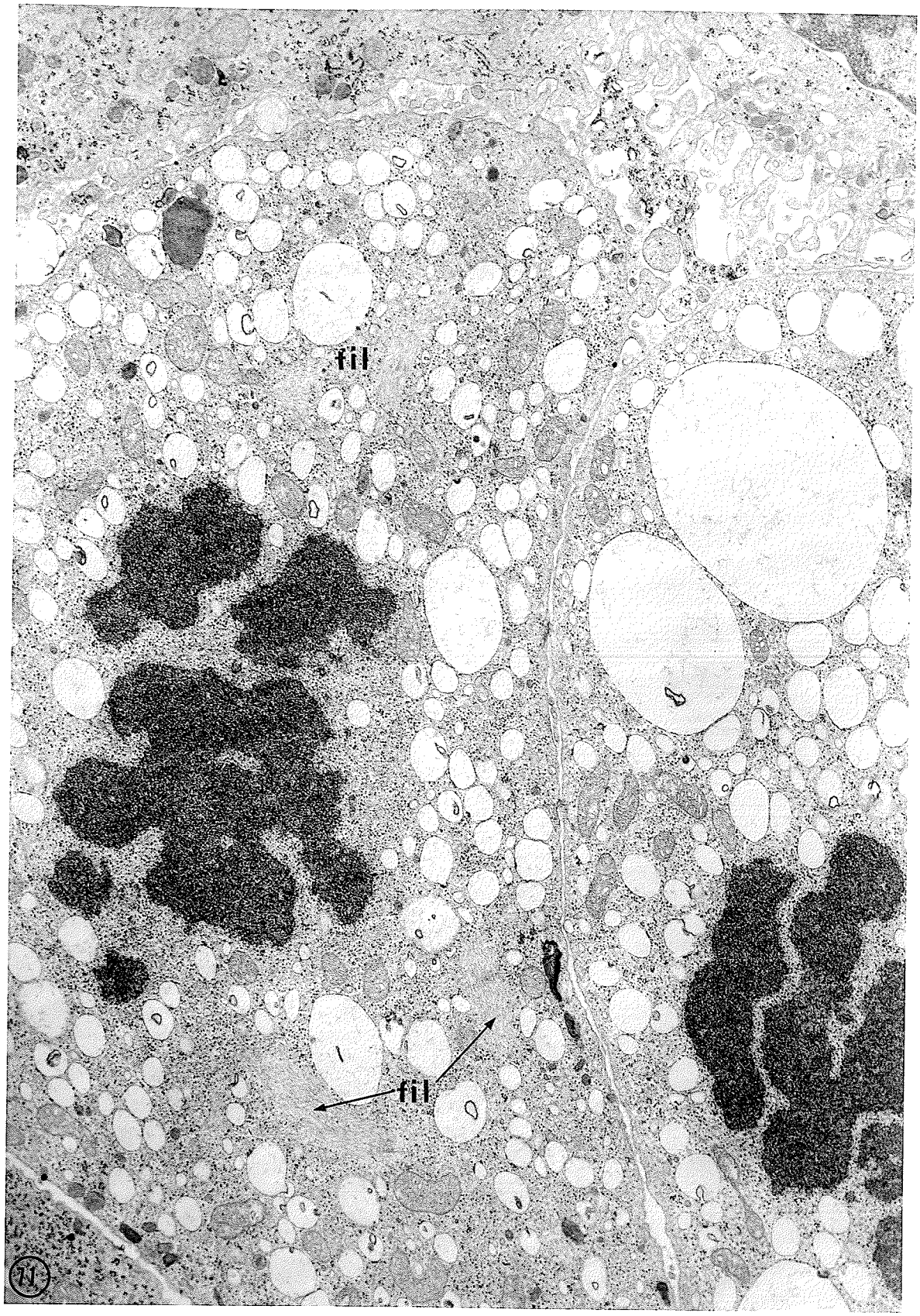

fil
fil

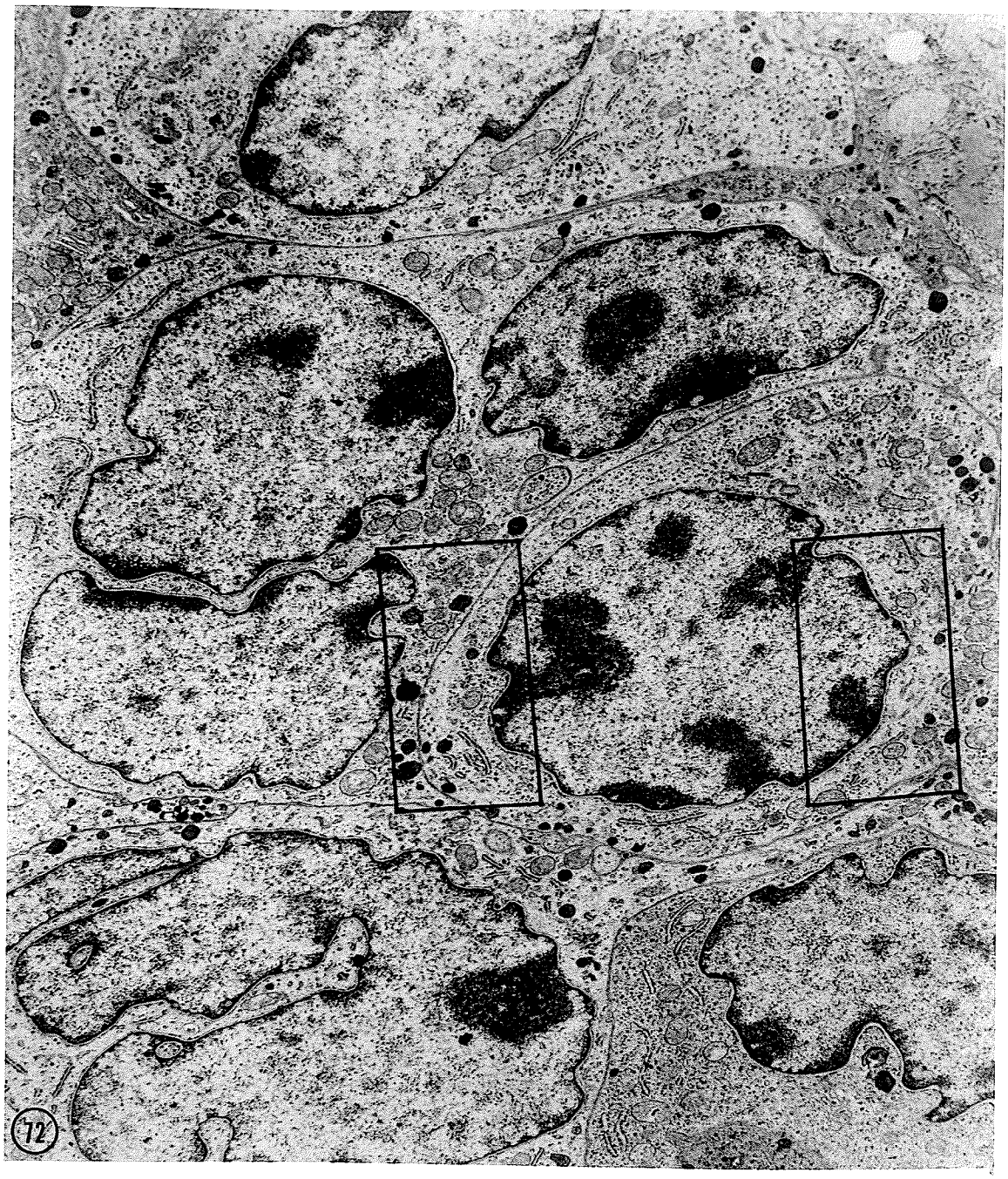
Figure 72, 73 & 74. EXPERIMENT C - INTERMITTENT DOSAGE - 2^{o} TREATED TUMOR

This micrograph shows a number of interphase cells illustrating the irregularity of the nuclei. The insets show the presence of perinuclear bundles of filaments (fil) in one of the interphase cells. Numerous previously described cell organelles and inclusions are also evident in many of the cells depicted in this figure.

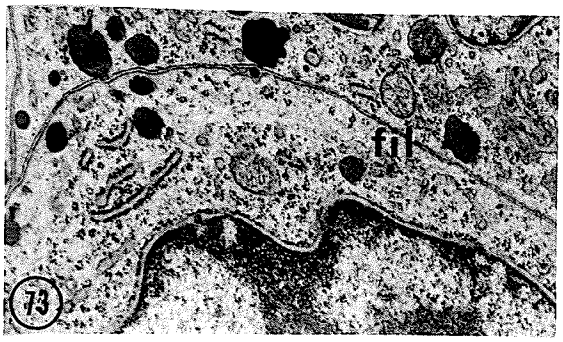
Fig. 72. X 9,120

Fig. 73. X 17,328

Fig. 74. X 17,328



72



73

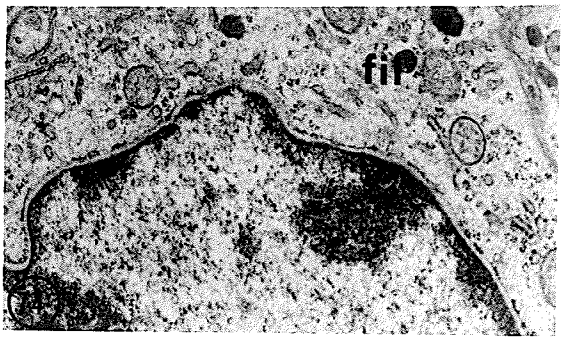
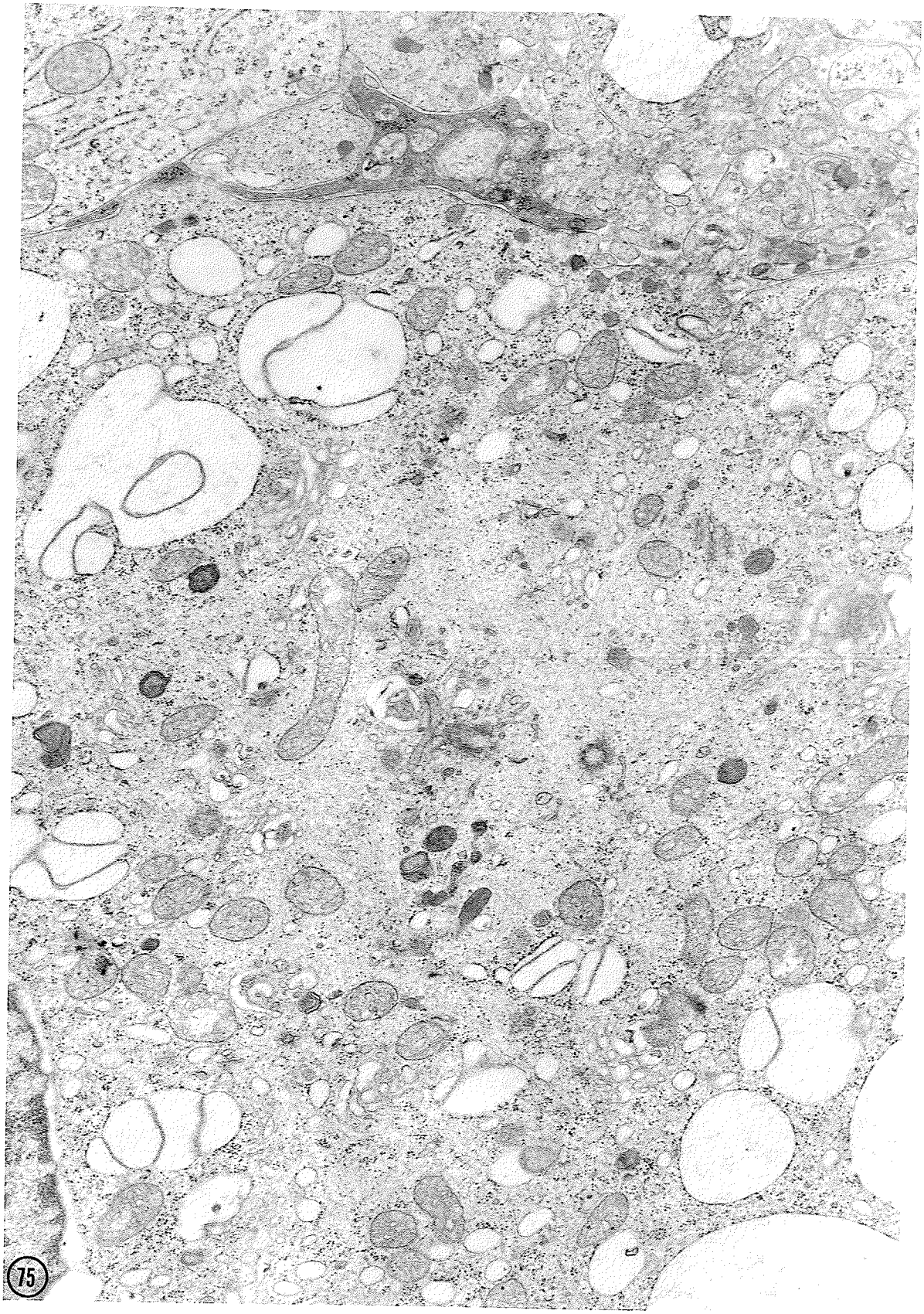


Figure 75. EXPERIMENT C - INTERMITTENT DOSAGE - 2^{o} TREATED TUMOR
Note a large amount of filaments within the cytoplasm of the cell. Present also in the cytoplasm are centrioles, mitochondria, Golgi complexes and dilated endoplasmic reticulum as well as melanin is various stages of melinization.

X 22,663

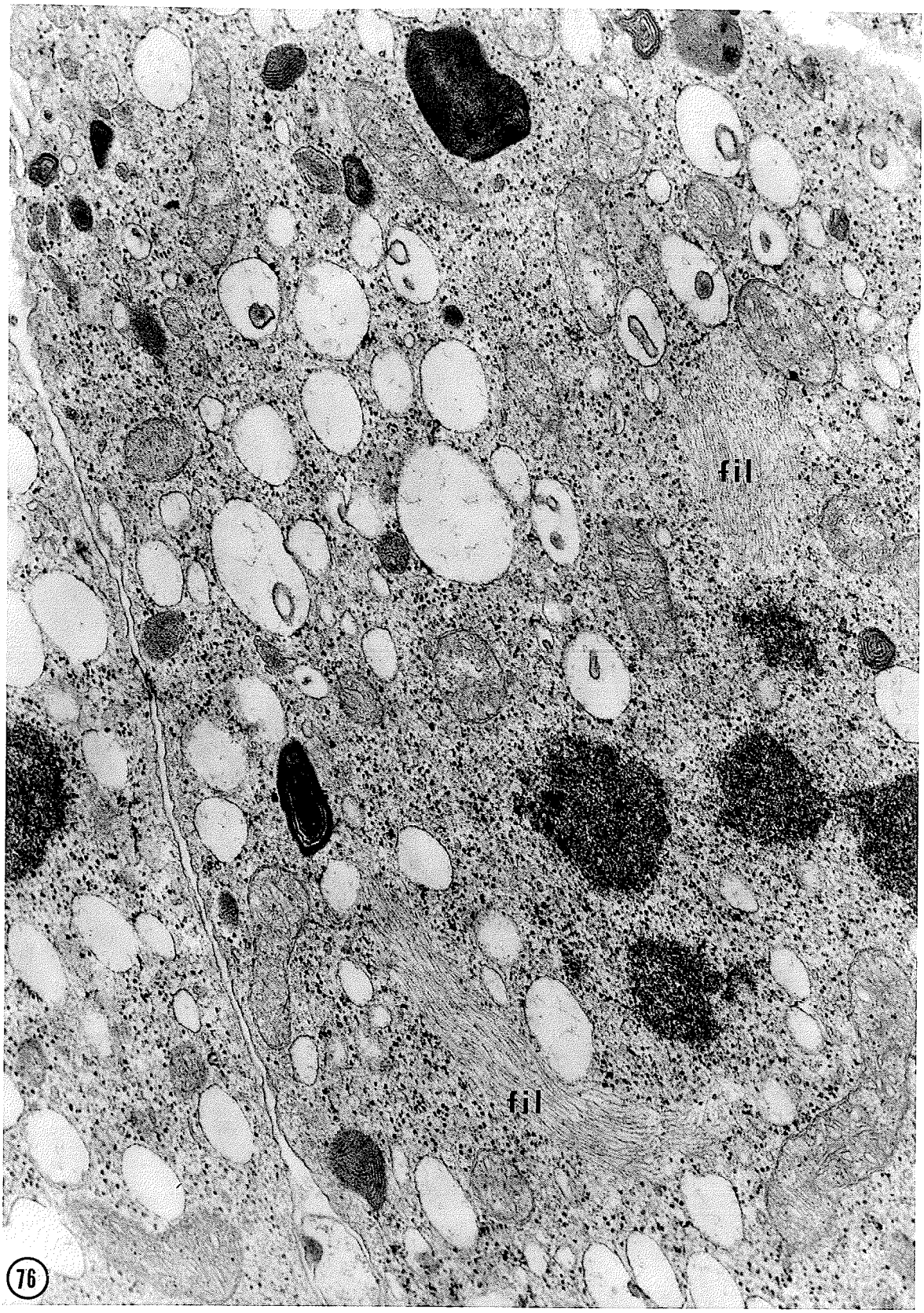


75

Figure 76. EXPERIMENT C - INTERMITTENT DOSAGE - GENERATION III - 2 weeks after second colchicine treatment

This micrograph shows portions of two mitotic cells containing dilated cisternae of endoplasmic reticulum. Within one cell are visable two large bundles of filaments (fil). Numerous premelanosomes, melanosomes and melanin granules are also evident, the major amount of which are located in the periphery of the cell.

X 36,708

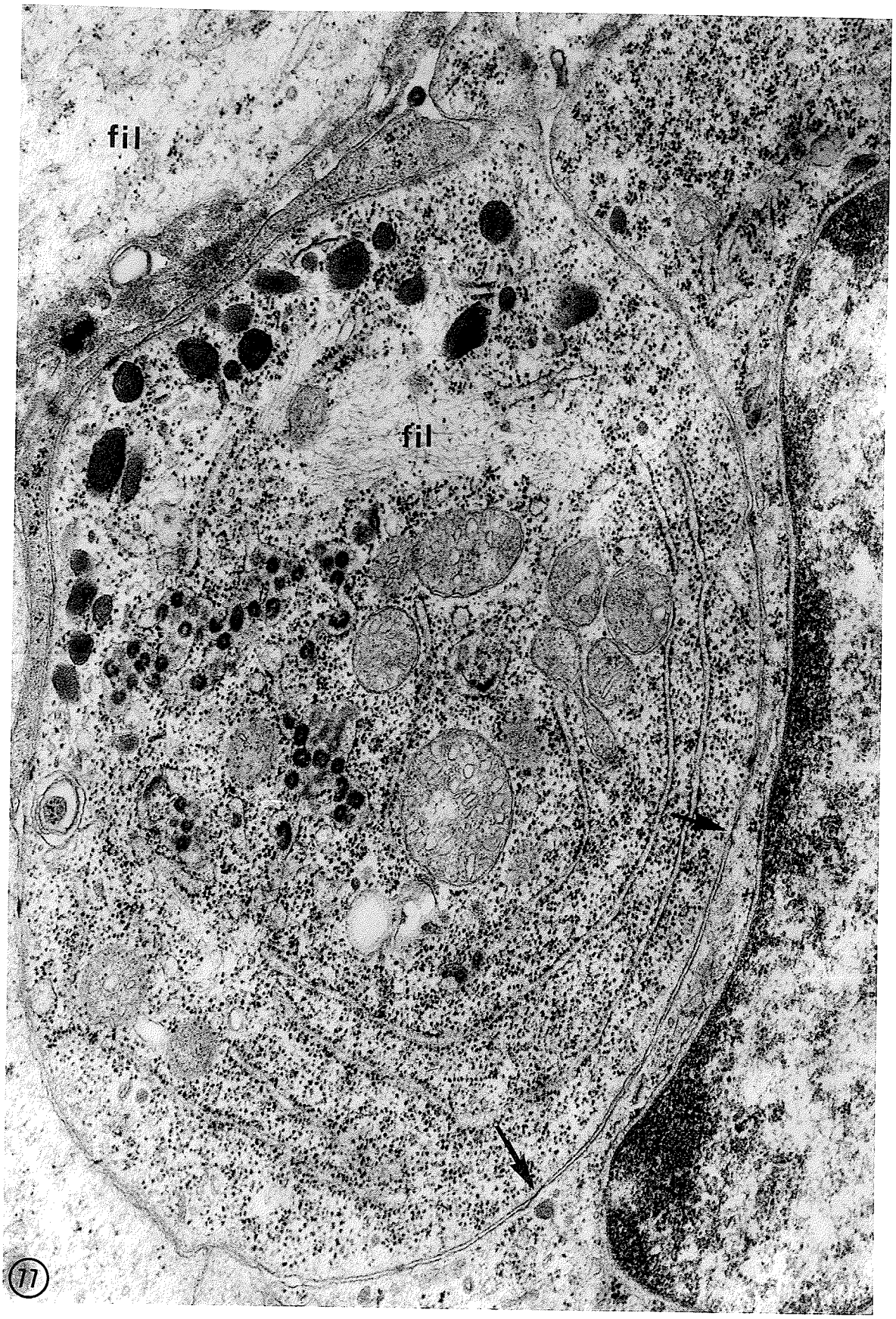


76

Figure 77. EXPERIMENT C - INTERMITTENT DOSAGE - GENERATION III - 2 weeks after second colchicine treatment

Present in this figure are portions of interphase cells, one containing filaments (fil) in bundle form, and the other with filaments (fil) permeating the cytoplasm. In addition, virus particles, tubulo-vesicular mitochondria, melanin and granular endoplasmic reticulum are present. Note the presence of junctional complexes (arrow) between the plasma membranes of the two cells.

X 35,112



11

Figure 78. EXPERIMENT C - INTERMITTENT DOSAGE - GENERATION III - 2 weeks after second colchicine treatment

In this figure are seen large amounts of filaments (fil) within the cytoplasm of this cell, the filaments being cut in both longitudinal and cross-section. Virus particles (arrows) can be seen to originate from the dilated cisternae of the endoplasmic reticulum.

X 28,842

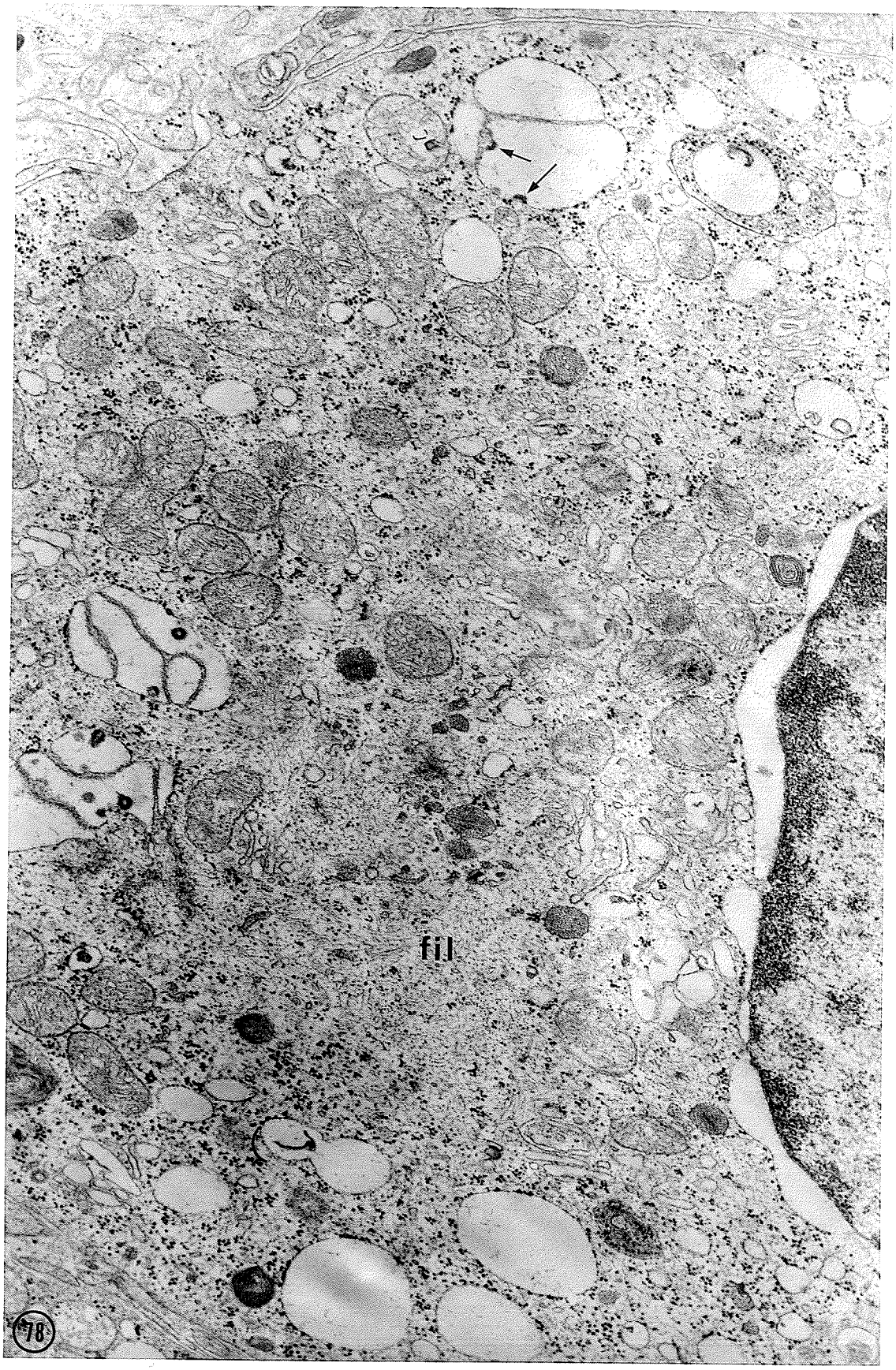

fil
78

Figure 79. EXPERIMENT C - INTERMITTENT DOSAGE - 3^{O} TREATED TUMOR
This micrograph shows a cell containing a large bundle of filaments, 35-50A^{O} in diameter. Numerous dilated cisternae of endoplasmic reticulum are seen, as are tubulo-vesicular mitochondria and melanin granules. The cytoplasm of an adjacent cell contains numerous filaments (fil) permeating the cytoplasm.

X 18,616

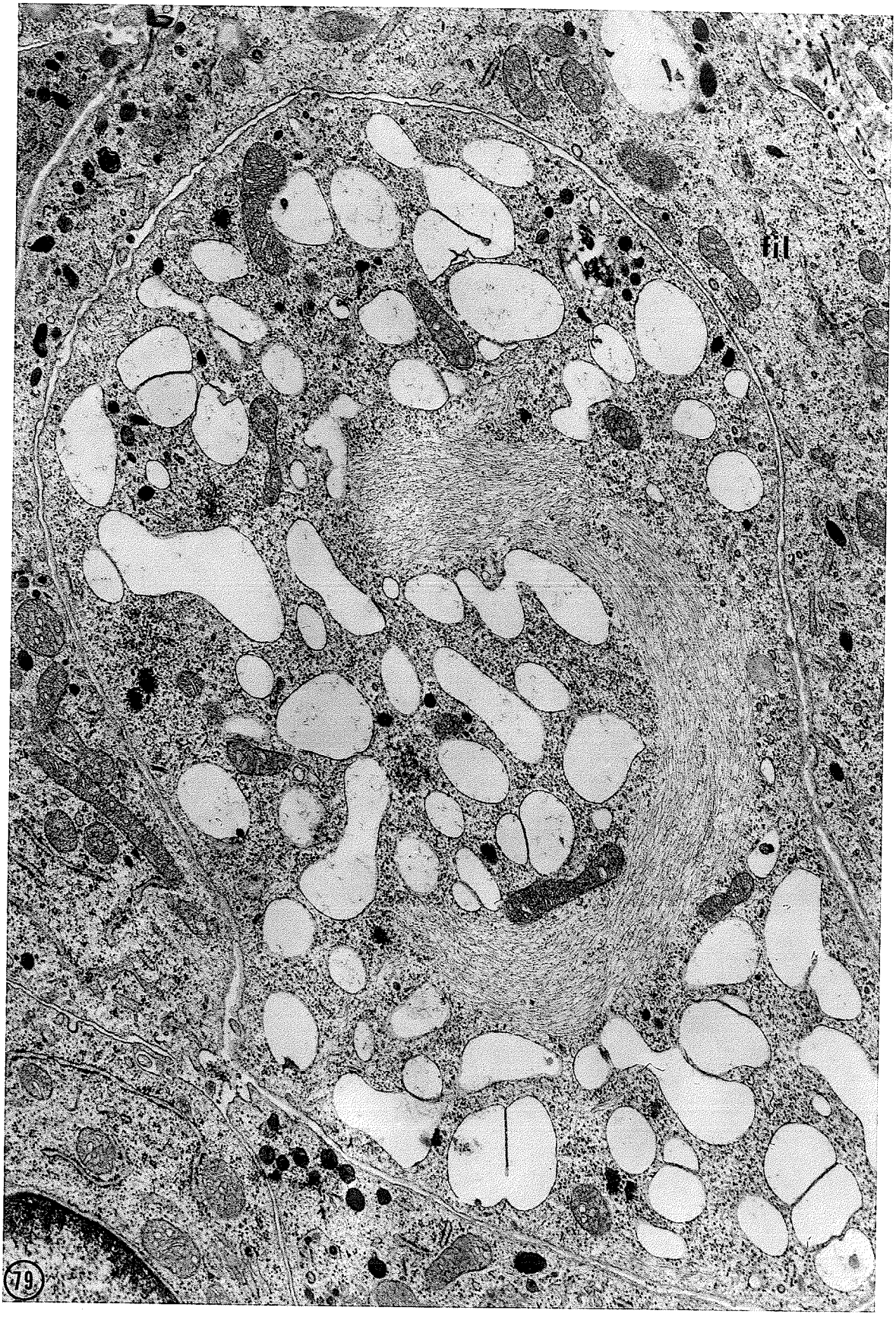
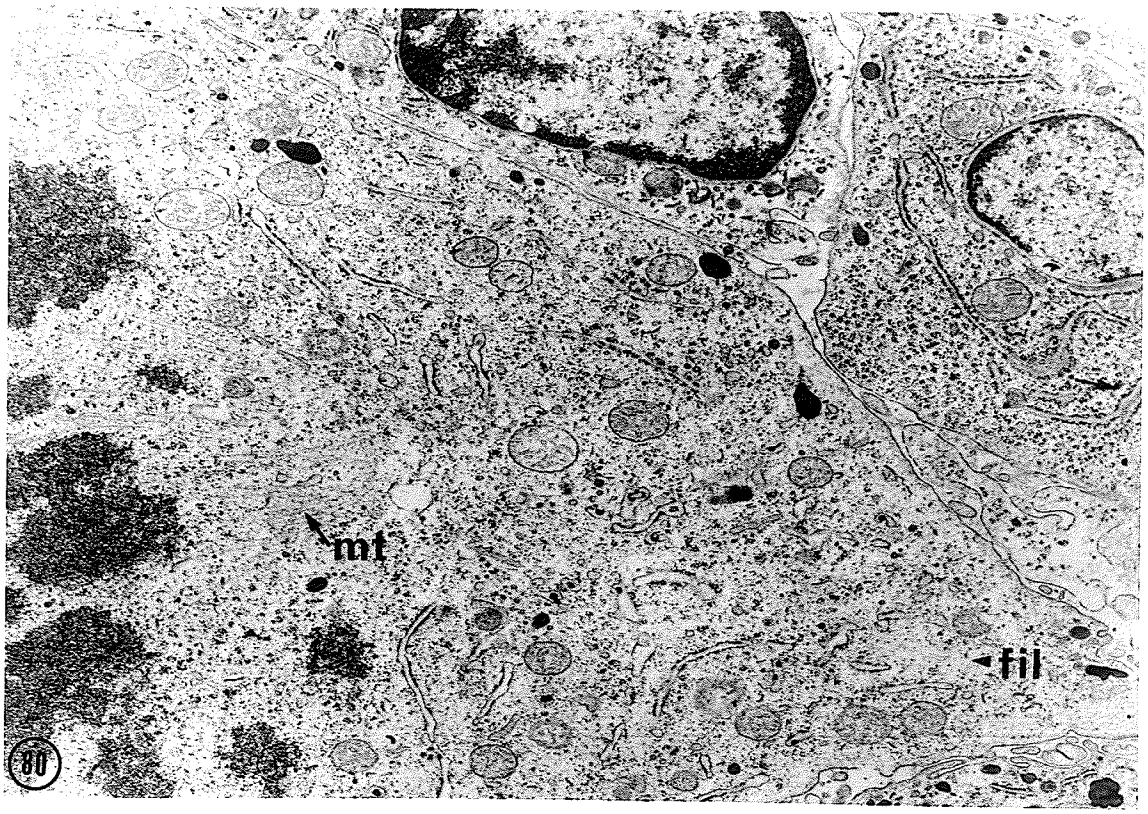

fil
79

Figure 80. EXPERIMENT C - INTERMITTENT DOSAGE - 3^{O} TREATED TUMOR
This low power micrograph shows a mitotic cell containing both microtubules (MT) and filaments (fil) serving to distinguish them in size and physical characteristics. Mitochondria and melanin granules are also seen within the cytoplasm of the mitotic cell.

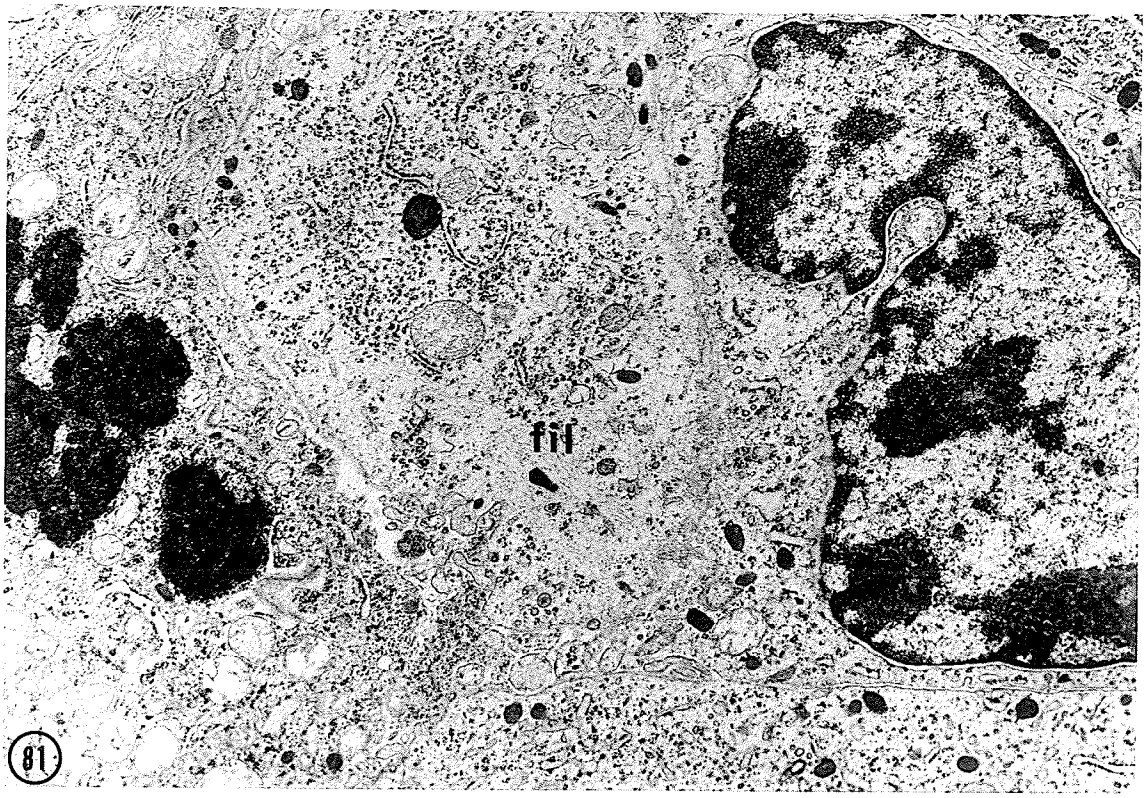
X 13,896

Figure 81. EXPERIMENT C - INTERMITTENT DOSAGE - 3^{O} TREATED TUMOR
This figure shows portions of a number of cells, one of which contains filaments (fil) permeating the cytoplasm. An interphase cell and a mitotic cell containing usual cytoplasmic organelles are also present.

X 12,848



80



81

Figure 82. EXPERIMENT C - INTERMITTENT DOSAGE - 3^{O} TREATED TUMOR
This high power micrograph demonstrates a large bundle of filaments within a cell. The filaments are sparsely interspersed with ribosomes. Melanin granules and mitochondria are generally excluded from the main body of filaments. The adjoining cells shows few filaments (arrows) permeating the cytoplasm.

X 38,304

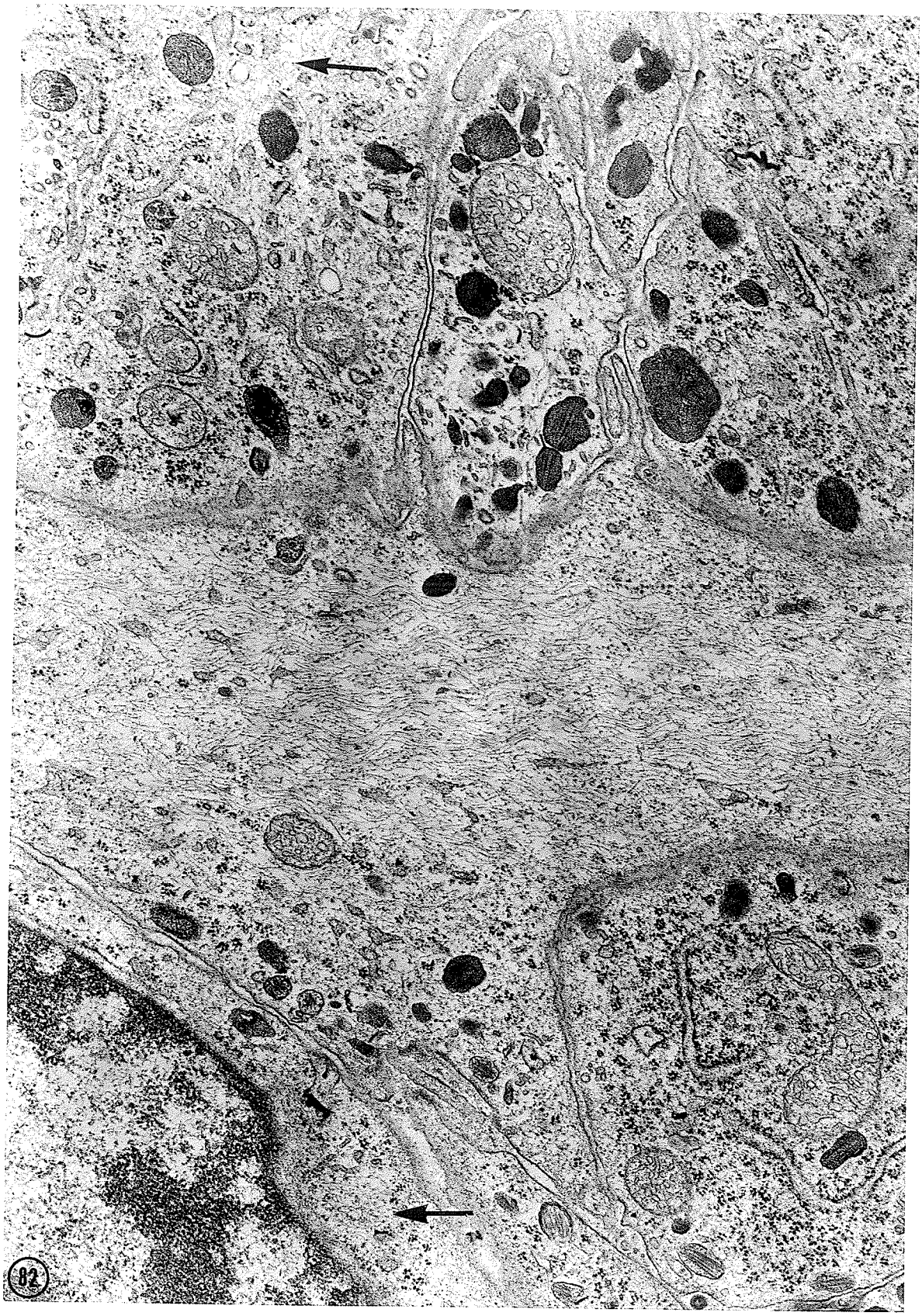
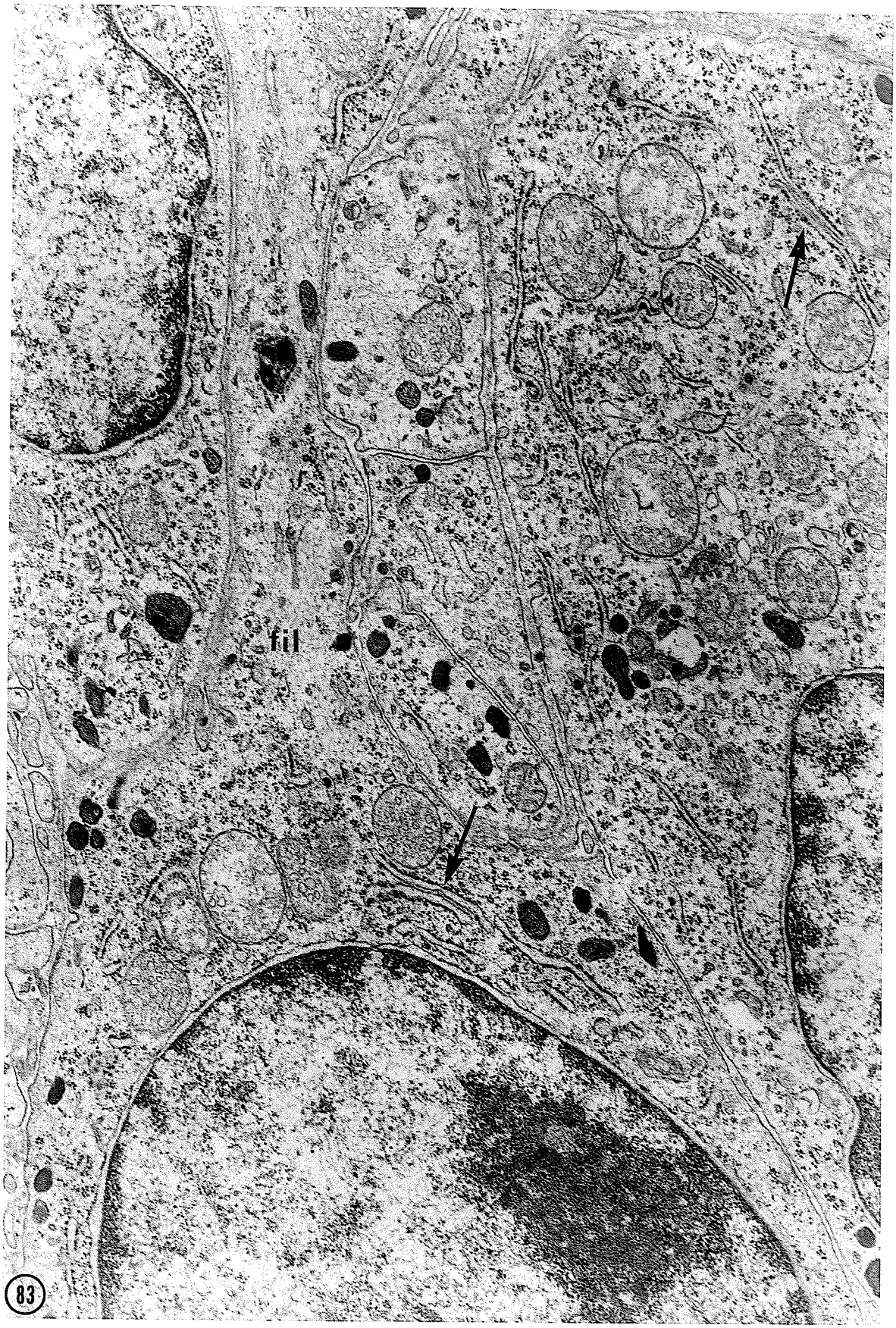

82

Figure 83. EXPERIMENT C - INTERMITTENT DOSAGE - 3^{O} TREATED TUMOR

An electron micrograph showing portions of several interphase cells, some of which contain elongated bundles of filaments (fil) within the cytoplasm. Note (arrows) the unusual configuration of rough encoplasmic reticulum. Similar patterns of endoplasmic reticulum were also seen in the other two experiments as well.

X 23,598

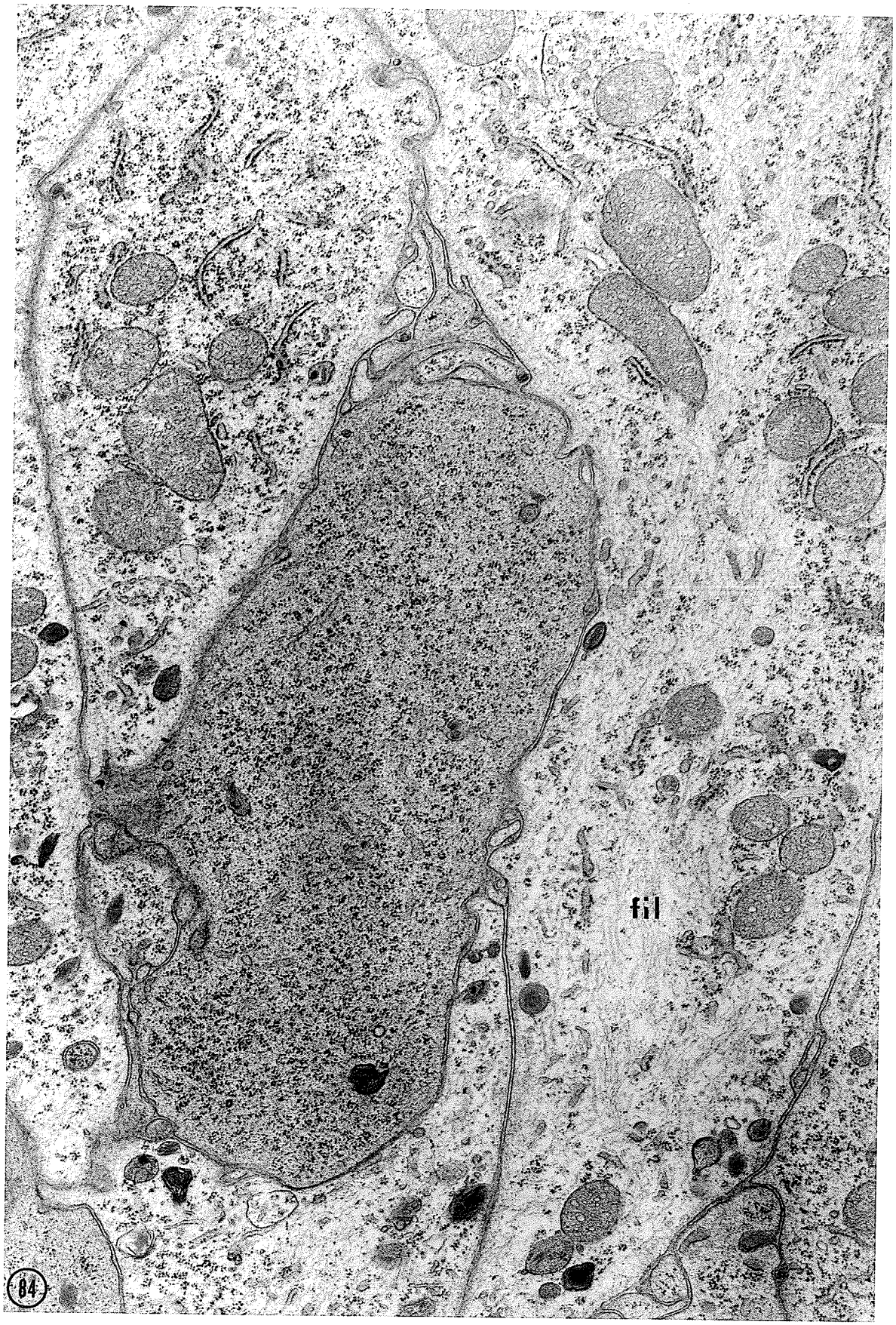


83

Figure 84. EXPERIMENT C - INTERMITTENT DOSAGE - GENERATION V - 2 weeks after third colchicine treatment

This electron micrograph shows a large bundle of filaments (fil), 35-50A^{o} in diameter, within the cytoplasm of a cell. Numerous mitochondria and melanin granules are also seen within the cell.

X 23,598



84

Figure 85. EXPERIMENT C - INTERMITTENT DOSAGE - GENERATION V - 2 weeks after third colchicine treatment

This figure shows portions of two cells which contain filaments (fil) within the cytoplasm. Tubulo-vesicular mitochondria are numerous, and the Golgi complexes are well-developed. Melanin in various stages of formation are evident throughout both the cells.

X 28,842

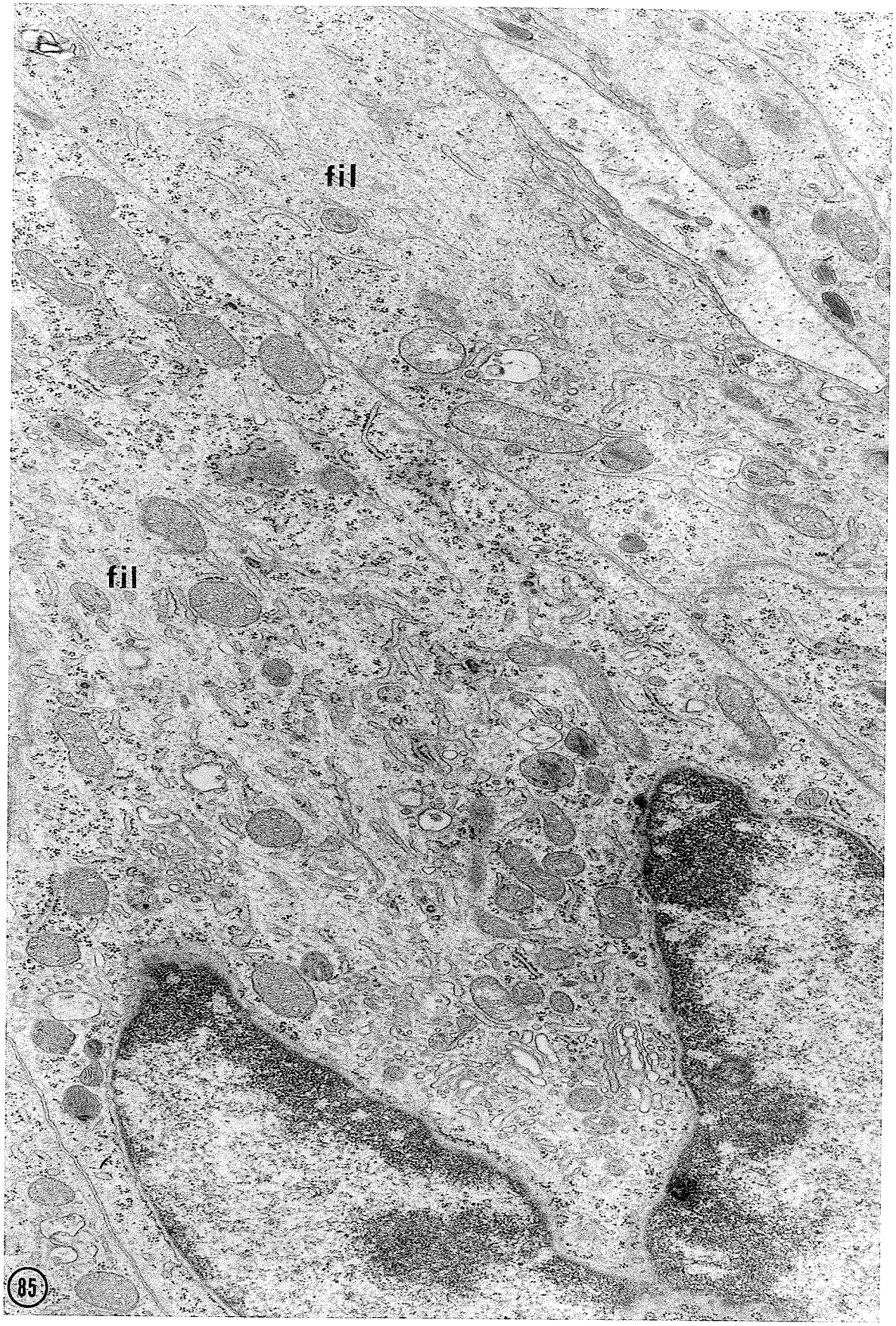

fil
fil
85

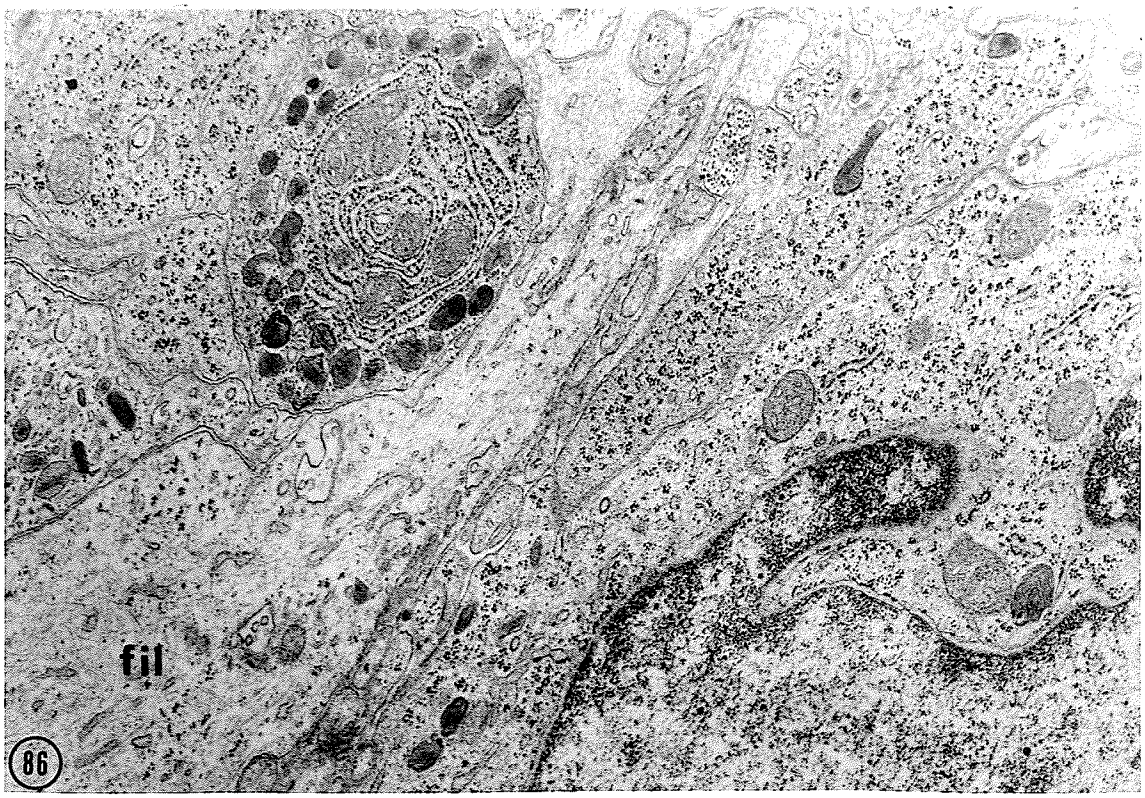
Figure 86. EXPERIMENT C - INTERMITTENT DOSAGE - GENERATION V - 2 weeks after third colchicine treatment

This low power micrograph shows a cell containing a large bundle of filaments (fil). A portion of a cell with a very well-developed endoplasmic reticulum and peripheral accumulation of melanin is seen in the upper left aspect of the figure.

X 18,616

Figure 87. EXPERIMENT C - INTERMITTENT DOSAGE - GENERATION V - 2 weeks after third colchicine treatment

In the lower part of the figure are seen two cells containing distinct bundles of filaments (fil). The cell located in the upper half of the figure, shows filaments permeating the cytoplasm. Also seen in this figure is a well-developed granular endoplasmic reticulum in one of the cells. Note that the melanin granules generally show 'margination'.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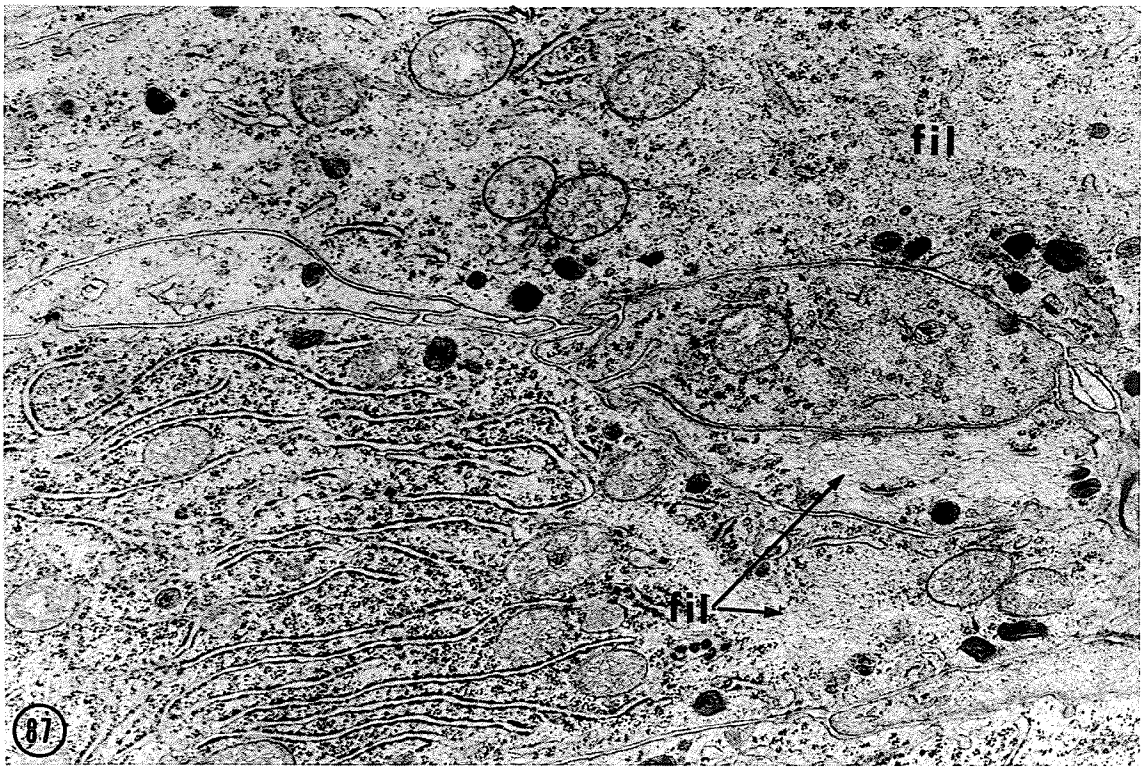
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DISCUSSION

The results of many experiments involving melanomas have found them to be a difficult group of cancers to deal with clinically. This observation is derived from the fact that both chemotherapy and radiation in the past have been shown to have little effect on the palliation or destruction of melanomas.

Chemotherapeutic agents may exert their action in one of three ways: one is to destroy the tumor cells; a second is to induce changes in tumor cells to make them more amenable to other forms of treatment such as chemotherapy with other drugs, irradiation, or a combination of the two; a third is to convert the energies of the tumor cell from one of multiplication to one of differentiation since the common denominator for both cell proliferation and differentiation is protein synthesis.

It has long been realized that differentiated cells do not divide and that dividing cells tend not to differentiate. In spite of this general statement which emphasized this antagonism between division and differentiation, differentiated cells are certainly capable of dividing with the exception of nerve cells and muscle cells. Hepatic cells in regenerating liver may undergo mitosis with little if any interruption of their normal function (Harkness, 1957). The most that one can say about antagonism of division and differentiation is that the more clearly a cell is differentiated, the less likely it is to divide, though this capacity is never lost completely (Swann, 1957, 1958). Swann (1958) described the process of differentiation as being the result of an induction, the essense of which is that a short-lived stimulus of some sort produces a relatively long-lasting effect on the

pattern of synthesis. Weiss (1949) suggested that the process of differentiation brings with it, or virtually consists of, the continuous elaboration of molecular novelties which have not been present from the beginning.

We shall initially discuss the significance of the light microscopic observations and subsequently proceed to a discussion of the electron microscopic findings. It is of importance at this point to discuss the significance of the values obtained by counting mitoses per hundred cells following the varying dose regimes used in the three experiments.

It has been previously shown by many authors including Inoue (1952), Swann (1953), Eigsti and Dustin (1955), Davidson et al., (1966), and Brinkley et al., (1967) that colchicine causes metaphase arrest. This arrest is thought to persist for about 15 hours after which the cell either degenerates or passes through the remainder of the cell cycle. The present results of colchicine administration on Harding-Passey melanoma showed an increase in percent mitosis in tumors after maximal dosage of colchicine as described in Experiment A (Text-Fig.1, p. 39). The values slowly declined from a high of 7.0% in Treated-tumor, to a low of 4.1% in Generation V. This gradual decline in percent mitosis can be attributed to the decrease in colchicine concentration in tumor cells of successive generations after colchicine treatment. Interestingly enough though, the values obtained in Generation V are still maintained above those observed in non-treated tumors.

Values obtained after minimal dosage - Experiment B, declined from a high of 5.9% mitosis in Treated-tumor to a low of 3.8% in Generation V, the latter value in the final generation being somewhat

closer to the non-treated values. This decline of % mitosis, as in Experiment A, is considered to be due to a decrease in concentration of colchicine in tumor cells of successive generations.

Intermittent dosage schedule of Experiment C produced significant changes in percent mitosis observed. As expected, Treated and Generation I values of 5.9% and 5.8% followed those of minimal dosage during the same time period since they both received the same dosage of drug. However, after 2^{o} Treatment, the percent mitosis observed was 23.8%. Although only a small dosage of colchicine was used, the effects of colchicine apparently were potentiated by being given in a second dosage regime. The percent mitosis observed in Generation III was 7.6%, showing a striking reduction from that observed in 2^{o} Treated tumor. This sharp drop in percent mitoses cannot be explained. When 3^{o} Treated tumors were observed, the values showed a slight rise to 9.8%. The generation V values of 7.5% remained well above those observed in non-treated tumor.

The results seem to indicate that colchicine not only causes immediate chromosomal arrest, but its effects are potentiated after a second treatment, and to a lesser degree after a third administration. One might further conclude from these results that there remains a certain dosage value of drug, in this case colchicine, after which no further treatment can beneficially influence the cells of a tumor population, possibly due to a built up resistance to the drug.

There have been several studies directed to elucidate the length of time that colchicine exerts its action on the mitotic cell. Eigsti and Dustin (1955) stated that colchicine causes chromosomal aberration and arrest for up to 10 hours after which the cell either recovers or

dies. Davidson et al., (1966) have stated that the effects of colchicine do not wear off for several hours, and metaphase arrests continue to occur. Kleinfeld and Sisken (1966) reported that HeLa cells arrested in metaphase up to 5 hours by colcemid were capable of reforming a normal functional spindle, but failed to recover if they were blocked for longer than 5-6 hours.

The percentage mitoses in generation V in both Experiment A treated with maximal dosage of colchicine and in Experiment B subjected to a minimum dosage were higher than in the untreated tumor indicating continuous but decreasing effect of the drug on the mitotic cell. Support to such a concept comes from the studies of Davidson et al., (1966) in which they felt that the mitotic index, the percentage of cells in division, is known to increase in actively dividing cell populations which have been treated with colchicine. The increase in mitotic index caused by the arrest of metaphase is maintained after treatment has ceased. Colchicine appears to have a stimulatory effect sometime during the mitotic cycle; it is this stimulation that results in the increase in the number of cells in division, seen 1-2 days after treatment.

The most significant ultrastructural change observed in the tumor cells of Harding-Passey melanoma following colchicine administration was the appearance of microfilaments 35-50Å in diameter not seen in the untreated tumor cells. They were found perinuclear in position as well as in the rest of the cytoplasm.

Experiment A results showed that the filaments which appeared following the treatment persisted in all five generations of the tumor without further treatment. The amount of filaments observed in cells,

and the frequency with which they occurred, gradually decreased from cells of generation I to generation V (Loader and Nathaniel, 1972; Nathaniel and Loader, 1972).

Minimal dosage therapy as shown by Experiment B also revealed that a smaller dosage of the drug colchicine could induce the formation of microfilaments in tumor cells. Although the amount of filament bundles found was diminished in comparison to the amount seen in maximal dosage therapy, the filaments presented similar features to those seen in maximal dosage treatment. The filaments also appeared up to five generations or 10 weeks following cessation of colchicine treatment.

Intermittent chemotherapy in Experiment C showed the most pronounced changes in morphology of the Harding-Passey melanoma. The first two generations resembled those found in minimal dosage treatment, as would be expected since the dosage regime at this point was identical. After the second treatment of colchicine, numerous cells were seen to contain filamentous bundles in both mitotic and interphase cells. This increase in the amount of filaments continued to be observed in the fourth generation. Further colchicine administration did not alter the morphologic picture (Loader and Nathaniel, 1973).

Other fine structural changes resulting from colchicine treatment of the Harding-Passey melanoma were the appearance of greater quantities of melanin granules and intercellular junctional complexes between tumor cells. The increase in melanin granules appeared to be more evident in tumors of Experiment A subjected to maximal dosage of colchicine than in tumors of Experiment B treated with minimum dosage. In Experiment C the increase in melanin appeared to be more evident after the second and third injection of colchicine. The melanin

granules in the cells of the three experimental series tended to have marginal localization. Intercellular junctional complexes were observed in the tumor cells of all the three experimental groups.

Although the appearance of filaments was used as the major criterion for indicating that differentiation was occurring after colchicine chemotherapy, the appearance of other factors helped to ascertain the differentiation process. One of these was the increased amount of melanin, with a tendency to marginate; and another the presence of junctional complexes.

Nathaniel, Friedman and Rychuk (1968) reported the presence of numerous microfilaments in tumor cells of the Harding-Passey melanoma in both mitotic and interphase cells. They observed that ribosomes were closely related to the filaments. However, these investigators did not study the long term effect of colchicine on the melanoma tumor cells and they were unable to ascertain whether the microfilaments observed were transitory in nature or more enduring.

The investigations carried out by the author have clearly shown that the altered morphologic differentiation induced in the tumor cells of the Harding-Passey melanoma was more enduring than initially presumed to be. These studies therefore considerably extended the original investigations of Nathaniel et al., (1968).

Friedman and Drutz (1958) studied the effects of irradiation on Harding-Passey melanoma. They observed that the irradiated tumor cells showed cellular enlargement and increased pigment production. Friedman and Drutz (1958) observed in a light microscopic study that colchicine caused deposition of melanin pigment granules in the peripheral portion of the tumor cell, which are in keeping with observations of the present study.

Microfilaments have been reported in a variety of cells performing several functions. The appearance of fine filaments in the cells of Harding-Passey melanoma following colchicine treatment is interesting. These filaments morphologically resembled tonofilaments of epidermal cells. The basal cells of the epidermis of adult Rana pipiens (Parakkal and Matoltsy, 1964), as well as the basal cells of human oesophagus and trachea (Rhodin and Reith, 1962), contain an abundance of similar filaments. Rhodin and Reith (1962) considered that tonofilaments played a role in keratin formation. Selby (1955) showed that the tonofibrils of the light microscopist, which are considered to contain a prekeratin material (Giroud and Leblond, 1951) are in fact bundles of smaller units called tonofilaments. In fact, even though much emphasis has been placed in the role of such tonofilaments in keratinization, filaments considered similar to these have been described in a variety of non-keratinizing cells (Leblond, Puchtler and Clermont, 1960).

The appearance of filaments under the influence of colchicine indicated that this drug elicited a different type of differentiation not normally observed in melanocytes. The association of ribosomes to these filaments could probably indicate their role in filament production.

It has been shown that some antimitotic agents can stop the division of cells without affecting their differentiation. The dichotomous effect of certain chemotherapeutic agents on growth has been reported by several investigators. It was found that synthesis of mucous and resultant differentiation of reserve cells into goblet cells continues to take place in the intestinal epithelium of the rat despite doses of irradiation, mustards or aminoprotein which inhibit division (Friedman, Sargent and Drutz, 1955). Friedman and Drutz (1961) reported that mechlorethamine

(nitrogen mustard) or irradiation dosage to rats and mice, permitted maturation of the sperm in the seminiferous epithelium while interfering with mitosis of more undifferentiated elements. Friedman and Drutz (1958) studied the effects of chemotherapy and irradiation on different transplantable tumors in mice. They found that irradiation and nitrogen mustard induced poorly keratinizing or transitional epidermoid carcinoma to produce more keratin, and melanomatous cells of Harding-Passey melanoma to become loaded with pigment.

Similar dichotomous effects have been reported in lower forms. Thus, ultraviolet irradiation inhibits DNA but not RNA synthesis in E. coli (Kelner, 1953), and mechlorethamine hydrochloride inhibits DNA but not RNA synthesis in E. coli (Herriot, 1951).

It has been observed that many of the cells in neoplasms do not die as a result of chemotherapy or irradiation. On the other hand these cells show alterations which include enlargement, hyperchromatic nuclei, and formation of giant and multinucleate elements. These bizarre changes are considered to indicate not increased malignancy, but inhibition of cellular division without interference with cellular synthesis and growth. Hall and Friedman (1948) in a study of serial specimens taken during the course of treatment of an undifferentiated transitional carcinoma in man by roentgen irradiation, observed the transformation of the tumor to a well differentiated and cornified epidermal growth. Thus, many of the effects of irradiation and chemotherapy on neoplasms in man are not necessarily mediated by necrosis, but by alteration of the cellular cycle of growth, division and differentiation.

The continued growth on the one hand and the inhibited division on the other hand are consistent with the view that synthesis of

deoxyribonucleic acid (DNA) can be affected without disturbing the synthesis of ribonucleic acid (RNA) and protein (Greenberg, 1955; Swann, 1957).

The presence of filaments which resembled tonofilaments and junctional complexes observed in colchicine treated tumor cells indicate possible similarities between the morphologically altered melanocyte and an epithelial cell.

The embryogenesis of melanocytes is far from settled. Rawles (1947) based on experimental embryological studies came to the conclusion that the melanocytes of most vertebrates are neuroectodermal in origin. On the basis of this, many investigators believe that it may also be so of the human melanocyte. Masson (1951) was of the opinion that melanocytes are "neural" in origin. However, Allen (1949) subscribed to the view that the melanocyte was epidermal in origin. The presence of microfilaments and junctional attachments which resemble similar structures in epidermal cells may indicate an attempt at transformation of a melanocyte to an epidermal cell. On the basis of the findings in these studies it appears that a relationship between the melanocyte and the squamous epithelial cell is plausible. Possibly the two conflicting theories can be reconciled by viewing the melanocyte as a neuroepithelial element altered by inclusion in the epidermis.

Filaments have also been observed to occur in other tumors. Molnar and Bekeski (1972) in ascites tumor cells, and Journey et al., (1968) in cultured cells after vincristine treatment noted the presence of filament bundles within the cytoplasm of cells. Robbins and Gonatas (1964) using spindle inhibitors such as vinblastine, noted an increase in the amount of 60-80Å fibrils in the cytoplasm of HeLa cells than

were normally observed in interphase cells. They often ran in discrete bundles parallel to the surface of the cell adjacent to the plasma membrane. Similar filaments 50-70Å in diameter were seen in the cytoplasm of cells of basal cell cancer (Flaxman, 1972). These cells further showed numerous cytoplasmic interdigitations and were connected to one another by desmosomes.

Intermittent form of chemotherapy has been found to be more effective than single dosage chemotherapy in animal and human neoplasms. Tumor cells in Experiment C showed a pronounced increase in amount of filaments observed as compared to Experiment A and B. The results of this present study suggest that the effects as produced by colchicine on the cell continue to act in some way after the initial metaphase block to induce the changes so characteristically seen. Bertalanffy and Gibson (1971) reported that a lower dosage of arabinosylcytosine (ara-C) reduced the daily mitotic rate of B-16 melanoma to a lower level than a higher dose.

Chemotherapy of tumors by a combination of anticancer drugs was found to be more beneficial than by treatment with a single chemotherapeutic agent in both human and animal neoplasms. A desirable feature of combination therapy was decreased toxicity. In this form of treatment the component drugs appear to potentiate each other's effects by synergism. Moon (1970) reported beneficial responses in 8 of 18 patients with malignant melanoma using a combination of 1-3, bis (2-chlorethyl)-1-nitrosourea (BCNU) and vincristine. Helson et al., (1972) observed that the combination of vincristine, cyclophosphamide and daunomycin administered to patients with disseminated neuroblastoma increased survival time in the absence of debilitating toxicity. Whitecar et al.,

(1972), Stolinsky et al., (1972), and Gardere et al., (1972), found moderate degrees of success using combinations of drugs in an attempt to eleviate human cancers.

Grindey et al., (1972) in mouse and Mashima et al., (1972) in rabbits found combination chemotherapy to be most effective in increasing survival time of tumor bearing host animals.

As the melanoma is a radio-resistant tumor, several researchers have attempted to make them more susceptible to irradiation by pre-treating them with chemotherapy and following it up by radiation. Studies by Cooper and Mashima (1972) have shown Fortners melanotic melanoma to be more radio-sensitive upon administration of chlorpromazine by masking the free nature of melanin. Demopoulas (1963) proposed that tyrosine activity might be necessary for the energy metabolism of the melanoma cell. He attributed the growth-inhibitory effect of phenyl lactate and pencillamine on S-91 mouse melanoma to the ability of these compounds to inhibit tyrosinase. On the other hand, the investigations of Woert and Palmer (1969) demonstrated that the carcinostatic action of chloropromazine is associated with an increase in tyrosinase activity in the B-16 and Harding-Passey melanoma. Similar antineoplastic activity of chlorpromazine on mouse sarcoma 37 has been reported by Belkin and Hardy (1957).

More recently investigators are exploring the potential of immunotherapy in the treatment of melanomas (Krementz et al., 1972).

A discussion on the evolution of the melanin granule is not within the scope of the present study. The observations in this study suggest that both the Golgi and endoplasmic reticulum are probably involved in the synthesis of these granules. Future radioautographic

studies using the precursor, of melanin, tyrosine, will be beneficial in understanding the origin of melanin granules.

Virus particles were observed to originate from the endoplasmic reticulum by a process of budding into the cisternae of the granular endoplasmic reticulum in all the generations of all three experiments. The significance of the Type A particles within this tumor is unknown. Many authors including Bernhard (1958, 1960), Parsons et al., (1960), Sobin (1964), Nathaniel et al., (1968) Novikoff et al., (1968), Volkman (1971), Dalton (1972), and Molnar and Bekeski (1972) have stated virus particles are definite cytoplasmic inclusions of many tumor types. Much work is presently being done in an attempt to better understand the relevance of these particles within a variety of tumors.

VI

SUMMARY

SUMMARY

1. The Harding-Passey melanoma when exposed to varying dose regimes of colchicine produces filaments 35-50Å in diameter.

2. The filaments were observed in both mitotic and interphase cells.

3. The filaments were observed to be closely associated with ribosomes.

4. Filaments both in bundle form, and permeating the cytoplasm, persisted through 5 generations of transplantation or ten weeks after cessation of colchicine therapy in maximal and minimal dosages.

5. Numerous bundles of filaments were observed in cells following a second low dosage intermittent therapy as shown in Experiment C.

6. The effects of colchicine were seen to substantially increase the number of mitotic cells in a population following a second intermittent low dosage injection.

7. Virus particles were seen to occur throughout all the Generations in all three experiments, and were observed to arise from the granular endoplasmic reticulum.

8. Melanin granules in various stages of development were observed in all three experiments, with melanin displaying the characteristic 'margination'.

VII

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