INTERNATIONAL ACADEMY OF PATHOLOGY MONOGRAPH

NEW CONCEPTS IN NEOPLASIA AS APPLIED TO DIAGNOSTIC PATHOLOGY

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New Concepts in Neoplasia as Applied to Diagnostic Pathology

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Foreword

This 27th monograph in the series *Monographs in Pathology* is the first in the series to be based on a course given at an International Congress, rather than at our annual meeting. Since this congress was hosted by this Division and the Long Course organized along the same lines as our previous Long Courses, it was felt appropriate to include this particular material in this series.

The Long Course presented at the XV International Congress of the International Academy of Pathology in September 1984 entitled "New Concepts in Neoplasia as Applied to Diagnostic Pathology" was considered to be timely because of the recent developments of these concepts. It was very ably organized and directed by Drs. Cecilia M. Fenoglio-Preiser and Ronald Weinstein. This publication gives us an opportunity to present up-to-date material by experts on the various topics in a manner which is more detailed, more extensive, and more documented than the oral presentations.

The Academy wishes to express its appreciation to Drs. Fenoglio-Preiser and Weinstein, to the other distinguished contributors to this monograph, and to the publisher, Williams & Wilkins, for valuable support and cooperation.

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Chapter 1

Introduction

CECILIA M. FENOGLIO-PREISER AND RONALD S. WEINSTEIN

Benign and malignant neoplastic proliferations occur in a population of cells which were previously normal and in some way have lost their usual regulatory mechanisms such that unrestricted growth occurs and tumors develop. Not all cells have the same potential to undergo neoplastic proliferations and, further, even a cell which may be rendered neoplastic relatively easily may not become transformed in every individual exposed to an oncogenic agent. In this volume, some specific oncogenic agents will be discussed. After considering current ideas on how cells become neoplastic, several chapters discuss the biology of the hallmarks of malignant growth, namely, tumor invasion and metastases. Then, tumor cell phenotype is considered from the standpoint of diagnostic pathology. The usefulness of several markers in identifying the progenitor cells of specific human malignancies is considered, as well as the use of cytoplasmic and cell surface markers in predicting the clinical course of the malignant dyscrasia in individual patients.

Cancer is clearly a multifactorial disease. Operationally, oncogenic agents can be subclassified into genetic, physical, chemical, infectious, and immunologic categories. There are specific associations between certain cancers and their presumed etiologic agents. Several examples exist of genetic disorders associated with an increased incidence of cancer. Specific chemicals are known to cause tumors both in experimental animals and in humans. Physical agents such as irradiation can irreversibly transform cells. Infectious agents such as certain viruses, both endogenous and exogenous, are oncogenic in experimental systems and are associated with some human malignancies.

Since the interaction of cells with dissimilar oncogenic agents can result in the production of a pathologically identical tumor, such as a squamous cell carcinoma, it is plausible that each of these agents may act through a common pathway, possibly the activation of an endogenous virus or an oncogene. Various aspects of this subject are explored in the chapters by Drs. Tomasi, Yunis, and Fajardo. This is followed by a presentation of the spectrum of lesions caused by one class of viruses, the papillomaviruses, by Dr. Lutzner.

Whatever the etiologic agent for a particular tumor in an experimental system or in man, tumors evolve over a period of time, and the individual cell populations comprising them apparently pass through various stages of abnor-

mal growth control regulation. It is also clear that even though a specific agent is oncogenic in some settings, most agents are unable to cause malignant proliferations in many cells lines or in all individuals, either due to unique interactions required of the agent with the progenitor cells, differences in host responses, or other factors. It is well established that some families are more prone to develop cancer than others. Some may have a definable genetic syndrome or karyotype abnormality as discussed by Listrom and Fenoglio-Preiser. Others may not have morphologically recognizable karyotypic abnormalities but may have chromosomes which are unusually susceptible or resistant to the mutagenic effects of a particular agent. This subject is explored by Dr. Yunis, especially with regard to fragile sites on chromosomes.

Mere exposure to potential carcinogens in the foods we eat or the air we breathe may be insufficient to cause cancer, until they are activated by enzymes to create an alkyl or aryl derivative, which then binds preferentially to DNA, RNA, or cell proteins. Radiation can interact with cells to cause lipid peroxidation of cell membranes, either the plasma membrane or membranes of individual organelles. Of greater importance to the cancer problem, radiation is known to induce chromosomal damage which may be manifested as deletions, gaps, or translocations within individual chromosomes which might directly activate oncogenes. The relationship of one type of physical injury, *i.e.*, ionizing radiation, to the subsequent development of neoplasia is discussed in detail in the chapter by Dr. Fajardo.

Tumors, once established, are influenced by growth factors, growth inhibitors, hormones, and biogenic amines. There are numerous growth promoters and modifiers. One which has generated a large literature and captured the imagination of the scientific community is so-called tumor angiogenesis factor. This is produced by successful neoplasms which have the capacity to induce the proliferation of endothelial cells to form new blood vessels, thereby allowing them to maintain sufficient access to oxygen and nutrients required for a sustained increase in tumor mass. It has been hypothesized that inhibition of tumor angiogenesis factor would stunt tumor growth and thus render malignant cell proliferations innocuous. In recent years, it has been suggested that angiogenesis factor is nonspecific, possibly restricting its potential clinical value. In a chapter on alternative approaches to restricting tumor dissemination, Drs. Kuettner and Pauli characterize endogenous stromal proteinase inhibitors that may partially explain differences in host susceptibilities to tumor invasion. It remains to be seen whether proteinase inhibitors are of therapeutic value. This is followed by a chapter by Drs. Liotta and Ras on the biology of tumor invasion, stressing the interactions of tumor cell surface receptors with extracellular matrix components.

A broad spectrum of nuclear, cytoplasmic and cell surface changes have been detected in neoplasms. Some, such as the laminin receptors described by Drs. Liotta and Ras may be mechanistically related to the invasive process whereas the expression of others may represent epiphenomena unrelated to tumor behavior but, nevertheless, of value for tumor diagnosis, classification, or as prognostic factors. The chapters by Dr. Neville and his associates and Dr. Rilke and associates describe the use of monoclonal antibodies to breast carcinomas in detecting micrometastases in bone marrow. This provides an im-

portant approach to the identification of patients with "dormant" carcinoma, undetectable by conventional histopathology. Drs. Weinstein, Schwartz, and Coon describe the prognostic significance of the deletion of normal epithelial cell surface components, the tissue blood group antigens, in patients who present with low grade, low stage urinary bladder transitional cell carcinoma, as well as the use of marker profiles for establishing the risk of recurrences and invasion. The chapter by Dr. Solcia and his associates outlines the use of antigenic markers in analyzing various neuroendocrine tumors. Finally, Dr. Triche summarizes the current state of the art in working up the ultimate challenge in tumor classification, the anaplastic tumor.

Chapter 2

Cellular Aspects of Neoplasia

MARGARET B. LISTROM AND CECILIA M. FENOGLIO-PREISER

Pathologists are characteristically able to distinguish between the neoplastic and nonneoplastic phenotype of a given cell population based on specific morphologic features. These features may be visualized using standard histological techniques (Fig. 2.1), ultrastructural analysis, chromosome preparations, or special staining techniques such as immunohistochemistry. In this chapter the morphologic, histologic, and cytologic features of neoplastic cells will be discussed organelle by organelle. No new cellular organelles are seen in neoplastic cells but quantitative and qualitative alterations may be present.[133]

NUCLEUS

General Morphologic Features

Often it is the nuclear characteristics that allow one to recognize the neoplastic phenotype (Fig. 2.1) and to distinquish benign from malignant cells. Criteria that are commonly present in neoplastic cells are an increased nuclear size, producing an increased nuclear cytoplasmic ratio (often with nuclear gigantism), and nuclear pleomorphism, correlating with an abnormal chromosomal number and an altered DNA content, prominent nucleoli, and peripheral chromatin clumping (Fig. 2.1). Abnormalities in nuclear size result from endoreduplication, true endomitosis, and a type of polytenization.[249] In addition, increased mitoses are often present, indicative of an actively proliferating cell population, even though the reproducibility of counting mitosis may be variable.[130, 222, 234]

Ultrastructurally, these nuclear abnormalities are even more prominent, with irregular distribution of the heterochromatin, prominent nucleoli, peripheral chromatin clumping, active mitoses, bizarre shapes (Fig. 2.2), and the presence of nuclear inclusions ranging from viruses to structures known as nuclear bodies (Fig. 2.3).[100, 233, 240, 243]

Many of the nuclear bodies have structures resembling nucleoli.[243] Some are similar to small nucleoli with a filamentous cortex. Others resemble ring-shaped nucleoli (Fig. 2.4) or nucleoli with separation of their components.[240] Others merely represent cytoplasmic inclusions.

In most cells the nucleolus disappears during mitoses; it is thought to disintegrate concomitant with dissolution of the nuclear membrane.[34] Those which

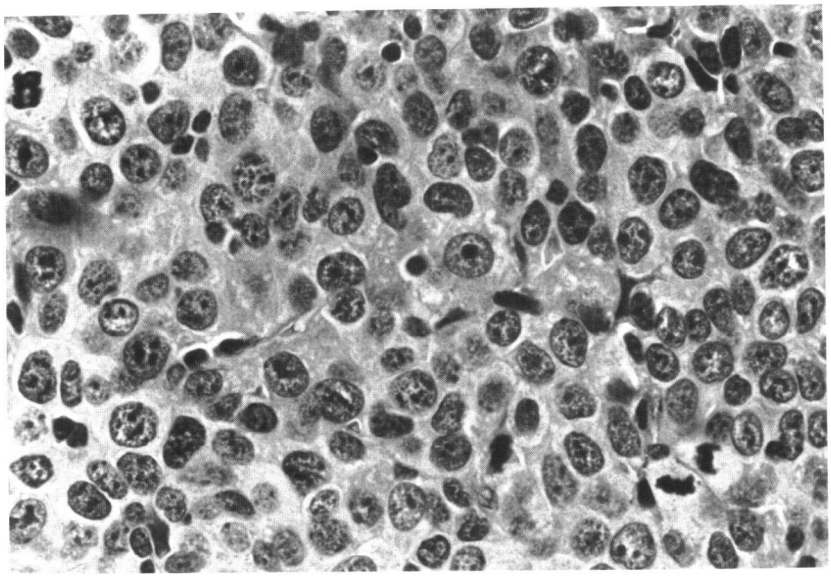

FIG. 2.1. Light micrograph of a malignant islet cell tumor. The cells are pleomorphic with many mitoses evident. In addition, peripheral nuclear clumping and prominent nuclei are noticeable. One would not have difficulty making a diagnosis of neoplasia on such a lesion.

TABLE 2.1. TUMORS CONTAINING INTRANUCLEAR RODLETS

Gliomas
Ependymomas
Pancreatic islet cell tumors
Parathyroid adenomas
Mycosis fungoides
Sarcomas
Neuroepithelial tumors

are retained and persist during metaphase and anaphase are referred to as persistent nucleoli. These may be seen in neoplastic cells,[148,225] and their number often reflects histological differentiation.[225]

Intranuclear rodlets are composed of proteinaceous material of uncertain origin and function[233] and are found in various tumors,[17,100,117,189,201,227,238,247] (Table 2.1). The rodlets are composed of individual filaments that measure up to 7–9 nm in width and are not restricted to neoplastic cells.[117] Intranuclear crystalline inclusions with a leaf-like, striated appearance ("zebra bodies") are inducible in tumor cells by viruses.[268] Intranuclear annulate lamellae may also be present.[96,133]

NUCLEAR MEMBRANE

By definition, human cells are compartmentalized into the nucleus (Fig. 2.1 and 2), which contains the genetic material and structures involved in transcription and processing of transcription products, and into the cytoplasm, which contains the translational apparatus, cellular organelles, and other

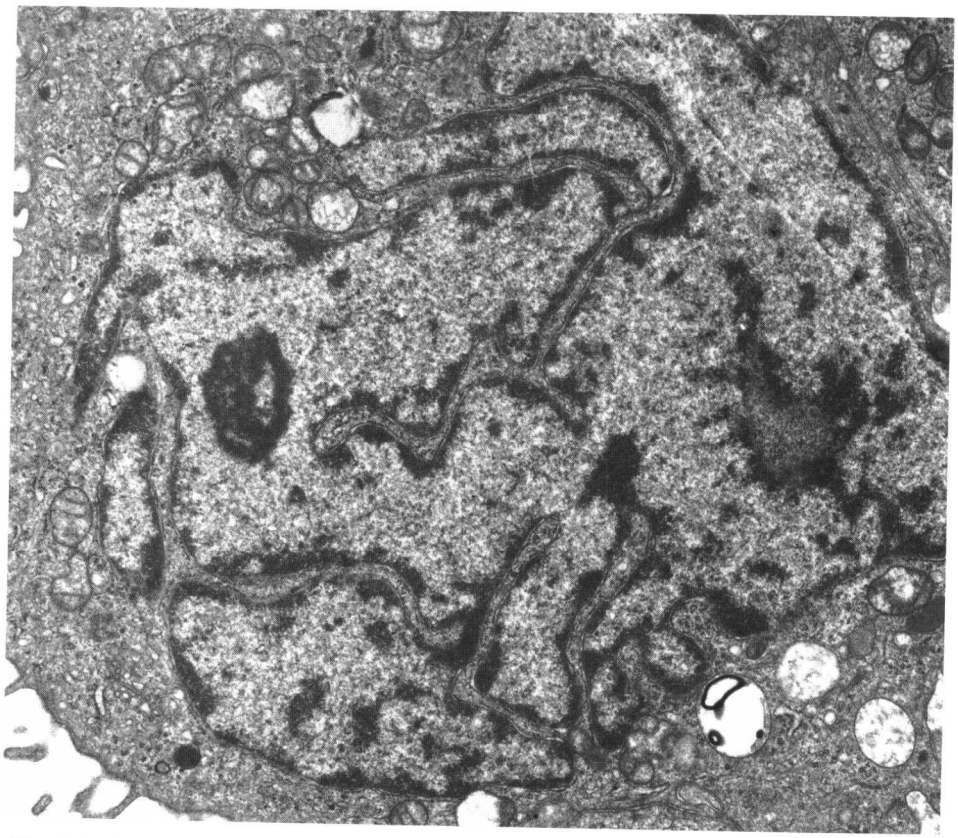

FIG. 2.2. Electron micrograph of an ovarian carcinoma cell. Notice the increased nuclear cytoplasmic ratio with the marked nuclear pleomorphism, cytoplasmic invaginations, peripheral nuclear clumping, and prominent nucleolus.

structures. This compartmentalization is maintained by the nuclear envelope, which is always present except during mitoses[86] (Fig. 2.1–2.5).

One important part of the nuclear membrane is the nuclear pore complex, which functions in nuclear-cytoplasmic exchanges. In some tumors nuclear pore complexes are significantly reduced. This observation has been used to explain the discrepancy that exists between active-appearing nuclei and an indolent-looking cytoplasm. The hypothesis is that there may be a disturbance of nuclear-cytoplasmic transport of proteins. It is further postulated that morphologic signs of nuclear hyperactivity may represent compensation for the nuclear membrane defect.[123]

In neoplastic cells the nuclear membrane may also show blebbing, pockets, and projections, particularly in lymphoma cells.[133] The fibrous lamina may also become quite prominent. This structure appears to be associated with some oncogenes.[75]

As noted above, neoplastic cells are often mitotically quite active. The nucleus undergoes profound alterations when cells enter mitosis: the nuclear envelope breaks down and chromatin condenses into individual chromosomes

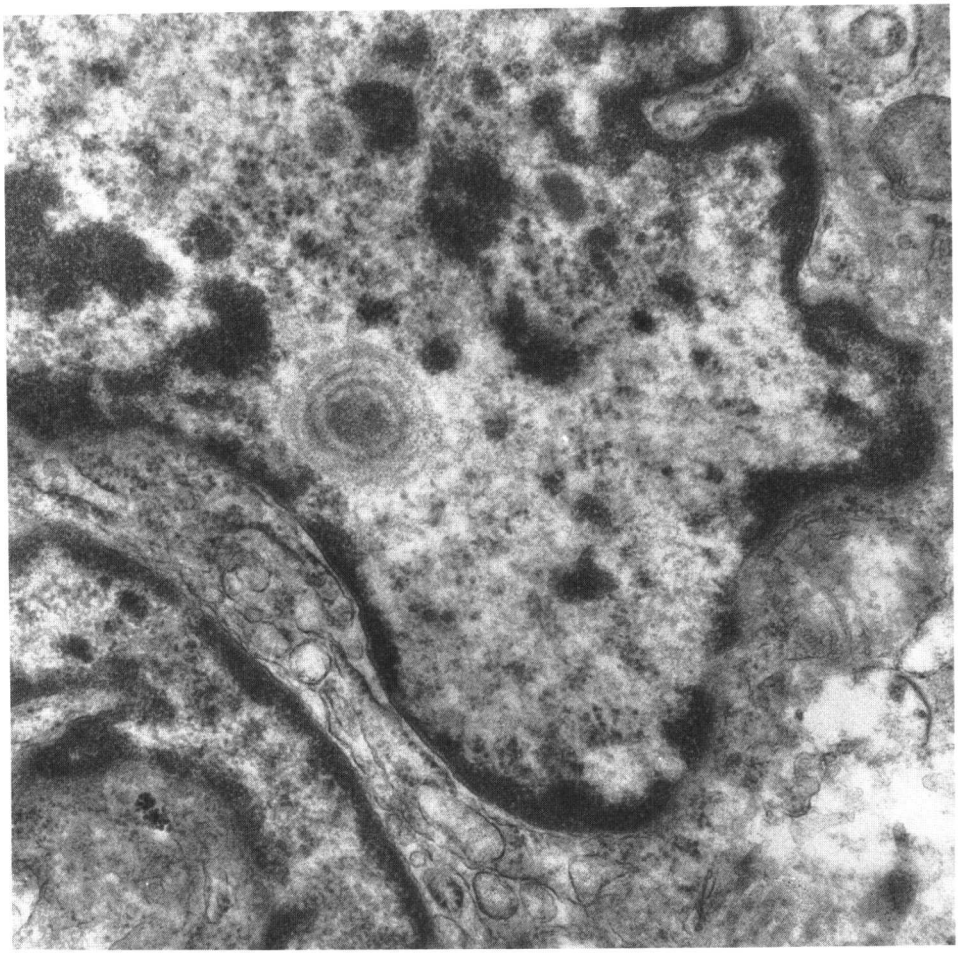

FIG. 2.3. Electron micrograph of an ovarian cancer. A prominent nuclear body is seen in the nucleus of the cell just left of the center of the illustration.

(Fig. 2.5). Certain proteins become associated with the condensed chromosomes.[196] There is (are) a specific protein(s) (phosphoproteins) that is (are) mitosis-associated.[62] It (they) may phosphorylate the nonhistone proteins necessary to initiate mitosis.[85]

NUCLEAR SEX CHROMATIN (BARR BODIES)

Barr bodies are frequently absent in the neoplastic cells of females; this may be due to: (1) the loss of the inactivated X chromosome; (2) the possibility that a previously inactive X chromosome may become activated by the malignant process; or (3) the presence of more than two X chromosomes. There is a direct relationship between ploidy and the incidence of normal single Barr bodies. Atkin[7] showed that approximately 8% of near diploid cervical cancers contain a normal single Barr body, whereas 20% of the aneuploid tumors had a low percentage of these structures. This contrasted with a low frequency of Barr

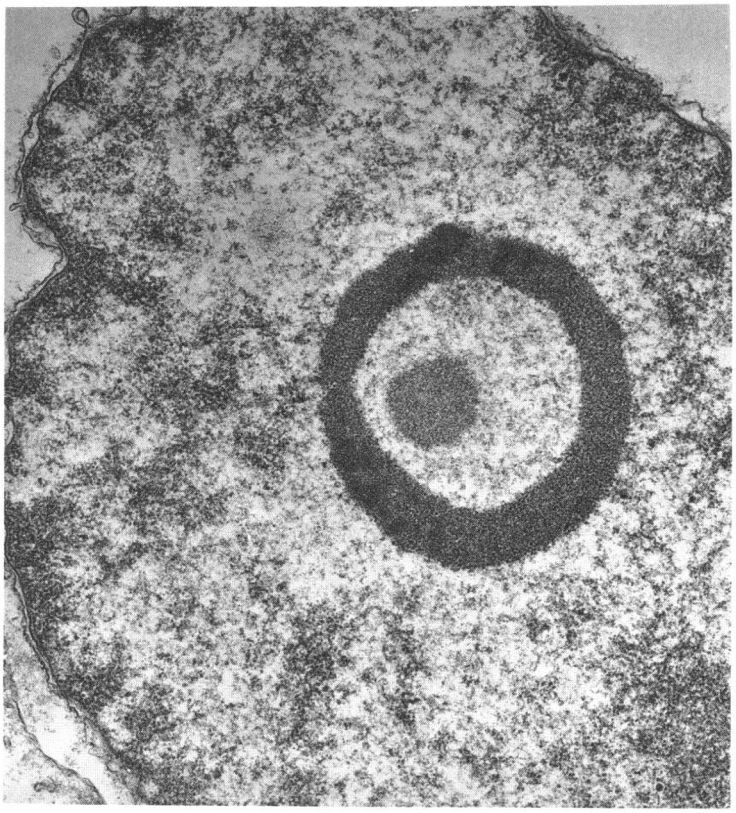

FIG. 2.4. Electron micrograph of a poorly differentiated carcinoma of the endometrium. Notice the prominent ring-shaped nucleolus.

bodies in tumors that had a near triploid pattern. This observation was also confirmed for breast cancer.[7] The relationship of the Barr bodies to the ploidy status has led investigators to suggest that the identification of the Barr body could be used as a prognostic factor in evaluating patients with cancer.[7, 8, 15, 126]

The concept that Barr body analysis could be useful diagnostically is not new and was first introduced in 1954 by Hunter and Lennox.[120] However, the usefulness of detecting these structures may be hampered by physiologic variations pertaining to sex chromatin frequency. For example, estrogen administration is capable of activating the X chromosome, thereby reducing the frequency of finding Barr bodies.[6, 16, 107, 202]

It now appears that in breast cancer patients, tumor grade is not directly related to the presence of these structures, although tumor cells with small, uniform nuclei, as in infiltrating lobular carcinomas, are more likely to have a higher Barr body frequency than cells with large atypical nuclei as seen in medullary carcinoma.[26, 29, 106, 132, 134] There is also a relationship between the age of the patient, the presence of metastatic disease, and the presence of sex chromatin. Finally, large numbers of Barr bodies are usually associated with high levels of estrogen receptors and other receptors in primary breast can-

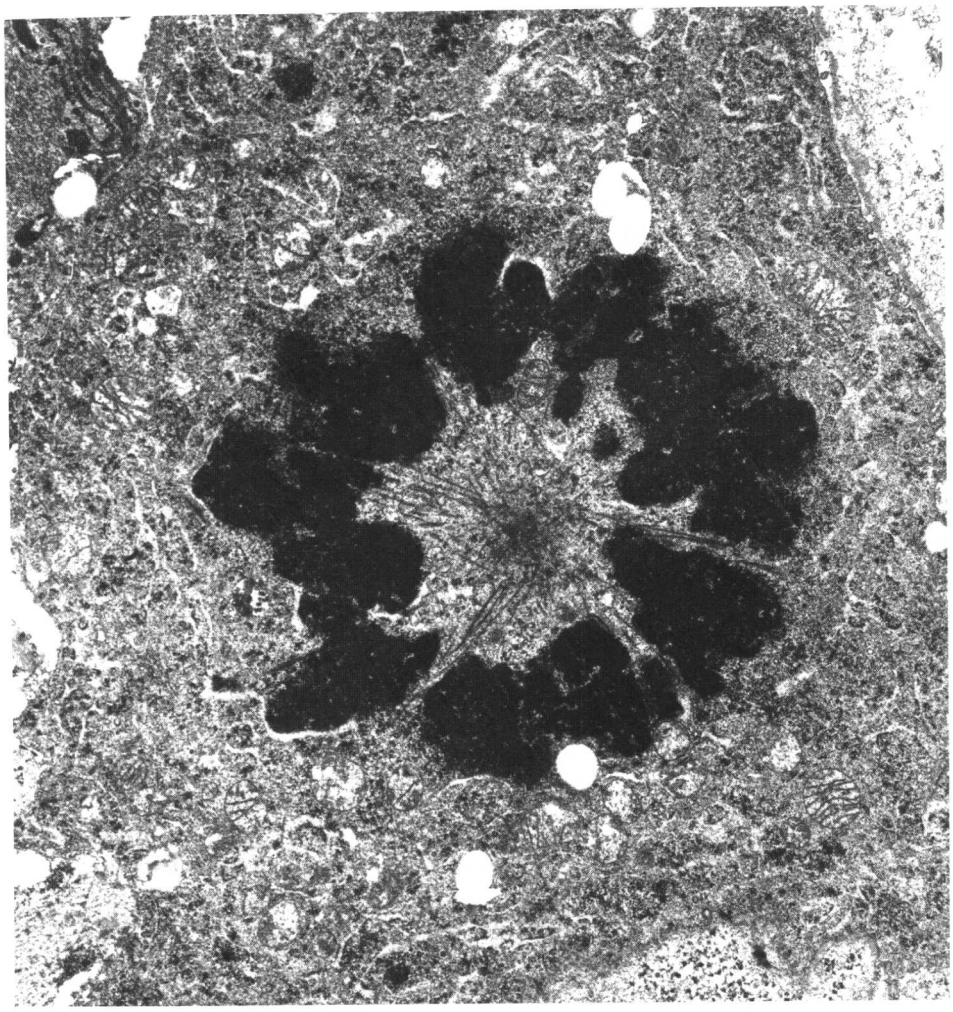

FIG. 2.5. Electron micrograph of a cell in mitoses. The prominent microtubular network is present in the middle of the condensed chromosomes getting ready to spread these into the mitotic spindle.

cers.[236, 239] Epidermoid cancers of the cervix and endometrial adenocarcinomas in patients with sex chromatin-negative tumors do more poorly over a 5-year period than do those with sex chromatin positive tumors.[41]

Occasionally, one finds the presence of a Barr body, and therefore a female karyotypic pattern, in males with tumors. A sex chromatin-positive melanoma and choriocarcinoma of the testis have been reported, as have male patients with lung cancers containing Barr bodies.[7, 111]

Neoplastic cells may contain more than one Barr body and some have as many as three. Tumors containing more than one Barr body are usually near tetraploid or hyperdiploid. Most near tetraploid tumors in females are sex chromatin-negative, either through the loss of an inactive X chromosome dur-