Ultrastructural Study of the Nuclei of Normal, Dysplastic, and Carcinomatous Epithelial Cells of the Human Cervix Uteri

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The nuclei of epithelial cells of the uterine cervix of normal women and of patients with various degrees of dysplasia, carcinoma in situ, and invasive carcinoma were studied by means of electron microscopy. Nuclear ribonucleoprotein components and chromatin were contrasted using preferential methods for RNA and DNA. Changes in the distribution of the extranucleolar ribonucleoprotein-containing structures were found, ranging from low-grade dysplastic lesions to invasive carcinoma. Compared with normal epithelial cells, dysplastic and neoplastic cells possess more nuclear bodies, as well as deep invaginations of the nuclear envelope and lobulations. Morphometric parameters estimated were nuclear volume, numerical density of perichromatin granules (PCG), and fraction of nuclear volume occupied by compact chromatin. The pattern of values of these parameters in the cell layers of normal cervical epithelium was disrupted in all the lesions. These data suggest that the processes studied induce early alterations in transcription and processing and/or exportation of mRNA to the cytoplasm. Two populations of cells were found in invasive carcinomas, one with large nuclei, sparse compact chromatin, and few PCG, and the other with small nuclei, abundant compact chromatin, and numerous PCG. Their morphologic features indicate that the former population is composed of relatively undifferentiated cells, while the latter is made up of well-differentiated cells which could be neoplastic or entrapped normal cells.

Keywords cancer, chromatin, differentiation, nuclear bodies, ribonucleoprotein

Squamous carcinoma of the uterine cervix is the most frequent carcinoma in women, in Mexico as well as in other underdeveloped countries [1]. Most invasive cancers are preceded by dysplastic alterations that are known to be the precursor stages of the cancerous transformation [2–4]. These stages are identified by an increase in nuclear size, increment in the number of mitotic divisions, and structural changes at the tissue level. The degree of these alterations allows distinction of three degrees of dysplasia: mild, moderate, and severe [3, 4]. The dysplastic alterations may revert to normal or progress to carcinoma in situ and to invasive carcinoma [2, 3].

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The ribonucleoprotein (RNP) components of the interphase nucleus of mammalian cells were clearly defined by Monneron and Bernhard [5] and later studied in detail with different methods (for reviews see [6, 7]). Perichromatin fibrils were found to contain pre-messenger RNA [8, 9] undergoing splicing [10, 11]. Perichromatin granules (PCG) are involved in storage of processed messenger RNA [12, 13]. Interchromatin granules are mainly composed of RNAs of low molecular weight rich in uridine (snURNAs) and their associated proteins, and they are related to the splicing of pre-messenger RNA [10].

The RNP components of the nucleus are sensitive to changes in the physiological states of the cell. The frequency and distribution of PCG display significant variations with changes in the transcription and transportation of RNA to the cytoplasm, as in modifications in the concentration of some hormones [14–17], or the changes of gene expression associated with cell differentiation and functional maturation during embryonic development [18–20].

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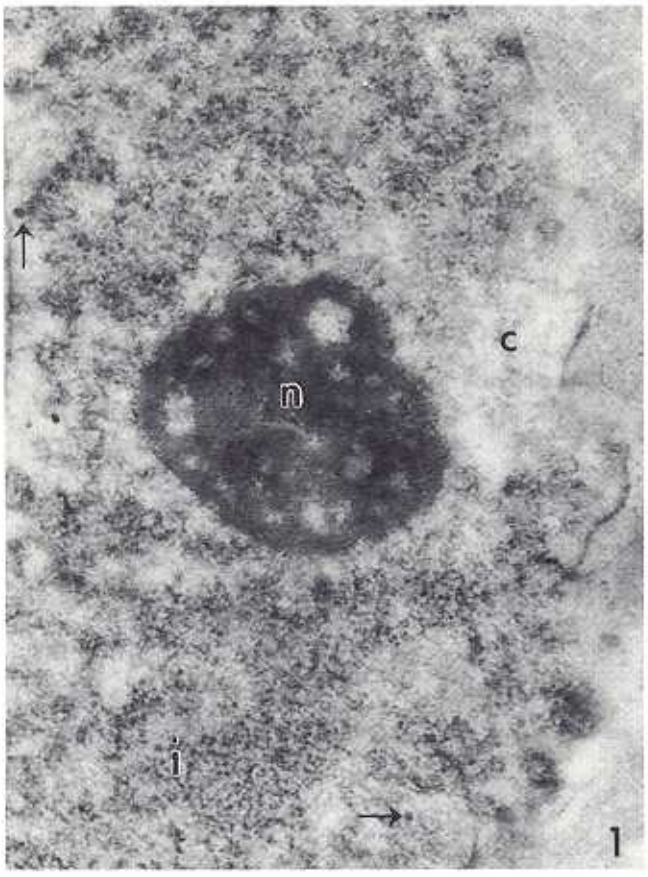

FIG. 1 *Nucleus of a normal basal cell of the cervical epithelium stained with EDTA procedure preferential for RNP-containing structures. The masses of compact chromatin* (c), *which form a discontinuous peripheral layer, appear clear gray with this staining procedure that darkens only the RNP-containing structures as the nucleolus* (n), *the clumps of interchromatin granules* (i), *and the perichromatin granules* (arrows), *×29,000.*

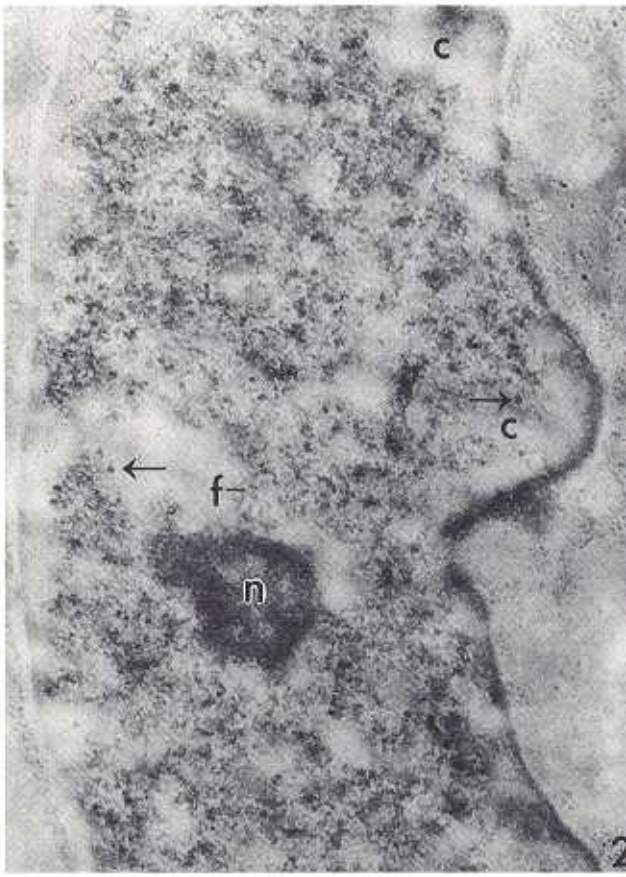

FIG. 2 *Nucleus of a normal basal cell stained with EDTA procedure. The masses of compact chromatin* (c) *are more abundant than in the preceding nucleus. The nucleolus* (n) *is smaller, and the perichromatin granules* (arrows) *are more numerous.* (f) *perichromatin fibrils, ×30,000.*

Nuclear bodies are 1- to 1.5-μm intranuclear spherical structures found in various normal and pathological cells [21]. They have been grouped as five types based on their size and the distribution of their fibrillar and granular components [22]. Nuclear bodies have been found to be particularly frequent in a number of human diseases [22, 23], including various carcinomas [22], leukemia, and viral diseases [21, 24]. A special type are the coiled bodies. They are spherical bodies, 0.3–0.5 μm in diameter, composed of tiny fibrils that are darkly stained by the uranyl acetate-EDTA-lead citrate procedure, which preferentially stains RNP [5, 25]. Immunocytochemical methods have revealed that an 80-kD protein is concentrated exclusively in coiled bodies [26]. A number of other antigens were also localized in these structures, including components of small ribonucleoproteins involved in the processing of mRNA and nucleolar RNA [27]. In situ hybridization demonstrated that a small nuclear RNA U7 is concentrated in coiled bodie and that these bodies often associate with specifi gene loci in interphase nuclei [28]. Coiled bodie are thought to be structures that have been con served by evolution because they are found in dif ferent animals and in plants [29].

The interphase nucleus of cancer cells has beer extensively studied [30, 31]. The normal RNP con stituents are slightly altered in cancer cells [31]. Th carcinogenic factors that participate in the genesi of squamous carcinoma of the cervix have beer intensely studied in recent years, especially the po tential for neoplastic transformation of human pap illoma viruses [32]. Several pieces of evidence in dicate that integration in the genetic material o types 16 and 18 papilloma virus has carcinogeni potential, because of the activity of viral oncogene (E6, E7) which degrade the product of anti oncogenes like p53 and cooperate with the Ras on cogene, acting in concert with other risk factors t produce neoplastic transformation [33].

Most cervical cancers are the final stage of a con tinuum of progressively more atypical changes

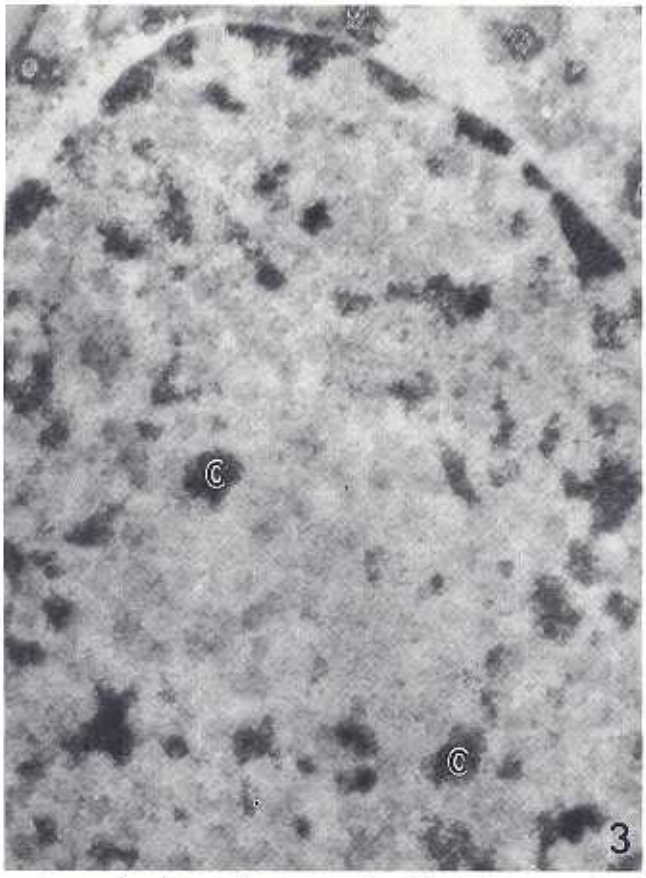

FIG. 3 *Nucleus of a normal epithelial cell stained with PTA method preferential for DNA. The masses of compact chromatin are darkly stained, ×24,000.*

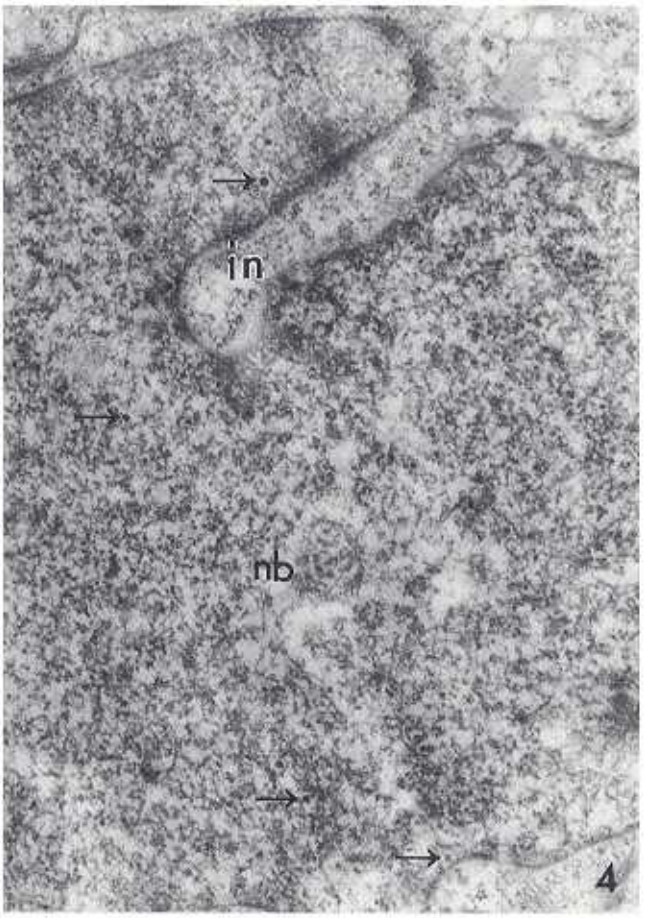

FIG. 4 *Basal cell of a mild dysplasia lesion stained with EDTA procedure. Numerous RNP-containing structures occupy most of the nuclear space. No compact chromatin masses can be seen. An invagination* (in) *deforms the nuclear shape. The arrows point to perichromatin granules.* (nb) *nuclear body type I, ×23,000.*

from atypical cells in the basal layers of the squamous epithelium (mild dysplasia) to full-thickness involvement in carcinoma in situ and infiltration to the subjacent connective tissue by an invasive carcinoma. During this sequence of abnormal cell maturation and differentiation, abnormalities in genetic expression must be acting, and they are probably related to morphological alterations in the nucleolus. With the objective of analyzing these nuclear morphologic abnormalities, we studied the interphase nuclei of normal, dysplastic, and neoplastic cervical epithelial cells with electron microscopy and preferential contrast procedures for RNP structures and chromatin.

MATERIALS AND METHODS

The control group was made up of 5 normal women who gave informed consent to participate in the study. The dysplastic lesions were grouped as mild (5 patients), moderate (7 patients), and severe (5 patients) according to standard diagnostic criteria. Eight patients with carcinoma in situ and 7 with well-differentiated invasive carcinoma were also studied.

The samples were tissue fragments obtained by biopsy. Small pieces were fixed in 2.5% glutaraldehyde in 0.16 M phosphate buffer at pH 7.3 for 2 h at room temperature. The samples from each patient were divided in two groups: Half were dehydrated and embedded in glycol methacrylate while the other half were dehydrated in ethanol and embedded in Epon. The contrast of RNP components was enhanced with the uranyl acetate–EDTA–lead citrate procedure preferential for RNP-containing structures [25]. Chromatin was stained in sections of glycol methacrylate embedded material with the aqueous phosphotungstic acid (PTA) method preferential for DNA, according to Vázquez–Nin et al. [34].

Morphometry

In each sample, all cells (not showing obvious artifacts) of the central part of the lesion in two consecutive squares of one section of one grid were selected for quantitative analysis. When more cells of the same patient were needed, at least 20 (1-μm-

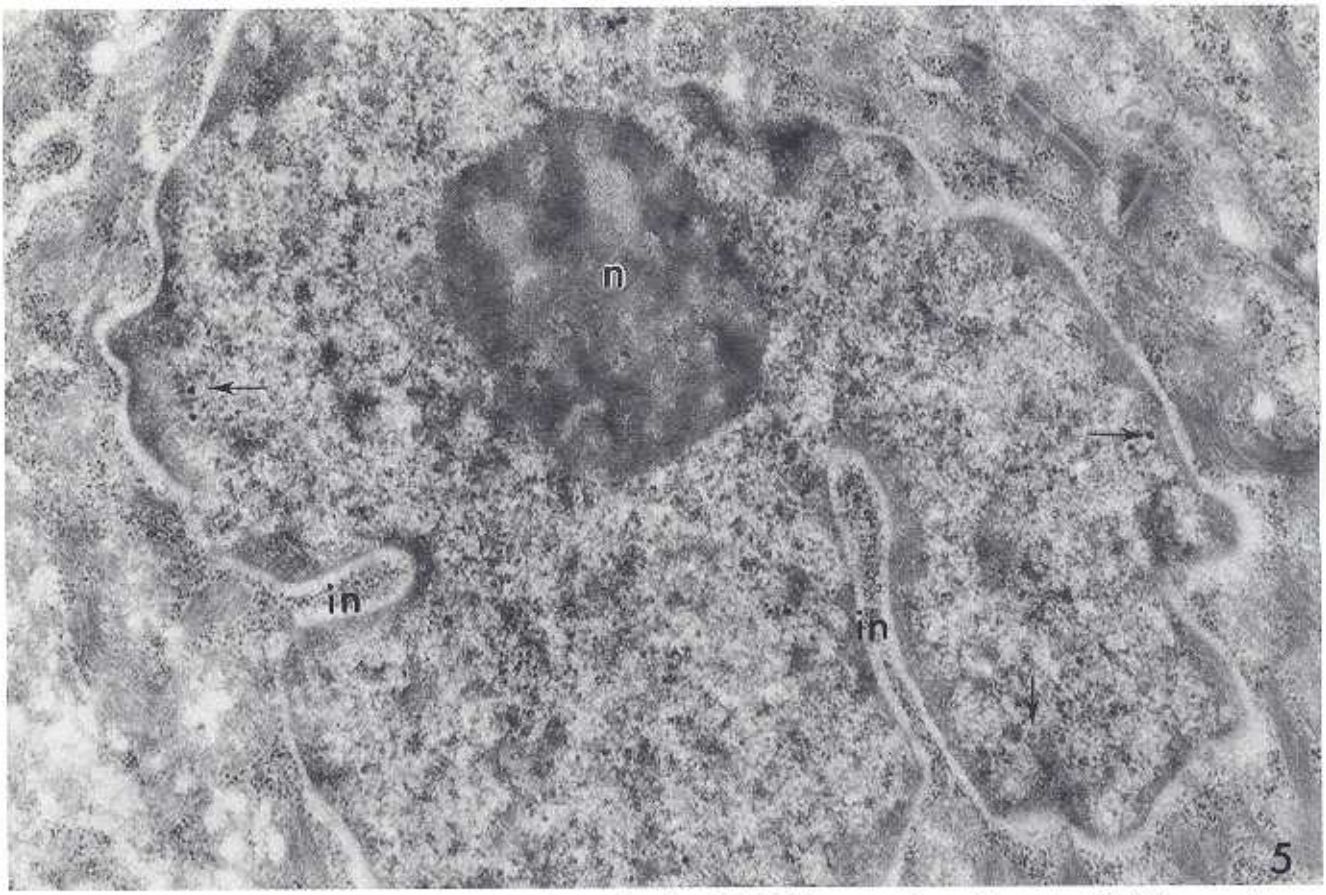

FIG. 5 *Nucleus of a severe dysplasia lesion stained with EDTA procedure. Abundant PCG* (arrows) *are located in the border of compact chromatin.* (n) *nucleolus,* (in) *profound invagination, ×25,000.*

thick) sections were discarded before a new round of thin sections was prepared. Photographs of cells located in the basal, middle, and superficial layers were taken at a fixed magnification (×12,500). In invasive carcinomas, the strata of the epithelium could be no longer recognized and the data of all nuclei were analyzed as a single group.

The numerical density of the perichromatin granules (Number of granules/Area of the nucleus) was estimated in electron micrographs at a magnification of ×35,000. The electron micrographs were digitized and calculations were performed using a personal computer provided with a digitizing board (Summasketch II, Summagraphics) and programs compiled in our laboratory. Surfaces were measured by traversing the boundary with a pointer. The program computed the area using the coordinates provided by the digitizing board as follows:

$$\text{Area} = \sum_{i=1}^{n} (X_{i+1} - X_i)(Y_{i+1} - Y_i)$$

where n is the total number of coordinates of points in the boundary, and i is the ordinal number of each point. The elemental trapezoid surfaces may be positive or negative.

Estimation of the relative volume of the compact chromatin was carried out by the areal fraction method [35], on sections stained with the PTA procedure. This fraction was obtained in each picture by dividing the sum of the areas of the masses of compact chromatin by the nuclear area. The areal fraction is an unbiased estimate of the fraction of the volume of the container (the nucleus) occupied by the content (the compact chromatin) [35]. In all cases, sections of nuclei presenting a diameter smaller than one-third of the average diameter (tangential sections) were not taken into account. Thirty electron micrographs of each type of cell from each patient were measured to estimate the statistical parameters.

Nuclear volume was determined by light microscopy using 1-μm-thick sections stained with toluidine blue. The largest and smallest diameters were measured by means of a micrometer eye piece, an oil immersion objective (×100), and an intermediate magnification lens. All calculations were performed with the aid of a personal computer. At least 60 nuclei of each type of cell from every patient were measured to estimate the statistical parameters of each group.

Estimation of the significance of the differences was carried out using the Student t test.

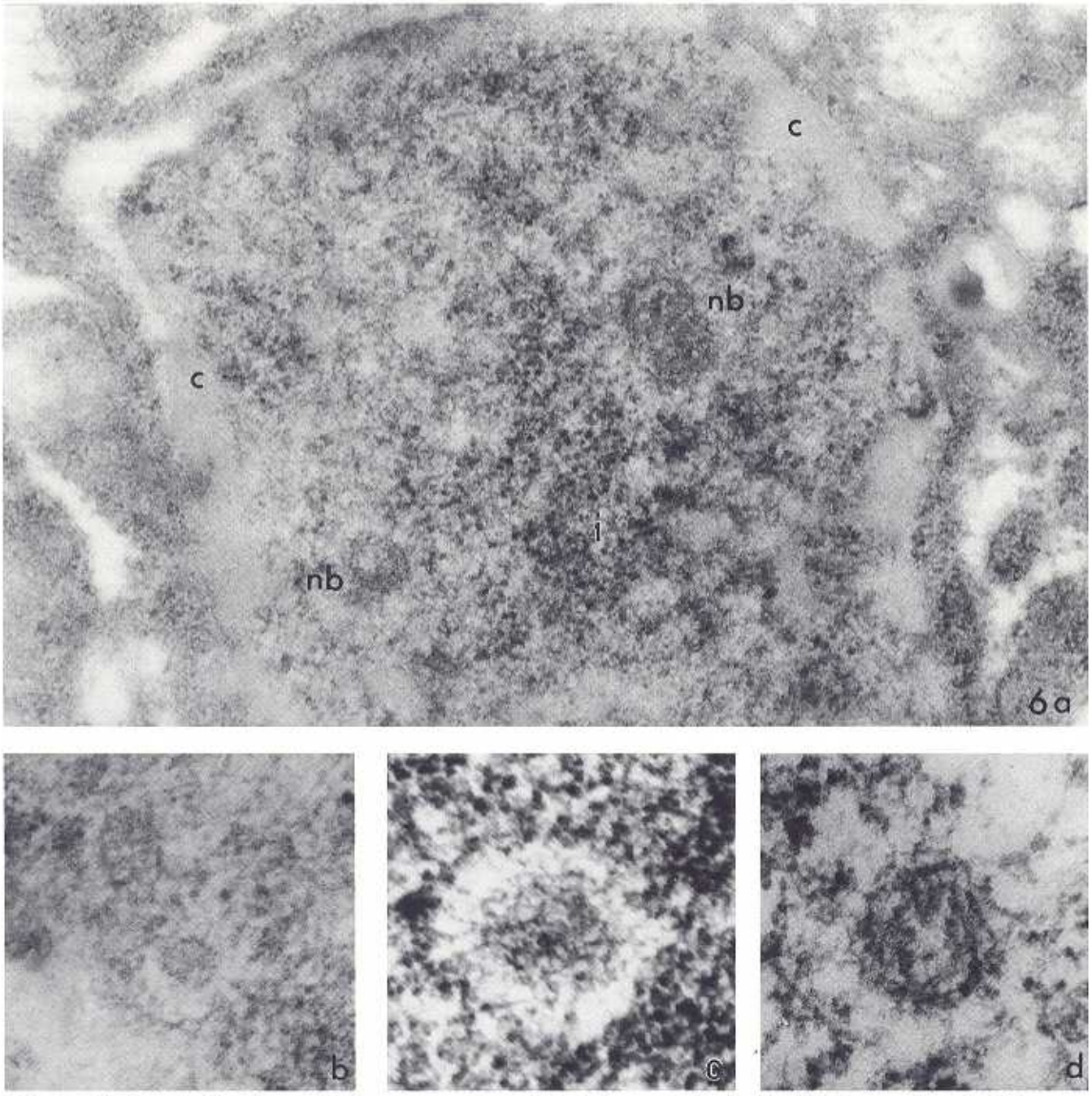

FIG. 6 *Nuclear bodies in nuclei from severe dysplasia lesions stained with EDTA procedure.* (a) *Two nuclear bodies of the type I showing positive stained fibrils* (nb). *Compact chromatin* (c) *is clear gray, clusters of interchromatin granules* (i) *are darkly stained, ×44,000.* (b) *Type I nuclear body. Very small peripheral granules are stained darker than the fibrils, ×90,000.* (c) *Type I nuclear body. The dark-stained fibro-granular material forming the body is continuous with gray fibrils crossing the clear halo to contact very dark stained granules surrounding the nuclear body, ×67,500.* (d) *Type II body composed by concentrically arranged EDTA positive fibro-granular material, ×69,000.*

RESULTS

Nuclear Ultrastructure

The nuclei of the cells of the basal and intermediate strata in the normal epithelium are elongated or spheroidal. Most of them have a discontinuous peripheral layer of compact chromatin, perichromatin granules located at the periphery of compact chromatin, clumps of interchromatin granules, and large nucleoli (Figure 1). Some of the basal cell nuclei are rich in compact chromatin and perichromatin granules (Figure 2). Nuclear bodies are in-

frequent in the nucleus of any type of normal cell. The PTA procedure shows abundant small masses of compact chromatin in contact with the nuclear envelope and distributed in the nucleoplasm (Figure 3).

The alterations in the ultrastructural features of dysplastic nuclei are described in basal and intermediate cells of the epithelium. Alterations in neoplastic lesions are better studied without reference to cell location.

The structure of the nuclei of the basal cells reveals the earliest dysplastic changes. In mild dysplasia, most of the nuclei of the basal layer present a thin, discontinuous peripheral layer of compact chromatin. RNP-containing structures are abundant and are dispersed in the interchromatin region. The RNP-containing fibrils and the perichromatin granules, which are normally found in the surrounding compact chromatin, appear distributed in the enlarged interchromatin. Most interchromatin granules are scattered, but small clumps can be observed in some sections. Type I nuclear bodies are frequently seen. Nuclear shape is altered by invaginations of the nuclear envelope (Figure 4). Nuclei with abundant compact chromatin, few RNP particles, and a small nucleolus are less numerous than in normal basal cells.

In moderate and severe dysplasia, these alterations become more intense. Nuclei with a small amount of compact chromatin become progressively less frequent, and nucleoli are more numerous and are larger than those of normal basal cells. The nuclei of polyhedral cells of the intermediate stratum of the epithelia of mildly dysplastic lesions are closely similar to the normal ones. However, sometimes perichromatin granules appear more widely dispersed by the interchromatin space than in normal cell nuclei. In moderate dysplasia, the invaginations of the nuclear envelope are prominent and frequent. The PCG are mainly located around the masses of compact chromatin, but some of them are dispersed in the nucleoplasm (Figure 5). Severe dysplasia is characterized by complex and profound invaginations of the nuclear envelope, and ample distributed perichromatin granules, one or several compact or nucleolonemal nucleoli, and frequent nuclear bodies (Figure 6).

Type I nuclear bodies composed of fibrillar material surrounded by a clear halo are common in cervical dysplasia and carcinoma. The fibrils of the nuclear body consistently stain with the EDTA procedure preferential for RNP-containing structures (Figure 6a–c). Type II nuclear bodies, formed by concentrically arranged fibrils darkly

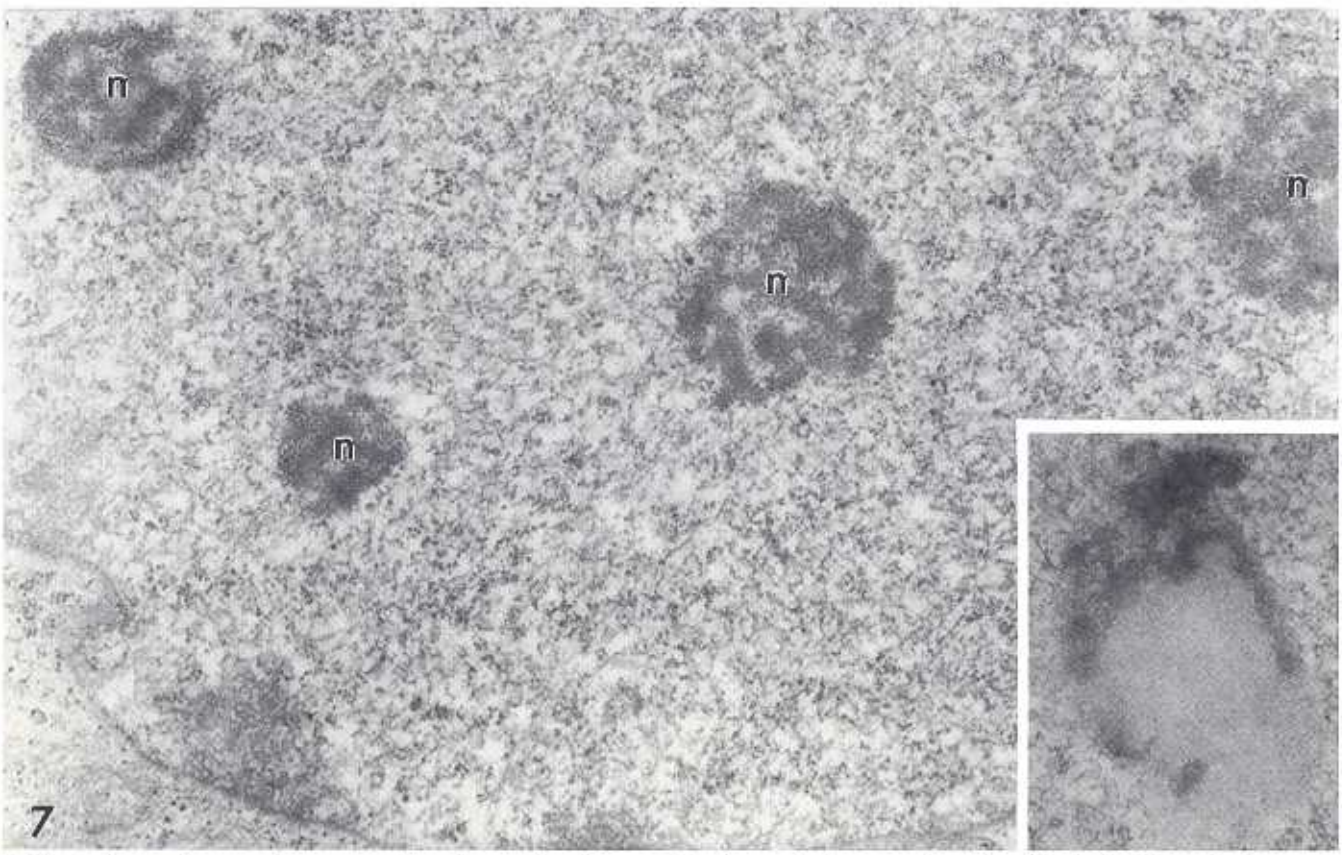

FIG. 7 *Invasive carcinoma cell nucleus showing multiple nucleolonemal nucleoli* (n). *EDTA staining, ×31,000. The inset shows a highly altered nucleolus formed by remains of fibrillar and granular components partially surrounding a large compact chromatin clump stained clear gray, ×26,500.*

stained by the EDTA procedure, were also observed (Figure 6d).

The nuclei in cells of carcinoma in situ are pleomorphic, and invaginations of the nuclear membrane can be so marked that they cause lobation of nuclei. Nuclear bodies are numerous and compact chromatin is frequently abundant. The perichromatin granules are distributed throughout the nuclear space, aggregates of interchromatin granules are conspicuous, RNP-containing fibrils are abundant, and types I and II nuclear bodies are frequent.

The nuclei in invasive cancer are characterized by a marked increase in pleomorphism, extreme nuclear membrane invaginations, and more than one nucleolus. Nuclear bodies are abundant, and multiple nucleoli with numerous fibrillar centers, large granular regions, and alterations in RNP distribution are common (Figure 7). Variations in the frequency of perichromatin granules, RNP fibrils, and compact chromatin produce marked changes among the cells. Some have large nuclei with few masses of compact chromatin and numerous RNP-containing structures other than PCG (Figure 8), while other nuclei are smaller with abundant masses of compact chromatin and few RNP other than PCG components (Figure 9).

Morphometry

Estimation of nuclear volume of the cells in the different strata of the normal cervical epithelium reveals that the flat cells of the superficial layer have significantly larger nuclei than polyhedral cells of the middle stratum and basal cells ($p < .001$) (Figure 10). In mild dysplasia, the nuclei of superficial cells are still significantly larger than those of basal cells ($p < .01$). In more advanced dysplasia and in carcinoma in situ, the differences disappear and the dispersion of data becomes larger. In well-differentiated invasive carcinomas, two populations with different nuclear sizes are clearly distinguished (Figure 11). One population of cells, which we name group A, have significantly larger nuclei than those in group B ($p < .01$).

If the nuclear volume of all strata of the epithelium of each patient is averaged, it can be seen that the nuclei of cells of dysplastic lesions and carcinoma in situ are larger than those of normal cells.

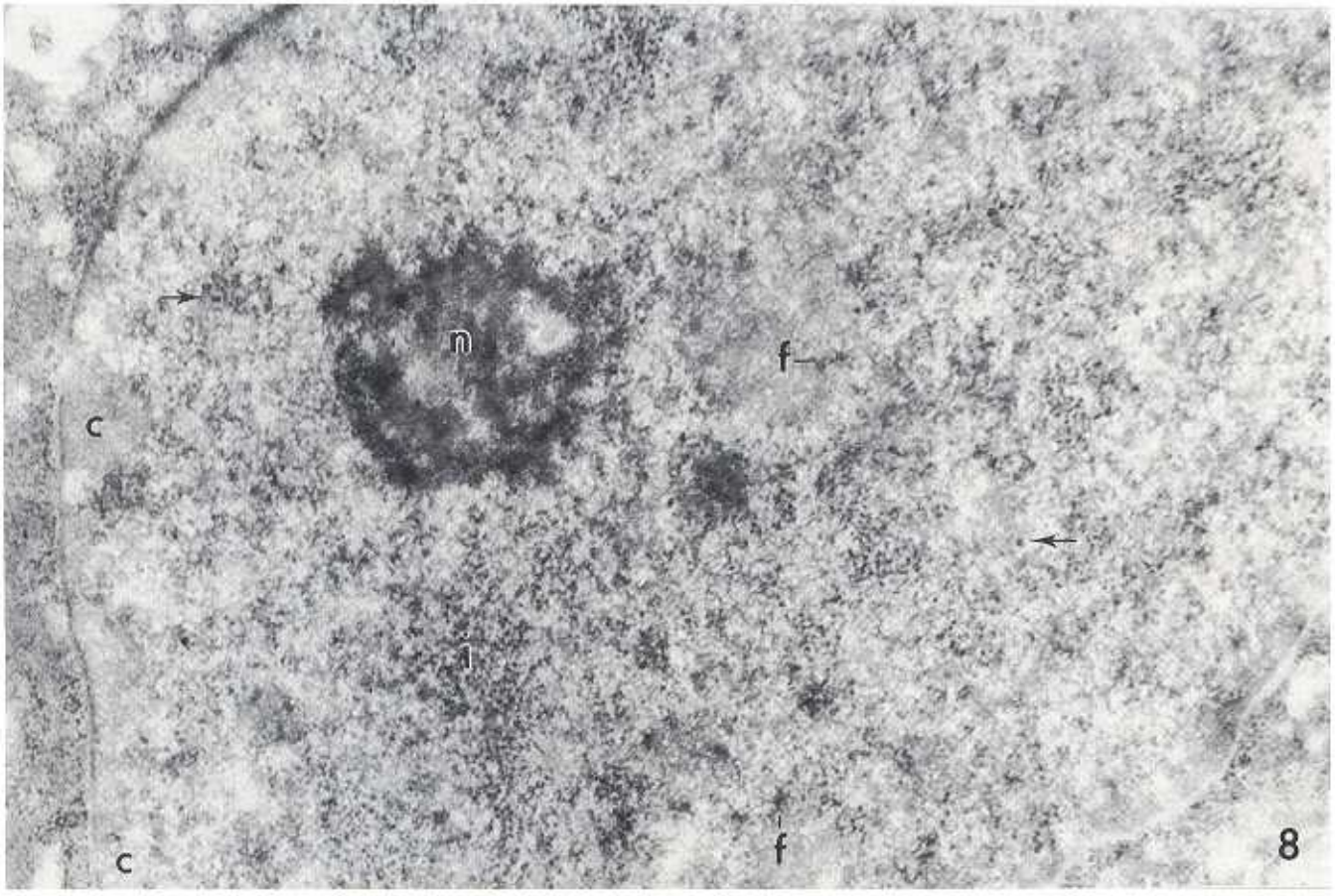

FIG. 8 *Invasive carcinoma cell nucleus stained with EDTA procedure. Isolated small masses of compact chromatin* (c) *are in contact with the nuclear envelope. RNP-containing particles are abundant in the nucleoplasm, but the perichromatin granules* (arrows) *are scarce.* (i) *interchromatin granules,* (f) *perichromatin fibrils,* (n) *nucleolus, ×31,000.*

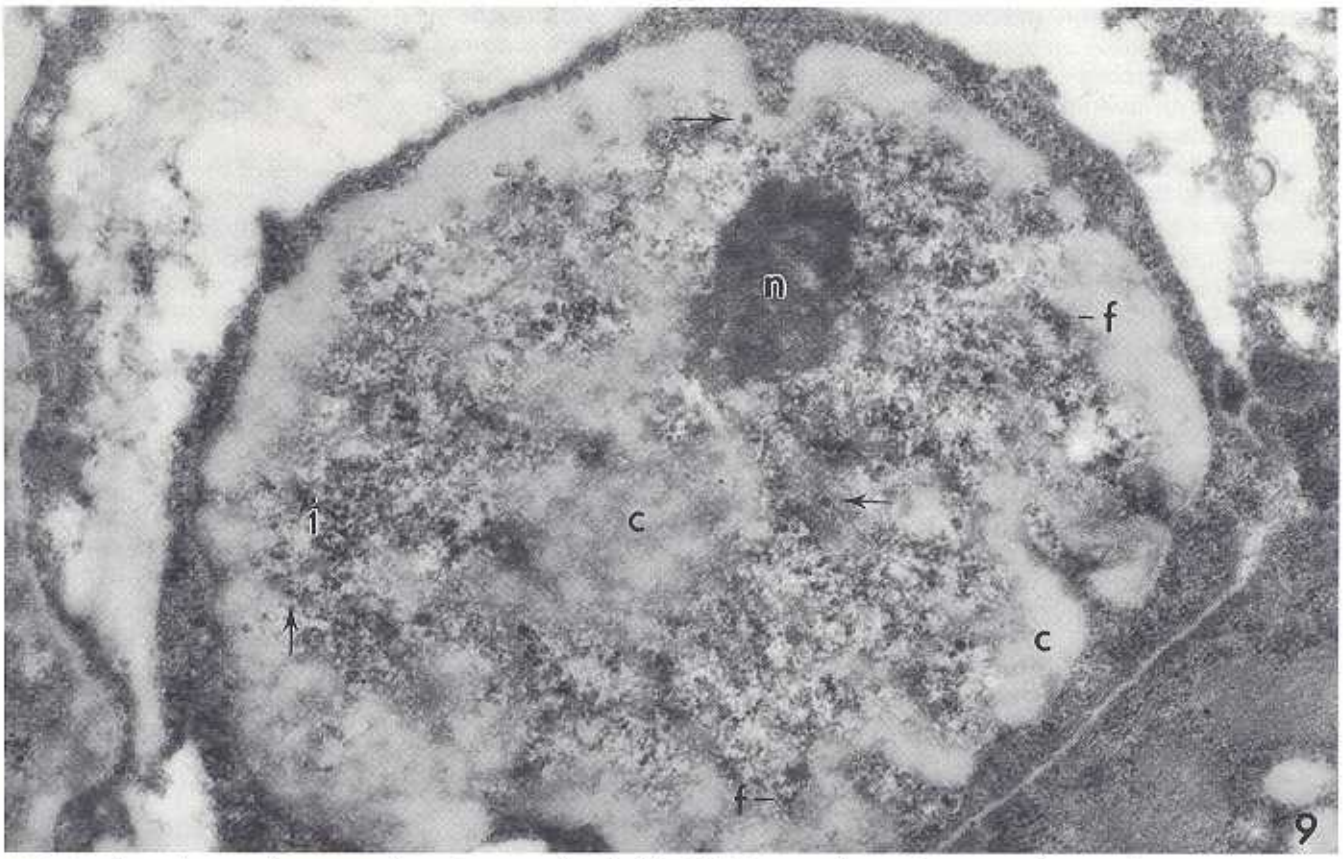

FIG. 9 *Invasive carcinoma cell nucleus stained with EDTA procedure. Compact chromatin masses stained clear gray* (c) *form a thick layer in contact with the nuclear envelope and also occupy a large part of the nucleoplasm. Interchromatin granules* (i) *and perichromatin fibrils* (f) *are scarce. Numerous PCG can be seen in the limit of the compact chromatin* (arrows). (n) *nucleolus, ×32,500.*

Both populations of cells of well-differentiated invasive carcinoma had smaller nuclei than the cells of carcinoma in situ.

Analysis of the numerical density of PCG in normal epithelia shows that the nuclei of the superficial cells are richer in these RNP structures than nuclei of the other strata ($p < .001$) (Figure 12). In dysplastic lesions, the cells of the different strata do not display significant differences ($p > .05$). The dispersion of data increases and the frequency of the granules decreases in carcinoma in situ (Figure 12). The frequency histogram of the numerical density of PCG in cells of invasive carcinoma lesions shows a bimodal distribution, indicating that these cells can be grouped in two populations with differing numerical densities of the granules (Figure 13). The two populations also show different nuclear sizes corresponding with the groups mentioned in the preceding paragraph. Cells in group B are richer in perichromatin granules than those of group A ($p < .001$).

The relative abundance of compact chromatin in the nuclei is estimated as the fraction of the nuclear area occupied by the total area of the masses of compact chromatin. If this parameter is assessed as an average of all cells of the epithelium, no significant differences can be found between normal epithelium and dysplastic lesions ($p > .01$) (Figure 14). The cells of intraepithelial carcinomas show significantly less compact chromatin and a larger dispersion of data (Figure 15). Evaluation of the fraction of the nuclear area occupied by compact chromatin in each stratum of the epithelium reveals that the basal cells have the highest concentration while the polyhedral cells of the middle layer have the lowest. This pattern of frequency of compact chromatin disappears in dysplastic and carcinomatous lesions (Figure 15).

The variability of the parameters studied in the nuclei of invasive carcinomas prompted a study of the correlation between nuclear volume, fraction of area occupied by compact chromatin, and the numerical density of PCG. This study revealed that group A cells with large nuclei (Figure 11) correspond to the group of cells with nuclei poor in PCG (group A in Figure 12), and to those cells with few masses of compact chromatin (group A in Figures 14 and 15). The group of cells that have small nuclei (group B in Figure 11) corresponds to those cells rich in PCG (group B in Figure 12), and to the cells

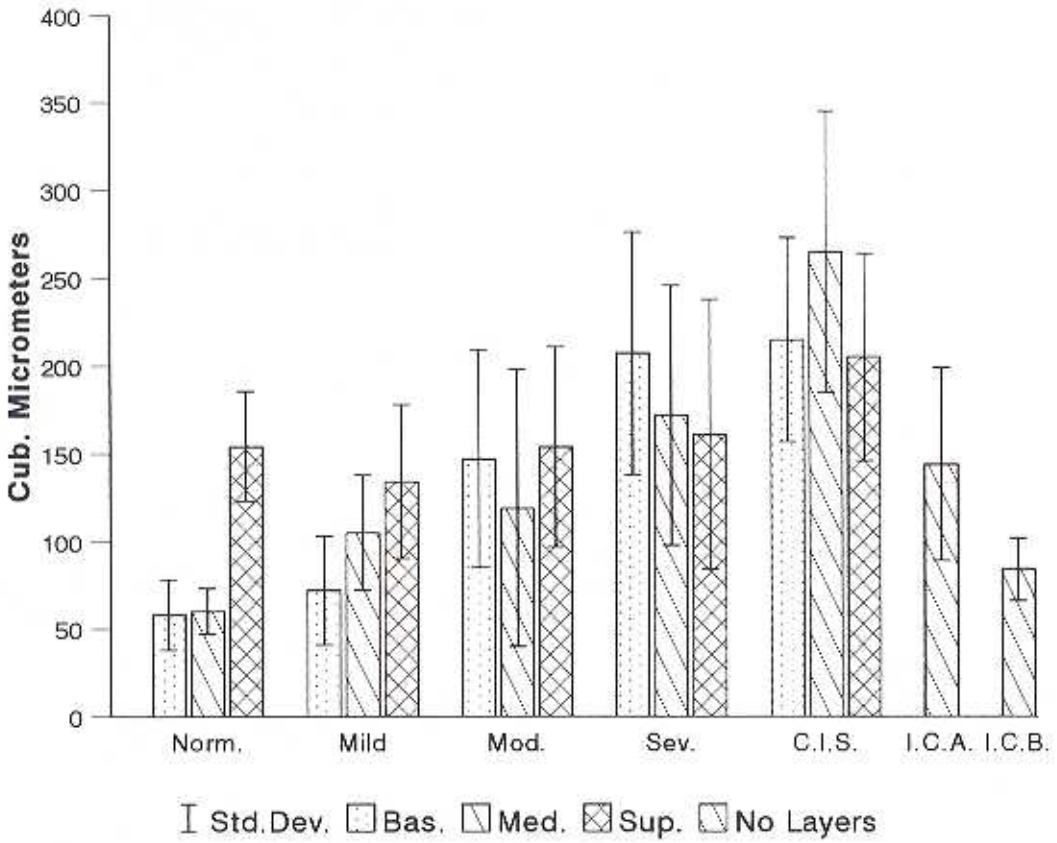

FIG. 10 *The differences in nuclear volume between superficial cells and cells of other layers can be seen in the normal epithelium. This pattern is only partially conserved in mild dysplasia, and has completely disappeared in the other types of lesions. Norm, normal epithelium; Mild, mild dysplasia; Mod, moderate dysplasia; CIS, carcinoma in situ; ICA, invasive carcinoma cell group A; ICB, invasive carcinoma cell group B; Std Err, standard error; Std Dev, standard deviation; Bas, basal cell; Med, intermediate cells; Sup, superficial cells; No Layers, in the samples of invasive carcinoma the altered cells were put together in one group regardless of their position in the epithelium.*

with abundant clumps of compact chromatin (group B in Figures 14 and 15).

DISCUSSION

Our results show that several nuclear features are altered in different stages of carcinoma-precursor lesions and in intraepithelial and invasive carcinomas. The changes are increases in the invagination of the nuclear membrane and in the size, structure, and number of nucleoli; altered distribution of RNP-containing extranucleolar structures; and less obvious quantitative variations such as augmentation of the number of nuclear bodies. Related changes include nuclear volume and the abundance of compact chromatin and PCG. The normal gradient of these parameters due to the continuous differentiation of the basal cells to polyhedral and to flat superficial cells becomes altered.

Our electron microscopic observations are in accordance with classical light microscope results [3, 4, 36] and previous ultrastructural studies of cancer cells [30]. Invaginations of the nuclear envelope are deeper and more complex in severe dysplasia than in normal cells or in mild dysplasia. The folds in the nuclear envelope cause the formation of nuclear lobules in invasive cancer cells. Besides these well-known alterations, study of the RNP-containing structures using a preferential staining method demonstrates alterations in the distribution of these structures in the early stages of dysplasia. The presence in the interchromatin space of the perichromatin granules and fibers, which normally surround compact chromatin, increases from mild dysplasia to frank carcinoma. These changes in the non-nucleolar RNP-containing structures are accompanied by a decrease in the amount of compact chromatin and increased extended chromatin as demonstrated by the use of a preferential staining method for DNA. The ensemble of the alterations in RNPs and chromatin are manifested in ultrastructural features that can be related to the

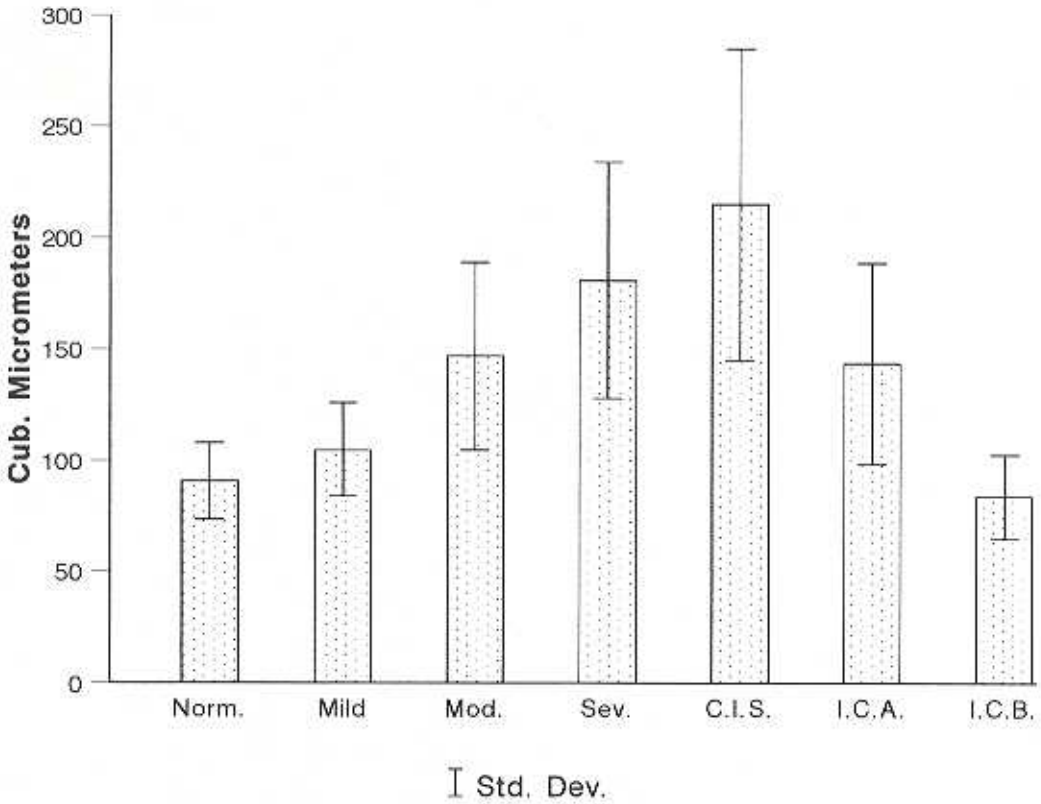

FIG. 11 *The nuclear volume of the ensemble of the cells of the epithelium increases between normal and carcinoma in situ. The cells of invasive carcinomas have nuclei smaller than those of the cells of intraepithelial carcinoma. See Figure 10 for abbreviations.*

progression of carcinoma-precursor to carcinomatous lesions.

Recent biochemical and morphological studies have shown that nuclear bodies are involved in acute promyelocytic leukemia. A protein (PML) related to the t(15;17) translocation of leukemic promyelocytes is bound to nuclear bodies, while the PML–Retinoic Acid Receptor fusion protein is scattered in multiple small nucleoplasmic clusters devoid of ultrastructural organization. Retinoic acid administration, which causes blast differentiation, results in the reaggregation of PML and other antigens onto the nuclear bodies [37]. In the present study, nuclear bodies were only found in small numbers in normal epithelial cells, but the frequency of nuclear bodies of types I and II increased sharply in severe dysplasia and in carcinoma. However, there was no elevation in the amount of coiled bodies. All the nuclear bodies studied contained fibrils and/or granules that stained darkly with the EDTA procedure, suggesting that type I and II nuclear bodies contain some RNP particles.

The variation in the amount of compact chromatin per nucleus in moderate and severe dysplasia correlates with the observation that hyperchromasia of dysplastic and carcinomatous cervical cell nuclei, which is due to polyploidism, can be found in only a percentage of cases [4]. Nuclear volume increases from normal nuclei to carcinoma in situ, but it is significantly lower in the cells of invasive carcinoma. These observations are in accord with measurements carried out using light microscopic preparations [38].

The normal epithelium presents a gradient of cell differentiation from basal cells to superficial cells and this process is accompanied by a sequence of variations in nuclear volume, frequency of perichromatin granules, and amount of compact chromatin. The flat cells of the superficial layers of the epithelium are terminally differentiated cells and mitosis occurs only in the basal layer. A high density of perichromatin granules is found in differentiated and functionally mature neurons [18, 19] and muscle cells [20], while their immature precursor cells contain significantly fewer granules. Changes in the number of perichromatin granules, nuclear volume, and amount of compact chromatin have been documented in defined stages of the differentiation of neurons and muscle cells [18–20]. In our study, we demonstrate that the superficial cells of normal cervical epithelium have significantly larger nuclei and a higher numerical density of perichromatin granules than the other layers, and this pattern was disrupted in all the lesions we studied. The sensitivity of this feature, which is evident in even the mildest preneoplastic alterations,

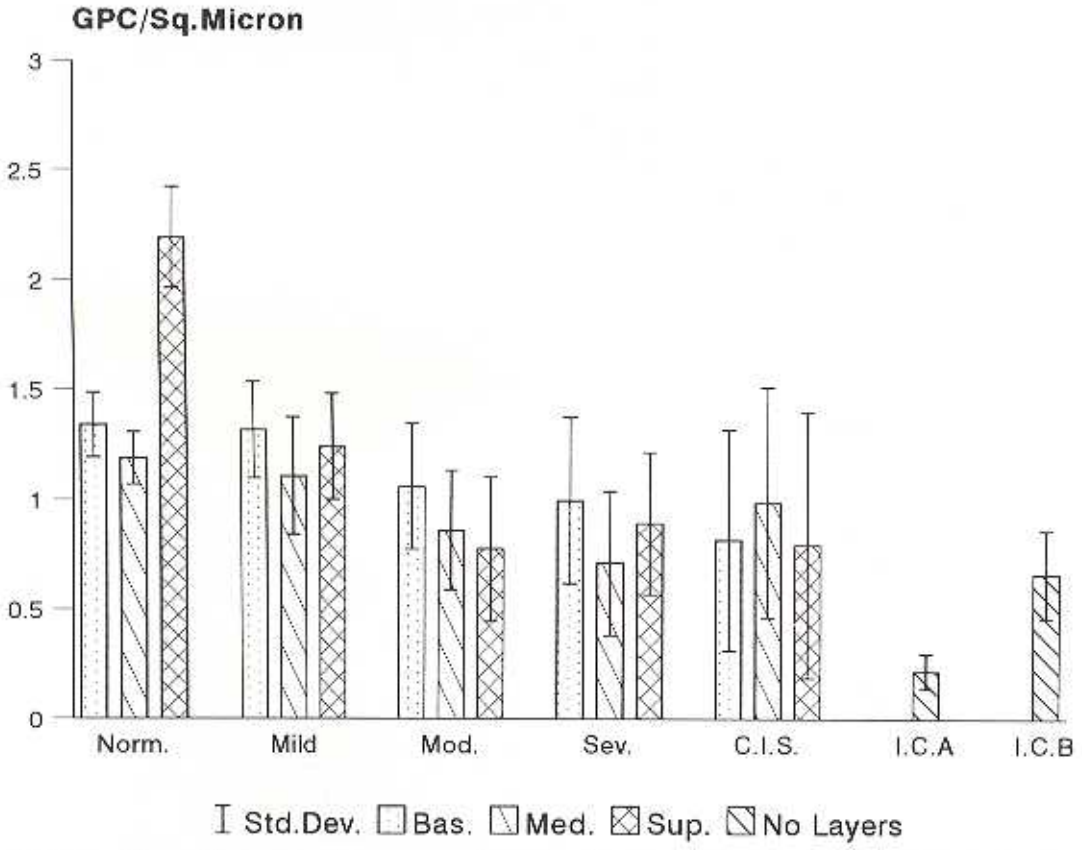

FIG. 12 *The difference of the numerical density of perichromatin granules between the superficial cells and the cells of the other layers of the normal epithelium is not conserved in non-normal epithelia. The standard deviations increase in severe stages of dysplasia and in the carcinoma in situ, indicating existence of cells in different cytophysiological states. In invasive carcinoma the cells in different stages of differentiation could be separated in two different populations* (group A and group B). *See Figure 10 for abbreviations.*

is probably related to early changes in the transcription, processing, and/or exportation of mRNA to the cytoplasm, which in turn alter the intranuclear storage of pre-mRNA or mRNA.

Alterations in the fraction of the nuclear volume occupied by compact chromatin do not correlate with the progressive changes occurring in the dysplastic cells. Furthermore, the nuclei of the intraepithelial carcinomatous cells have a smaller amount of compact chromatin than normal and dysplastic epithelial cells. Significant variations in the relative frequency of compact chromatin were found among patients who had similar dysplastic or carcinomatous lesions. These results correlate with findings in a detailed study of the DNA content of nuclei in different cervical cancer-precursor lesions [4]. The frequency of polyploid cells was not found to augment with the increasing severity of the lesion, and polyploid, euploid, and aneuploid DNA patterns could be found in lesions of the same degree of severity [4].

In the present study, two populations were distinguished among the cells of well-differentiated invasive carcinomas. Those in our group A had large nuclei with sparse compact chromatin and abundant RNP-containing structures, but few PCG. Group B was composed of cells with small nuclei, abundant compact chromatin, sparse RNP-containing particles, and numerous PCG. The morphologic and morphometric characteristics of these groups indicate that the cells of group A are relatively undifferentiated, comparable to blast cells in embryonic development. The features of the cells of group B correspond to morphologically and functionally mature cells such as the flat cells in the superficial layer of the epidermis. It is noteworthy that in electron micrographs of material stained with the EDTA procedure for RNP particles, the cells with abundant PCG have fewer RNP-containing structures (perichromatin fibrils and interchromatin granules) than those with few PCG. This observation rules out the possibility that the increased number of PCG could be due to a general increase in extranucleolar transcription.

Since perichromatin granules are composed

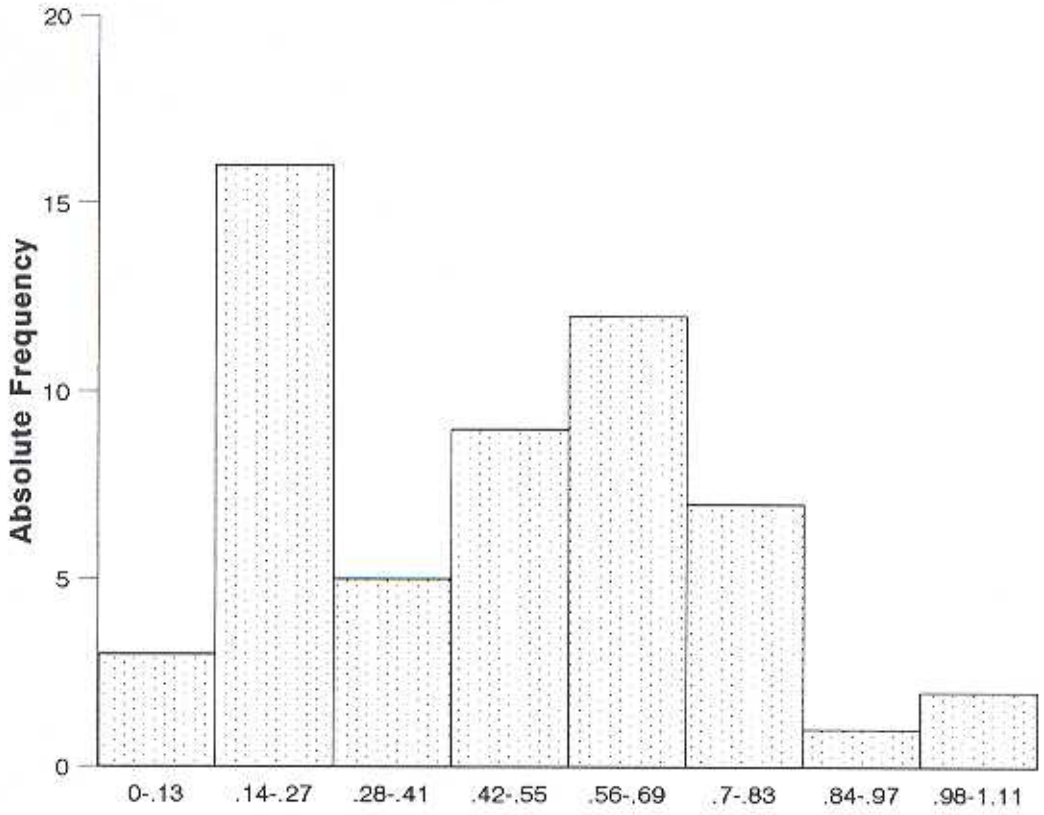

FIG. 13 *The bimodal distribution of the frequency of cells with different numerical density of PCG reveals the existence of two cell populations.*

of mRNA and associated proteins, the changes in the number of perichromatin granules are due to modifications in the rates of transcription and exportation of mRNA to the cytoplasm [12, 13]. Stem cells, such as matrix cells of the embryonic nervous system and myoblasts, have very few perichromatin granules [18, 20]. These observations suggest that in certain cytophysiological states, most mRNAs are not stored inside the nucleus as mRNA-containing granules. The sharp increase in the number of perichromatin granules observed during embryonic development in motoneurons [18, 19] and in skeletal muscle [20] are simultaneous with synaptogenesis. Evidently, it is the acquisition of mature functional features, and not exit from the mitotic cycle or commencement of the synthesis of special proteins, that brings about a modification in the relative rate of transcription and exportation of some gene products, causing the intranuclear storage of specific mRNAs. Collectively, these results strongly suggest that the two populations of cells revealed in invasive carcinoma correspond to mature and immature cells.

REFERENCES

1. Guerra G, Banaza S, Morales V. *Anuario Estadístico.* México: Secretaría de Salud, Dirección General de Epidemiología; 1990.
2. Scully ER. Definition of precursors in gynecologic cancer. *Cancer.* 1981;48:531–537.
3. Robbins S. *Patología humana.* México: Iberoamericana; 1990.
4. Fu SY, Reagan WJ, Ralph MR. Definition of precursors. *Gynecol Oncol.* 1981;12:220–234.
5. Monneron A, Bernhard W. Fine structural organization of the interphase nucleus of mammalian cells. *J Ultrastruct Res.* 1969;27:266–288.
6. Fakan S, Puvion E. The ultrastructural localization of nucleolar and extranucleolar RNA synthesis and distribution. *Int Rev Cytol.* 1980;65:255–299.
7. Puvion E, Moyne G. In situ localization of RNA structure. In: Busch H, ed. *The Cell Nucleus.* New York, E.U.A: Academic; 1981;8:59–115.
8. Bachellerie JP, Puvion E, Zalta JP. Ultrastructural organization and biochemical characterization of chromatin RNA-protein complexes isolated from mammalian cell nuclei. *J Microsc.* 1975;126:77–85.
9. Fakan S, Puvion E, Spohr G. Localization and characterization of newly synthesized nuclear RNA in isolated hepatocytes. *Exp Cell Res.* 1976;99:155–164.
10. Fakan S, Leser G, Martin T. Ultrastructural distribution of nuclear ribonucleoproteins as visualized by immunocytochemistry in thin sections. *J Cell Biol.* 1984;98:358–363.
11. Fakan S, Leser G, Martin T. Immunoelectron microscope visualization of nuclear antigens within spread transcription complexes. *J Cell Biol.* 1986;103:1153–1157.
12. Vázquez-Nin GH, Bernard W. Comparative ultrastructural study of perichromatin and Balbiani ring granules. *J Ultrastruct Res.* 1971;36:842–860.
13. Vázquez-Nin GH, Echeverría OM, Fakan S, Leser G, Martin T. Immunoelectron microscope localization of snRNPs in the polytene nucleus of salivary glands of *Chironomus thummi. Chromosoma.* 1990;99:44–51.
14. Vázquez-Nin GH, Echeverría OM, Molina E, Fragoso J. Ef-

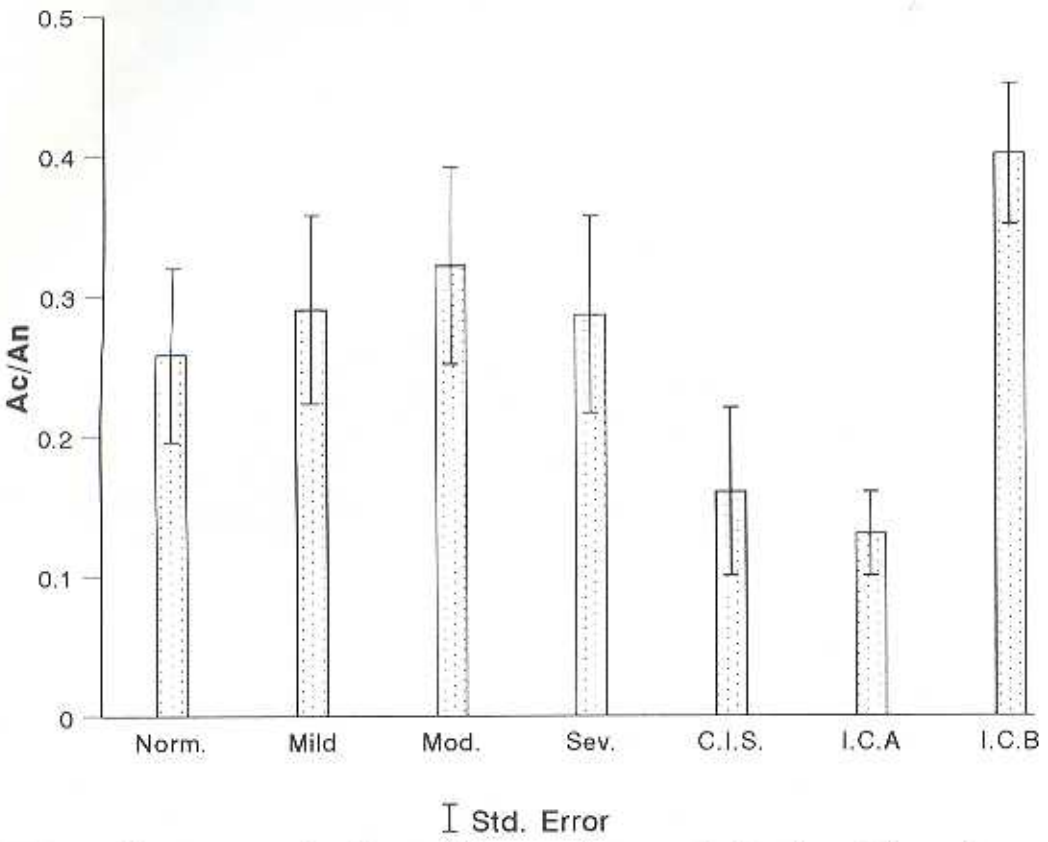

FIG. 14 *There are no significant differences between the fraction of the nuclear space occupied by compact chromatin in normal epithelial cells and in dysplastic epithelial cells. The two groups of cells found in invasive carcinoma present very different amounts of compact chromatin. Ac, area of compact chromatin; An, nuclear area. See Figure 10 for other abbreviations.*

fects of ovariectomy and estradiol injection on nuclear structures of endometrial epithelial cells. *Acta Anat.* 1978; 102:308–318.
15. Vázquez-Nin GH, Echeverría OM, Pedron J. Effects of estradiol on the ribonucleoprotein constituents of the nucleus of cultured endometrial epithelial cells. *Biol Cell.* 1979;35:221–228.
16. Echeverría OM, Zavala G, Benítez A, Vázquez-Nin GH. Changes during estral cycle in the nucleus of endometrial cells of the rat. *Biol Cell.* 1980;39:139–142.
17. Echeverría OM, Pagán-Santini R, Vázquez-Nin GH. Effects of testosterone on nuclear ribonucleoprotein components of prostate epithelial cells. *Biol Cell.* 1991;72:223–229.
18. Vázquez-Nin GH, Ortega-Rangel JA, Echeverría OM. Nuclear aspects of neuroblast differentiation in the chick embryo. *Biol Cell.* 1980;39:143–146.
19. Vázquez-Nin GH, Ortega-Rangel JA, Echeverría OM, Parra MR, Jiménez-García LF. Changes in nuclear ribonucleoprotein constituents and chromatin disposition during neuronal differentiation and maturation. *Biol Cell.* 1983;48:17–24.
20. Zavala G, Aguilar X, Jiménez-García LF, Echeverría OM, Vázquez-Nin GH. Changes in the ribonucleoprotein constituents of the nucleus during the differentiation of muscle cells in the chick embryo. *Biol Cell.* 1992;76:159–165.
21. Krishan A, Uzman B, Hedley-Whyte ET. Nuclear bodies: a component of cell nuclei in hamster tissues and human tumors. *J Ultrastruct Res.* 1967;19:563–572.
22. Bouteille M, Kalifat SR, Delarue J. Ultrastructural variations of nuclear bodies in human diseases. *J Ultrastruct Res.* 1967;19:474–486.
23. Popoff N, Stewart S. The fine structure of nuclear inclusions in the brain of experimental golden hamsters. *J Ultrastruct Res.* 1968;23:347–361.
24. Baines JD, Jacob RJ, Simmerman L, Roizman B. The herpes simplex virus 1 UL11 proteins are associated with cytoplasm and nuclear membranes and with nuclear bodies of infected cells. *J Virol.* 1995;69:825–833.
25. Bernhard W. A new procedure for electron microscopical cytology. *J Ultrastruct Res.* 1969;27:250–265.
26. Andrade LEC, Chan EKL, Raska Y, Peebles CL, Roos G, Tan EM. Human autoantibody to a novel protein of the nuclear coiled body: immunological characterization and cDNA cloning of p80-coilin. *J Exp Med.* 1991;173:1407–1419.
27. Raska Y, Andrade LEC, Ochs RL, et al. Immunological and ultrastructural studies of the nuclear coiled body with autoimmune antibodies. *Exp Cell Res.* 1991;195:27–37.
28. Frey MR, Matera G. Coiled bodies contain U7 small nuclear RNA and associate with specific DNA sequences in interphase human cells. *Proc Natl Acad Sci USA.* 1995; 92:5915–5919.
29. Moreno Díaz de la Espina S, Sánchez-Pina A, Risueño C, Medina FJ, Fernández-Gómez E. The role of plant coiled bodies in the nuclear RNA metabolism. *Electr Microsc.* 1980;2:240–241.
30. Bernhard W, Granboulan N. The fine structure of cancer cell nucleus. *Exp Cell Res.* 1963;9(Suppl):19–53.
31. Bernhard W. Ultrastructure of the cancer cell. In: Lima de Faria A, ed. *Handbook of Molecular Cytology.* Amsterdam: North Holland; 1969:687–715.
32. Bonfiglio TA, Stoler MH. Papillomavirus and cancer of the uterine cervix. *Hum Pathol.* 1988;19:621–622.

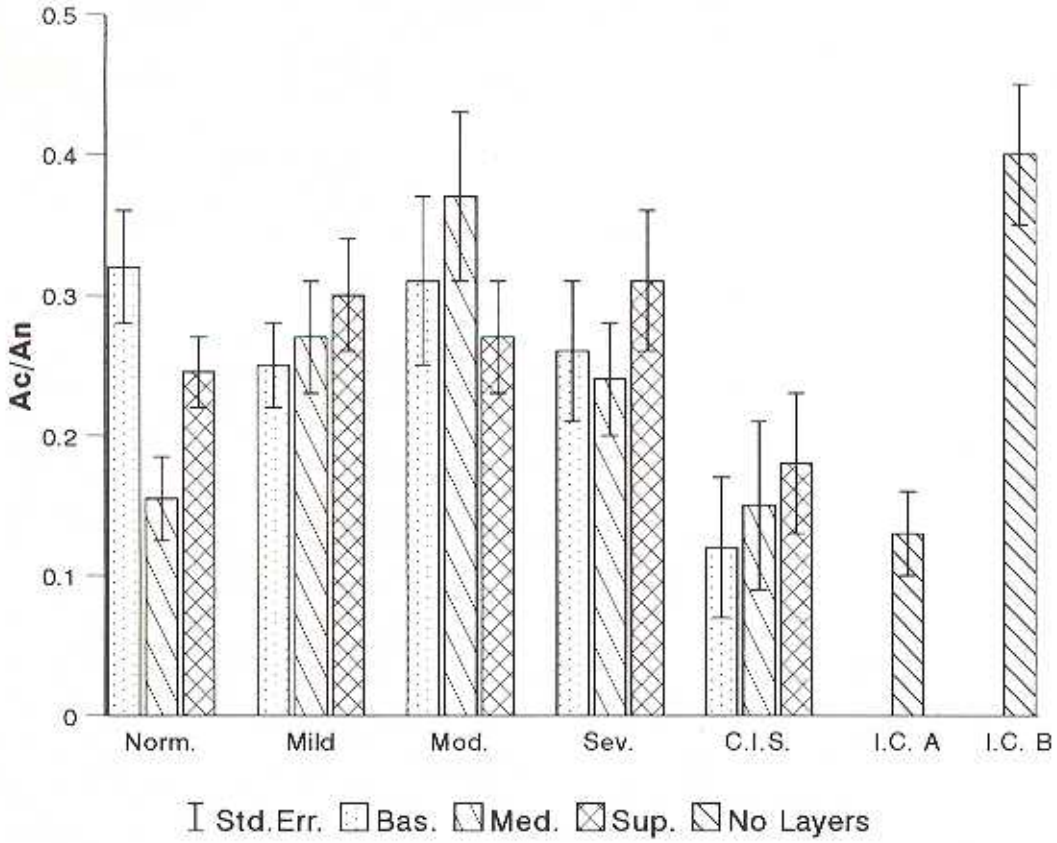

FIG. 15 *In the normal epithelium, the nucleus of the basal cells presents the highest content of compact chromatin and that of intermediate cells the lowest. This pattern of compact chromatin distribution is altered in dysplastic and carcinomatous lesions. See Figure 10 for abbreviations.*

33. Storey A, Pim D, Murray A, Osborn K. Comparison of the in vitro transforming activities of human papillomavirus types. *EMBO J.* 1988;6:1815–1820.
34. Vázquez-Nin GH, Chávez B, Tomás-Martin C. A preferential method for chromatin in electron microscopy. *J Microsc.* 1973;16:243–246.
35. Underwood E. *Quantitative Stereology.* London: Addison–Wesley; 1970.
36. Balanguero-Lladó L. *El Carcinoma in Situ del Cuello Uterino.* Barcelona: Espaxs; 1971.
37. Kokan MHM, Puvion–Dutilleul F, Guillermin MC, et al. The t(15;17) translocation alters a nuclear body in a retinoic acid-reversible fashion. *EMBO J.* 1994;13:1073–1083.
38. Hillemanns HG, Prestel E. Karyometrie mit dem Teichengrossenanalysator (TGZ) am beispiel des Cervixcarcinoms und seinen *Vorstufen Z Krebsforch.* 1968;71:316–319.