Squamous differentiation in carcinoma *in situ* of the cervix uteri

A cyto-histological correlation of malignant intraepithelial lesions with invasive carcinoma

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SYNOPSIS   Cervical biopsies from 97 women with malignant cells in cervical scrape smears have been studied. Forty-eight patients had invasive squamous carcinoma, and 49 had intraepithelial lesions. Of these, six had dedifferentiated carcinoma *in situ*, nine had the differentiated lesions generally known as 'severe dysplasia', and 29 had both; the severity of the dysplasia remained doubtful in five patients.

The 'severe dysplasia' was compared with invasive carcinoma. A cellular analysis of the biopsies showed that the two categories have a number of features in common, chiefly the presence of atypical and normal mitoses, nucleoli, horn cells, and giant cells. The stratification of the epithelium in 'severe dysplasia' is invariably abnormal and the architecture closely resembles that of invasive carcinoma. The cells in the smears from 'severe dysplasia' are similar to those in invasive carcinoma.

It is suggested on the basis of these observations that a 'severe dysplasia' should be interpreted as a differentiated carcinoma *in situ*.

The concept of carcinoma *in situ* of the cervix uteri has been accepted for some sixty years, but no agreement exists on the histological appearances of the lesion.

The aim of the present study is to compare the morphological appearances of intraepithelial lesions of the cervix in scrape smears and histological sections with those of invasive carcinoma, special attention being directed to intraepithelial lesions with squamous differentiation.

MATERIALS AND METHODS

The material derives from cases seen at the Royal Free Hospital between 1958 and 1964, where cytological examination revealed cells diagnostic or suggestive of malignancy.

The cytological preparations were cervical scrape smears processed by the Papanicolaou technique. They were obtained from patients with a clinical diagnosis of cervical carcinoma and from women attending the gynaecological clinics. The smears were rescreened.

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Ninety-seven cases with unequivocal 'malignant' cells in which adequate biopsy material was available were selected for study. A record was made of the variety of malignant cell types encountered in the smears, *e.g.*, differentiated squamous, partially differentiated, and dedifferentiated cells of squamous origin.

The quality of the biopsy material varied. In overt carcinoma punch biopsies taken before the first radium insertion were used. Where the presence of carcinoma was suggested by the cytological examination, a cone biopsy was done with some exceptions in the earlier years, when a punch or shallow ring biopsy was performed. Hysterectomy specimens were available in a proportion of the cases.

The histological material was fixed in 10% formol saline and the sections were stained with haematoxylin and eosin. The biopsies were studied afresh without reference to the original diagnosis. Special attention was directed to the quantity and arrangement of keratin, the presence of large partially differentiated cells, horn cells, cells with bizarre shaped large nuclei, and giant cells. The presence of nucleoli and of abnormal and normal mitoses and their position in the epithelium were noted.

Only those lesions in which the full thickness of the squamous epithelium was replaced by dedifferentiated cells were at first classified as carcinoma *in situ*; slight surface flattening was present in some. Intraepithelial

lesions displaying squamous differentiation were initially placed in the category of dysplasia and further subdivided into those that resembled squamous carcinoma and those that did not resemble squamous carcinoma and remained doubtful. Mild dysplasias are not included in the present study, as the result of the cytological examination was equivocal or negative. The cytological and histological appearances of the intraepithelial lesions were compared with those of invasive carcinoma.

RESULTS

The findings in the 97 cases are shown in Table I.

TABLE I

CERVICAL LESIONS (1958-1964)

Type of Cervical Lesion	Number of Cases
Carcinoma	48
Intraepithelial carcinoma	44
Doubtful intraepithelial carcinoma	5
Total	97

INVASIVE SQUAMOUS CARCINOMA Forty-eight cases of invasive squamous carcinoma were analysed. The tumours were divided into three groups. The 11 dedifferentiated tumours were either monomorphic (Fig. 4), or contained a mixture of small and slightly larger, round, oval or spindle-shaped cells. The 11 differentiated tumours were characterized by the presence of keratin (Fig. 12). Twenty-six tumours were partially differentiated and had a pattern midway between the two previous groups (Figs. 2, 6, 7, and 10). They contained a mixture of cell types. Small cells were found together with large cells, islands of prickle cells and scattered horn cells lay adjacent to islands of dedifferentiated cells, tightly packed spindle cells lay alongside areas of keratin.

One tumour with dedifferentiated and partially differentiated areas (Fig. 15) appeared morphologically to be intraepithelial, but the glands were involved to a depth of 7½ mm. from the ectocervical surface. Some doubt persisted about the invasive nature of this tumour which, however, is included in the group of partially differentiated invasive carcinoma (Reagan and Hicks, 1953).

The smears reflected the histological patterns. The dedifferentiated cells (Fig. 4 inset), whether small or medium sized, were mostly round or oval with poorly defined cell borders, grossly enlarged nuclei, and disordered chromatin arrangement. The differentiated tumours exfoliated bizarre-shaped cells of the type commonly referred to as tadpole, fibre, and snake cells. Most of these had clearly defined cell borders, dense cytoplasmic texture, and darkly staining nuclei. The moderately differentiated tumours exfoliated both types of cells and other cells of intermediate type and consequently presented the most pleomorphic picture (Figs. 2 and 9 inset).

INTRAEPITHELIAL CARCINOMA Forty-four lesions were considered malignant and five doubtful. A dedifferentiated carcinoma *in situ* (Fig. 3) was present in 35 cases but was accompanied by differentiated 'dysplastic' intraepithelial lesions (Figs. 1, 5, and 8) in 29 of these. Nine cases showed only differentiated 'dysplastic' lesions (Figs. 11 and 13). These 'dysplastic' lesions were considered malignant on the basis of an analysis of the cell elements in the biopsies (Table II) and because of their architectural resemblance to invasive carcinoma. They were finally classified under intraepithelial carcinoma in the mixed and differentiated groups.

The differentiation took various forms. In one variant, the entire thickness of the epithelium was replaced by abnormal cells of different shapes and sizes, arranged in a haphazard manner with numer-

TABLE II

CELLULAR ANALYSIS OF CERVICAL BIOPSIES

| | Carcinoma (48 cases) | | | Intraepithelial Lesions | | | |
| | | | | Carcinoma (44 cases) | | | |
	Dedifferentiated (11 cases)	Moderately Differentiated (26 cases)	Well Differentiated (11 cases)	Dedifferentiated (6 cases)	Mixed (29 cases)	Differentiated (9 cases)	Doubtful (5 cases)
Keratin	0	11	11	0	17	5	4[2]
Horn cells	2	20	11	0	17	7	4
Large cells	8	26	10	4	29	6	4
Small cells	8	19	6	5	27	5[1]	3
Giant cells	4	17	8	0	19	7	5
Normal mitoses	8	20	7	1	27	5	3
Atypical mitoses	9	21	8	2	26	6	1
Nucleoli	7	21	9	1	23	6	5

[1]In four of the five cases only scanty small cells were found.
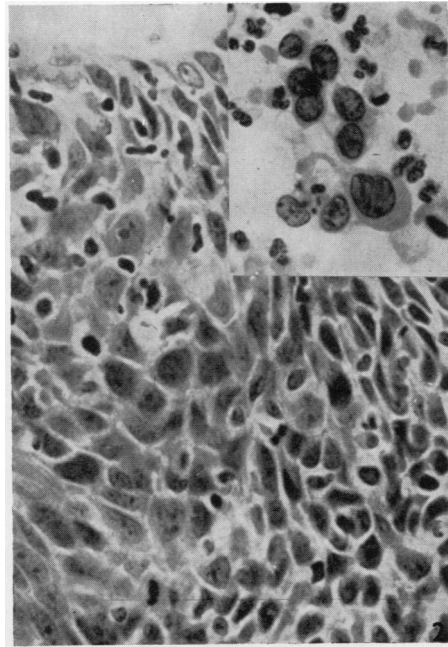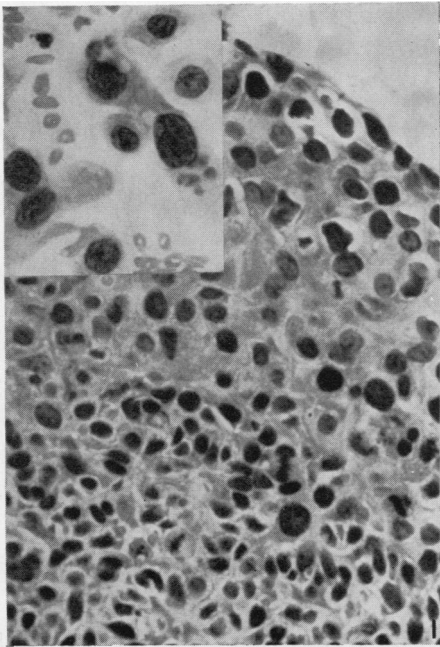[2]The amount of keratin in all the four cases was small.

FIG. 1. *Differentiated carcinoma* in situ. *Compare the cellular pleomorphism with that in Fig. 2.* (*Haematoxylin and eosin* × *360.*) *Inset: cells in a smear.* (*Papanicolaou* × *360.*)

FIG. 2. *Moderately differentiated invasive carcinoma.* (*Haematoxylin and eosin* × *360.*) *Inset: a mixture of dedifferentiated cells and one 'tadpole' cell in a smear.* (*Papanicolaou* × *360.*)
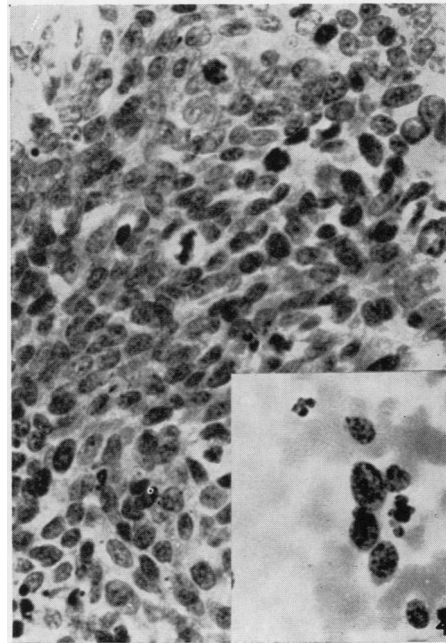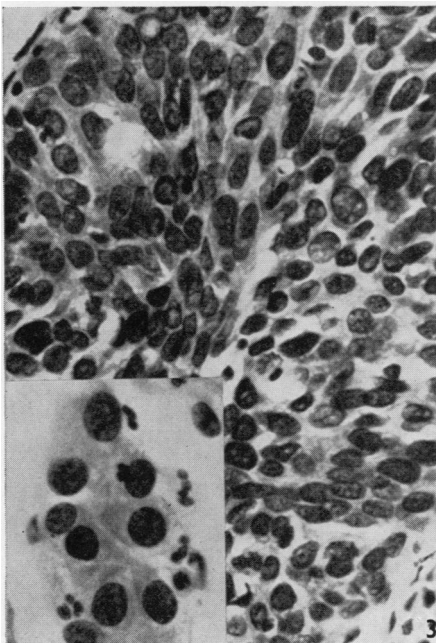

FIG. 3. *Dedifferentiated carcinoma* in situ. (*Haematoxylin and eosin* × *360.*) *Inset: cells in a smear.* (*Papanicolaou* × *360.*) *The cells both in the section and the smear resemble those in Fig. 4, but contain more cytoplasm.*

FIG. 4. *Dedifferentiated invasive carcinoma.* (*Haematoxylin and eosin* × *360.*) *Inset: cells in a smear.* (*Papanicolaou* × *360.*)
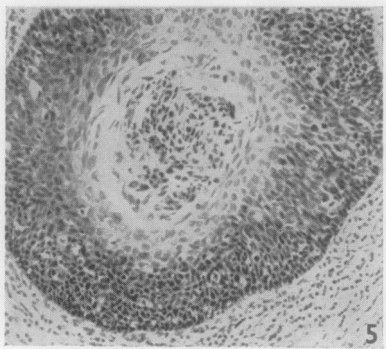
FIG. 5.   *Wide basal cell layer in an abnormally stratified intraepithelial carcinoma involving a gland. (Haematoxylin and eosin × 120.)*
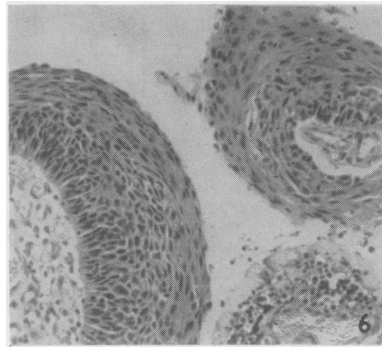
FIG. 6.   *Stratification in three layers in an invasive carcinoma. Compare with Fig. 5. (Haematoxylin and eosin × 120.)*
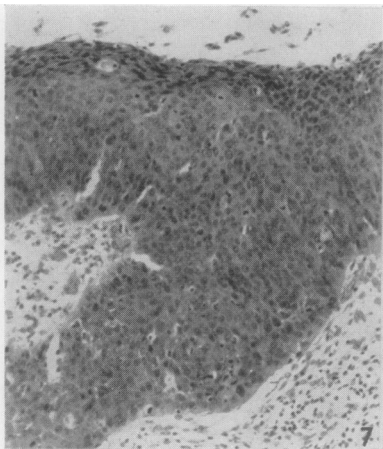

FIG. 7.   *Moderately differentiated invasive carcinoma composed of polygonal 'mid-zone' cells, scattered horn cells, and a marked surface zone of flattened cells. (Haematoxylin and eosin × 120.)*
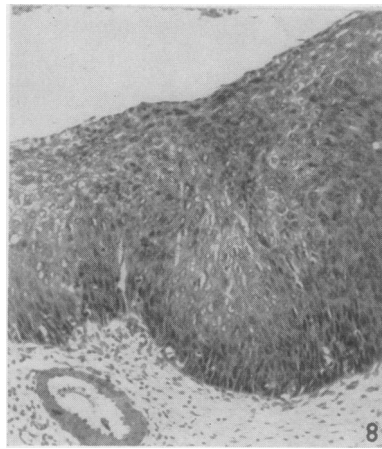
FIG. 8.   *Partially differentiated carcinoma* in situ *with a narrow surface zone of flattened cells. Compare with Fig. 7. (Haematoxylin and eosin × 120.)*

ous mitoses scattered throughout the epithelium. Even in this variant, the larger cells were concentrated in the superficial part of the epithelium in a semblance of stratification (Fig. 1 cf. Fig. 2). A second variety was characterized by a wide zone of polygonal cells and, distinctly demarcated on the surface, thin parakeratotic cells with dark, spindle-shaped nuclei. Normal superficial polyhedral cells were absent (Fig. 5 cf. Fig. 6). In the third variant, a normal looking basal layer was surmounted by a wide zone of parabasal cells. These were tightly packed and displayed a 'random swirling pattern' (Old, Wielenga, and von Haam, 1965). Parakeratotic cells constituted the superficial zone of the epithelium

(Fig. 8 cf. Fig. 7). A fourth variety displayed clearer differentiation and stratification. It contained a basal layer composed of cells with darkly staining upright nuclei, which in places extended into the overlying layer of larger and polygonal parabasal cells. There were elongated and flattened cells near the surface. The polygonal parabasal cells disrupted the basal layer in places and giant and multinucleated cells were present at various levels (Figs. 9 and 11, cf. Fig. 10). In the fully differentiated variety of carcinoma *in situ,* the abnormalities were most evident in the epithelium extending into gland clefts (Fig. 13). The random scattering of keratin, horn cells, and multinucleated giant cells was identical with that
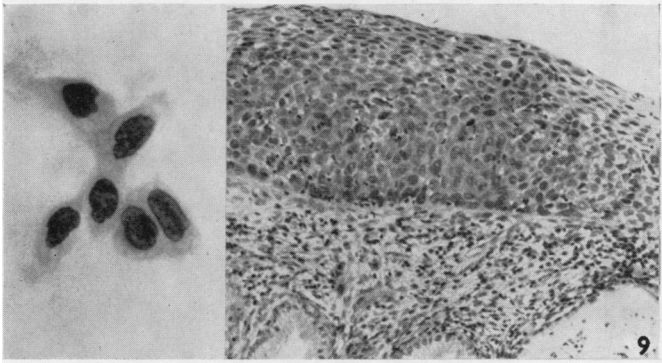
FIG. 9. *Differentiated carcinoma* in situ. *Note deep layer of round cells with pale nuclei and surface layer of flattened parakeratotic cells with dark nuclei. (Haematoxylin and eosin × 90.) Inset: cells in a smear. (Papanicolaou × 360.)*
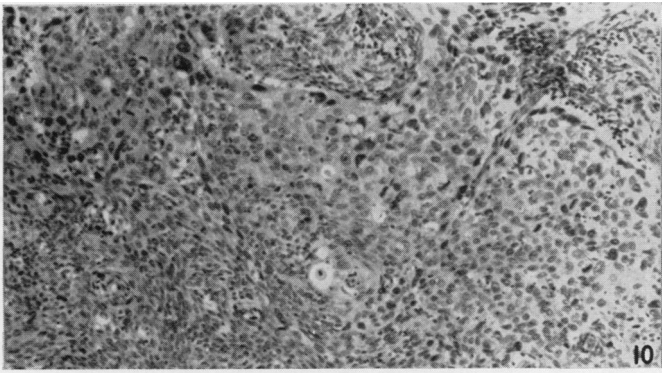

FIG.10. *Moderately differentiated invasive carcinoma from deep part of the case illustrated in Fig. 9. Note the two cell types, which were also present in the surface intraepithelial carcinoma. (Haematoxylin and eosin × 120.)*
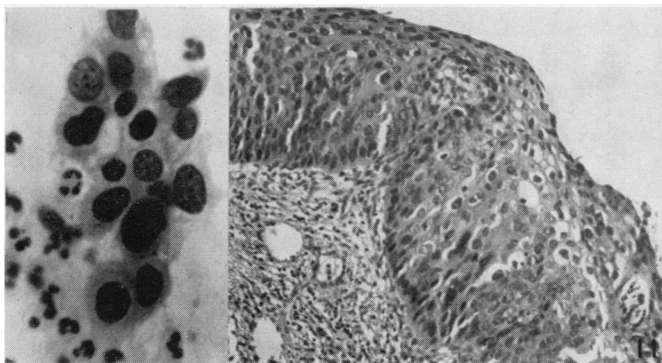

FIG. 11. *Differentiated carcinoma* in situ. *(Haematoxylin and eosin × 90.) Inset: cells in a smear. (Papanicolaou × 360.) Note the similarities with the lesions in Figures 9 and 10.*

seen in well-differentiated invasive carcinoma (Fig. 12).

Benign basal cell hyperplasia seen in the gland clefts and lining epithelium of the cervical canal in healing erosions (Meyer, 1941a) was distinguished by the well-organized architecture of the epithelium and the normal morphology of the constituent cells (Fig. 14).

TABLE III

HISTOLOGY OF CERVICAL CARCINOMA[1]

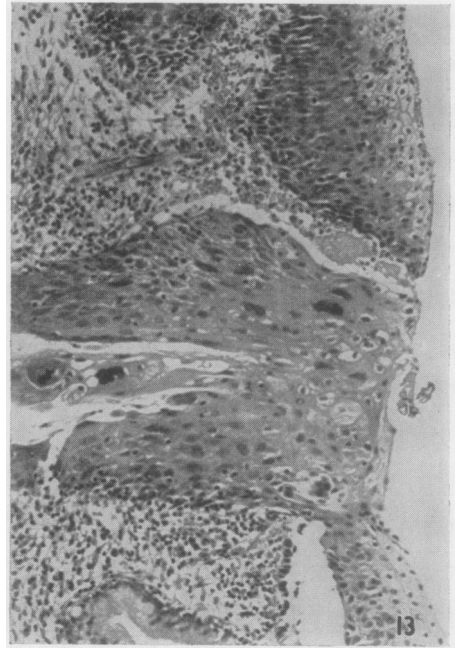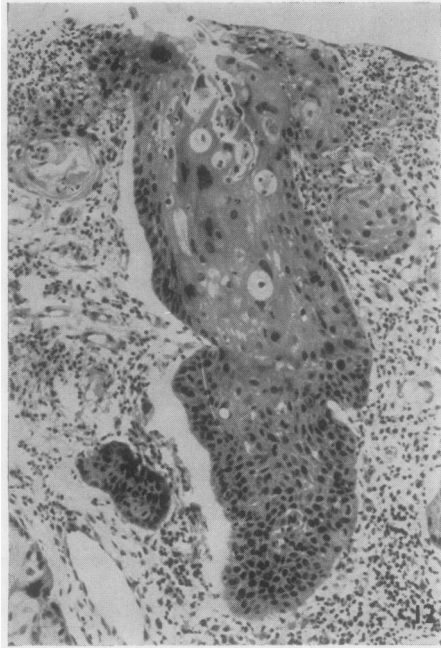| *Invasive Carcinoma (48 Cases)*		*Intraepithelial Carcinoma (44 Cases)*	
Dedifferentiated	11	Dedifferentiated	6
Moderately differentiated	26	Mixed	29
Well differentiated	11	Differentiated	9

[1]Doubtful cases are not included.

FIG. 12. *Well-differentiated invasive carcinoma with prickle cells, horn cells, giant cells, and keratin. Compare with the differentiated intraepithelial carcinoma in Fig. 13.* (*Haematoxylin and eosin* × *90.*)

FIG. 13. *Differentiated carcinoma* in situ *extending into a gland cleft, indistinguishable frcm the well-differentiated invasive carcinoma in Fig. 12.* (*Haematoxylin and eosin* × *90.*)
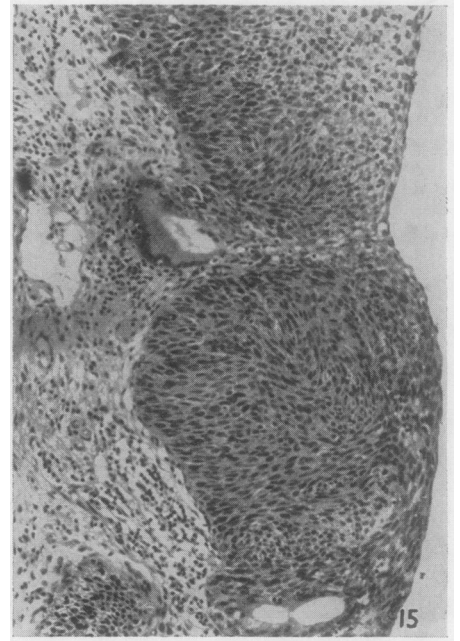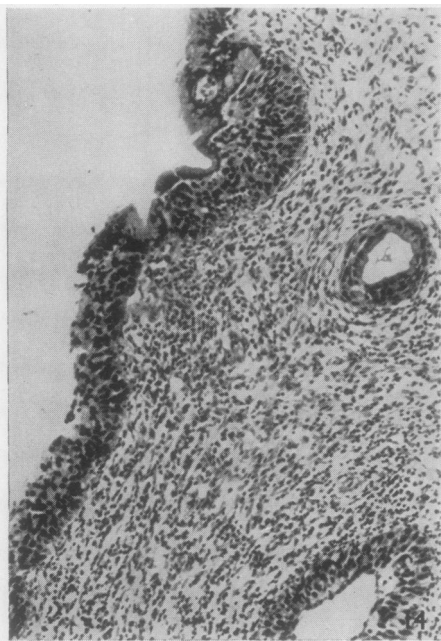

FIG. 14. *Hyperplasia of subcylindrical basal cells in the cervical canal and adjacent glands in a healing erosion.* (*Haematoxylin and eosin* × *90.*)

FIG. 15. *Dedifferentiated and partially differentiated areas in the tumour which involved glands to a depth of 7·5 mm. and represents the one doubtful case of invasive carcinoma.* (*Haematoxylin and eosin* × *90.*)

The frequency of the three groups of intraepithelial carcinoma paralleled that of the three groups of invasive carcinoma (Table III).

DISCUSSION

The biological behaviour of any one case of carcinoma *in situ* of the uterine cervix may be uncertain (Petersen, 1955; Peckham and Green, 1957; Old and Jones, 1965), but in view of the inability of the pathologist to recognize the lesions that will progress to invasion and metastasize, adequate treatment of all patients with carcinoma *in situ* is essential to 'prophylaxis'. In 1910, Rubin stated that 'unless we can decide upon the determining features of the diagnosis of a cancerous epithelium, it is evident that we may never hope to improve our prophylactic therapy for carcinoma'.

The definition of carcinoma *in situ* by the International Committee on Histological Terminology for Lesions of the Uterine Cervix (1962) as 'surface epithelium in which, throughout its thickness, no differentiation takes place' except possibly for some slight flattening on the surface, is obviously unacceptable to several workers (Fluhmann, 1961; Koss and Durfee, 1961; de Brux and Dupre-Froment, 1965; Old *et al.*, 1965). These workers extend the concept of carcinoma *in situ* to include some lesions with squamous differentiation, lesions which, according to the International Committee's definition, fall into the category of dysplasia. The present study stresses the need to remove these lesions from the group of 'dysplasia' with its overtones of relative benignity, into that of carcinoma *in situ*. We agree with Koss and Durfee's (1961) suggestion that *in situ* epidermoid carcinoma is best defined as an alteration of surface epithelium which histologically resembles cervical carcinoma. This would also appear to have been the opinion of Schiller (1933), Meyer (1941*b*), and Ewing (quoted by Hertig and Young, 1952).

A good correlation exists between the cytological and histological findings in invasive carcinoma of the cervix (Reagan and Moore, 1952; Graham, 1963; Wachtel, 1964) and in the dedifferentiated carcinoma *in situ* (Reagan and Hamonic, 1956). In the so-called dysplasia, confusion arises between a positive cytological diagnosis and apparently negative subsequent histological findings. In our study 48 invasive carcinomas and 49 intraepithelial lesions were found to have 'malignant' cells in the cervical smears. An analysis of the biopsies has shown that intraepithelial lesions with varying degrees of squamous differentiation have a number of cellular features in common with keratinizing and moderately differentiated squamous carcinoma, chiefly the presence of atypical and normal mitoses and nucleoli scat-

tered through the epithelium, horn cells, and giant cells with bizarre or multiple nuclei. The stratification is invariably abnormal. A study of the histological pattern of these lesions reveals similarity to invasive carcinoma to a degree which makes a distinction between the two difficult. In addition, the malignant cells seen in the smears from these cases resemble those seen in cases of moderately differentiated carcinoma. Both abnormal and normal mitoses are encountered with comparable frequency in invasive carcinomas and in differentiated intraepithelial lesions, whereas they were present in only two out of six cases of dedifferentiated carcinoma *in situ*. Similar observations are reported by Reagan, Seidemann, and Saracusa (1953) who found that atypical mitoses are uncommon in carcinoma *in situ* but are more often seen in the lesion they term dysplasia. Old *et al.* (1965) found that mitotic activity may be 'singularly sparse or unrecognizable' in their gamma or basaloid form of carcinoma *in situ*.

Numerous mitoses and particularly atypical forms are generally considered to be a significant feature of intraepithelial carcinoma (Hertig and Young, 1952; Haines and Taylor, 1962). Recent studies have shown chromosomal abnormalities in dysplasia as well as in carcinoma *in situ* and in invasive cervical carcinoma (Spriggs, Boddington, and Clarke, 1962; Wakonig-Vaartaja and Kirkland, 1965). Kirkland (1966) reports that chromosomal abnormalities are reflected in abnormal mitoses and has suggested that lesions containing such mitoses should be diagnosed as carcinoma *in situ* regardless of the apparent overall pattern. If atypical mitoses, which feature prominently in invasive carcinoma, are significant, many lesions diagnosed as severe dysplasia must qualify as carcinoma *in situ*.

We cannot provide data about the biological behaviour of the differentiated intraepithelial lesions studied, such as has been gathered by Fennel (1956), Peckham and Green (1957), Koss and Durfee (1961), and Old and Jones (1965). The group of carcinoma *in situ* should, however, include, apart from the dedifferentiated lesions, lesions with squamous differentiation which look malignant on cytological and histological examination. This would help to rationalize the aims of the current cytological screening programmes and possibly to avoid withholding treatment in a group of women at risk.

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