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<https://escholarship.org/uc/item/64v0h180>

Journal

International Journal of Gynecological Pathology, 3(1)

ISSN

0277-1691

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Publication Date

1984

DOI

10.1097/00004347-198403010-00007

Peer reviewed

Evaluation of Squamous Epithelium in Adenoacanthoma and Adenosquamous Carcinoma of the Endometrium: Immunoperoxidase Analysis of Involucrin and Keratin Localization

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Summary: A study was undertaken to determine whether immunoperoxidase stains for keratin and involucrin, the latter a protein present in cells of stratified squamous epithelium that have differentiated beyond the basal stage, distinguish any differences in squamous cells present in the adenoacanthoma from those in the adenosquamous carcinoma of the uterine corpus. Forty-eight tumors were studied, of which 33 were adenoacanthomas and 15 adenosquamous carcinomas. The patients with adenoacanthomas were slightly younger (mean 61.5 vs. 64.5 years) and had tumors that were generally better differentiated than the adenosquamous carcinomas. The squamous epithelium in every tumor, regardless of histologic type, stained positively for keratin. There were no obvious differences in staining when tumors were stratified for histologic type, grade, or location within the tumor. The glandular portion of both tumor types stained irregularly, but nonetheless positively, for keratin in 71% of the cases. Involucrin was detected in 57% of adenoacanthomas and 87% of adenosquamous carcinomas. The deeper or more central portion of the squamous morules stained only if the more superficial or peripheral areas were positive. The extent of the involucrin staining was less in the adenosquamous carcinomas than in the adenoacanthomas. The glandular component of the tumors did not stain for involucrin. It is concluded that no qualitative differences in the staining reactions with respect to keratin and involucrin distinguish the adenoacanthomas from the adenosquamous carcinoma. These findings support the argument that the adenoacanthoma and adenosquamous carcinoma represent a spectrum of squamous differentiation in a single tumor type. **Key Words:** Adenoacanthoma—Adenosquamous carcinoma—Endometrium—Involucrin—Keratin—Immunoperoxidase analysis.

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The pathogenesis and biologic importance of squamous epithelial differentiation within endometrial adenocarcinomas (adenoacanthoma and adenosquamous carcinoma) have remained controversial. By current definitions adenoacanthoma refers to the presence of well-differentiated squamous cells with minimal nuclear atypia, usually proliferating within a gland lumen (morule) or on the surface of the endometrium (1,2). Adenosquamous carcinoma refers to a squamous component with a marked degree of cytologic atypia, or to any neoplastic squamous component, regardless of the degree of differentiation, that invades the endometrial stroma or myometrium in the absence of a malignant glandular component (1-3). According to one school of thought, the squamous component of the adenoacanthoma is metaplastic and has no bearing on the ultimate prognosis of the patient. This school designates the adenoacanthoma as "adenocarcinoma with squamous metaplasia"; another school contends that the squamous component in the adenoacanthoma is neoplastic and can act in an aggressive fashion (1,3,4). Most investigators feel that the squamous component in the adenosquamous carcinoma is malignant.

A relatively recent concept proposes that adenoacanthomas and adenosquamous carcinomas represent a spectrum of squamous differentiation of a single tumor ranging from well-differentiated to poorly differentiated neoplastic squamous elements (2,4); the degree of squamous cell differentiation is thought to generally parallel the degree of glandular differentiation. Consequently, to circumvent the subjectivity entailed in the interpretation of the degrees of "benignancy" or "malignancy" of the squamous element, endometrial tumors have been graded for prognostic purposes by evaluation of only the glandular component.

Recent applications of immunocytochemical techniques have been useful in the identification of cellular components or their secretory products as markers of both cellular differentiation and function. Two recently utilized markers of squamous differentiation are involucrin and keratin. Involucrin is a protein present in squamous cells that have differentiated beyond the basal stage (suprabasal differentiation). It is involved in the terminal differentiation of the keratinocyte (5) and is incorporated into the membrane "envelopes" which constitute the protective integument produced by involuting squamous epithelium. Both tissue culture and histologic studies of surgical biopsy specimens indicate that both dysplastic and malignant squamous cells have defective involucrin expression. Accordingly, this protein should be useful for the assessment of differentiation of squamous cells in endometrial neoplasms; the more highly differentiated neoplasms might be expected to contain more frequently demonstrable involucrin in comparison with more poorly differentiated squamous neoplasms in which involucrin should be rarely demonstrable or absent. Keratin is believed to be present in virtually all epithelial cells and in greater amounts in squamous tumors, primarily in the form of tonofilaments (6). The present study was therefore undertaken to investigate the patterns of involucrin and keratin expression in adenoacanthomas and adenosquamous carcinomas of the endometrium.

MATERIALS AND METHODS

All cases of adenoacanthoma and adenosquamous carcinoma recorded between

1976 and 1981 in the files of the Massachusetts General Hospital were retrieved and reviewed. All cases were used where the microscopical findings met the criteria of adenoacanthoma and adenosquamous carcinoma (2), paraffin blocks were available, and the tumor in the block measured at least 1×1 cm. If two or more biopsies or a hysterectomy specimen were available for a patient, the specimen with the greatest quantity of tissue showing squamous differentiation in the block was chosen. Utilizing all materials, 48 cases proved acceptable for study.

The methodology for the immunoperoxidase reactions for both keratin and involucrin has been described previously (7,8). In brief, sections were incubated for 1 h at room temperature with rabbit antisera against either keratin (dilution 1:100; obtained from Dakopatts, Santa Barbara, CA) or involucrin (dilution 1:1,000). The sections were then washed in buffer and incubated with antirabbit antisera (dilution 1:100). After another wash in buffer, the sections were incubated with peroxidase rabbit-antiperoxidase complexes (dilution 1:20; obtained from Accurate Chemical, Westbury, NY). Antibody localization was determined by reaction with diaminobenzidine, following which the sections were counterstained with methyl green.

The results of staining for involucrin and keratin were recorded as either present or absent in both glandular cells and in the squamous cells forming intraglandular morules. In the latter group, preferential staining of either peripheral or central areas of morules was noted; for purposes of comparison, they were grouped, respectively, as superficial or deep. Occasionally, adenoacanthomas may form sheets of squamous cells near the endometrial surface. The cells exposed to the endometrial cavity were recorded as superficial cells; those adjacent to the glandular portion of the tumor were recorded as deep.

The histologic type of tumor was based on hematoxylin and eosin staining and was determined independently from the results of immunoperoxidase staining for involucrin and keratin. The tumors were graded according to a modification of the Broders system, the details of which are given elsewhere (9). In general, the Broders grade 1 or 2 refers to a tumor that is well differentiated and corresponds to a tumor of grade 1 by the FIGO method. Grade 3 of Broders is a moderately differentiated tumor with both glandular differentiation and solid areas, corresponding to FIGO grade 2. Broders grade IV designates a poorly differentiated tumor growing in solid sheets usually without glandular differentiation; this grade corresponds to FIGO grade 3.

RESULTS

The 48 tumors with materials suitable for immunohistochemical staining consisted of 33 adenoacanthomas and 15 adenosquamous carcinomas (Table 1). The mean age of the patients with adenoacanthomas was 61.5 years, which was 3 years younger than the mean age of patients with adenosquamous carcinoma ($p = \text{NS}$). The adenoacanthomas were generally better differentiated with a mean grade of 2 in contrast to the mean grade 3 of the adenosquamous carcinomas ($p < 0.001$).

The squamous epithelium in all the tumors stained diffusely for keratin (Figs. 1-4). The intensity of the stain was similar in the morules, the surface squamous

TABLE 1. Immunohistochemical staining of 48 tumors, 33 adenoacanthomas, and 15 adenosquamous carcinomas

	Adenoacanthoma	Adenosquamous carcinoma
Number	33	15
Age range (yr)	31-88 (mean 61.5)	41-89 (mean 64.5)
Grade 1	11	0
2	18	2
3	5	11
4	0	2
Keratin staining		
Squamous epithelium	33/33	15/15
Glandular epithelium	24/33	10/15
Grade 1	10/11	—/—
2	11/18	2/2
3	3/5	7/11
4	—/—	2/2
Involucrin staining		
Squamous epithelium		
Superficial	20/33	13/15
Grade 1	5/11	—/—
2	10/17	2/2
3	5/5	9/11
4	—/—	2/2
Deep	15/33	11/15
Grade 1	5/11	—/—
2	7/17	2/2
3	3/5	8/11
4	—/—	1/2
Glandular epithelium	0	0

epithelium, and in the squamous component of the adenosquamous carcinomas. The glandular portions of both the adenoacanthomas and adenosquamous carcinomas stained weakly for keratin in a patchy fashion in 34 of 48 (71%) of cases (Fig. 5). Because of variable expression of keratin in formalin-fixed tissues, only correlations based on qualitative data were made.

Involucrin was detected in 20 of 33 (61%) adenoacanthomas and 13 of 15 (87%) adenosquamous carcinomas (Figs. 1-4). In all cases, staining in the central component of the squamous morules was positive only if the more peripheral squamous cells were positive. The staining was intracytoplasmic and the intensity of the involucrin staining was subjectively less in adenosquamous carcinomas than adenoacanthomas. There was no correlation between the involucrin staining and the grade of the glandular component of the adenoacanthoma. The glandular component of the tumors did not stain for involucrin.

DISCUSSION

Historically, adenoacanthomas and adenosquamous carcinomas have been distinguished as separate clinical entities because the two tumors have a different prognosis. Some investigators have ascribed the poor prognosis associated with adenosquamous carcinoma to the malignant squamous component. In most series women with an adenoacanthoma have a 5-year survival of 83-93% in comparison

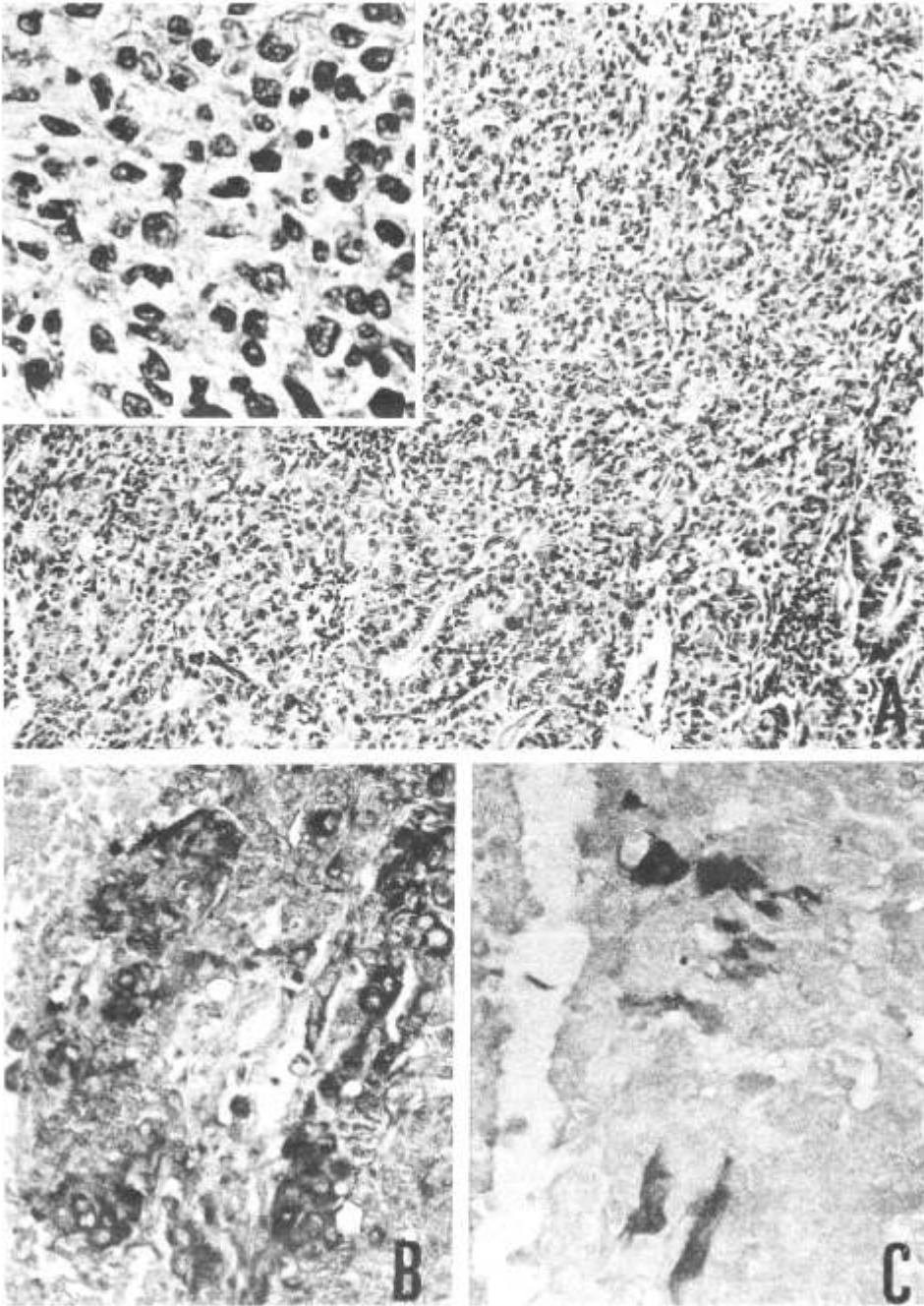


FIG. 1. A: Adenosquamous carcinoma, characterized by sheets of poorly differentiated squamous cells admixed with poorly differentiated glandular tissue. Inset: detail of squamous epithelium. Squamous cells stained for keratin (**B**) and involucrin (**C**) (H&E $\times 100$, $\times 400$; keratin $\times 200$; involucrin $\times 200$).

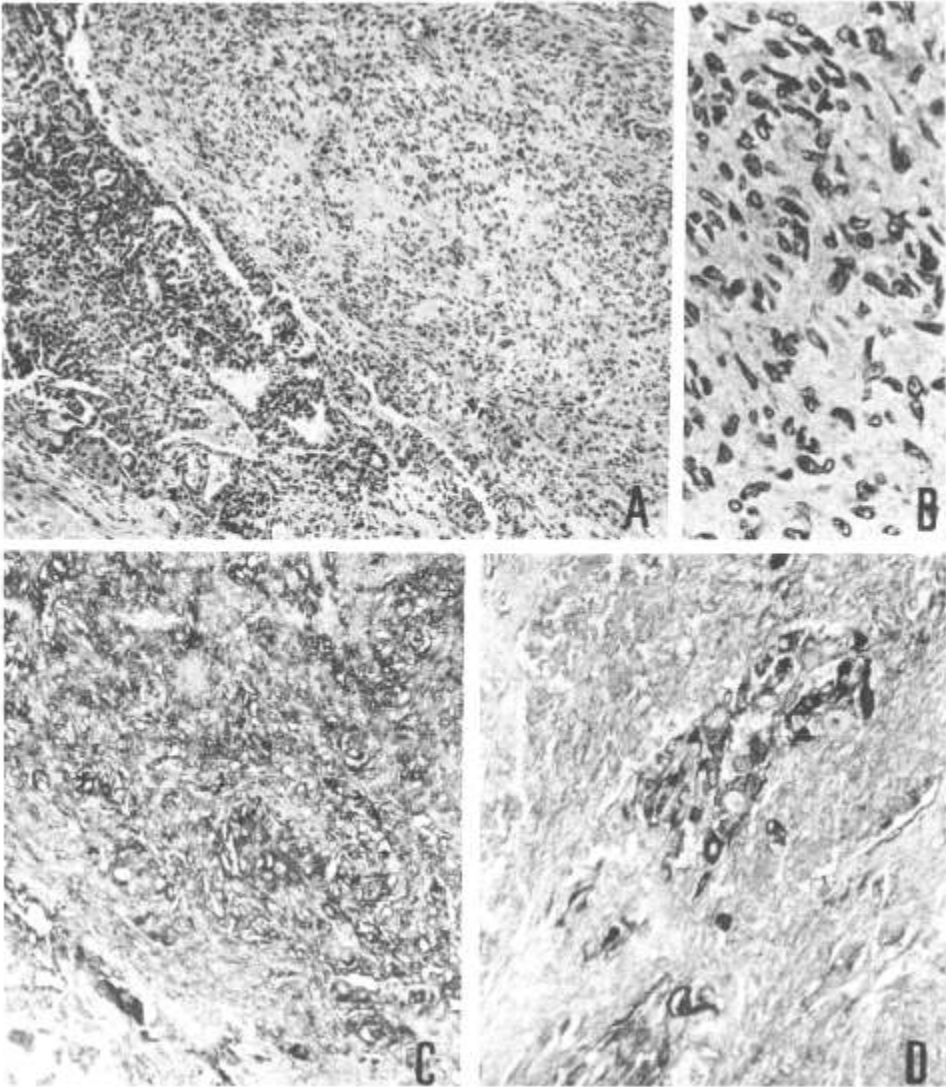


FIG. 2. A: Adenosquamous carcinoma, characterized by poorly differentiated squamous cells admixed with poorly differentiated glandular tissue invading myometrium. B: Detail of squamous epithelium. Squamous cells stained for keratin (C) and involucrin (D) (H&E $\times 100$, $\times 200$; keratin $\times 200$; involucrin $\times 200$).

with 35–80% for those with adenosquamous carcinoma (1,2,10–13). Recently, two groups of investigators have shown the difficulty in determining the degree of differentiation of the squamous cells in these tumors (4,12). This may be due partly to the inherent subjectivity in grading the squamous component. In our experience there is some variability in the degree of squamous differentiation among endometrial adenocarcinomas with squamous differentiation. Conversely, some adenosquamous carcinomas exhibit areas that are relatively well differentiated. In accordance with accepted principles, the tumor is graded by the most

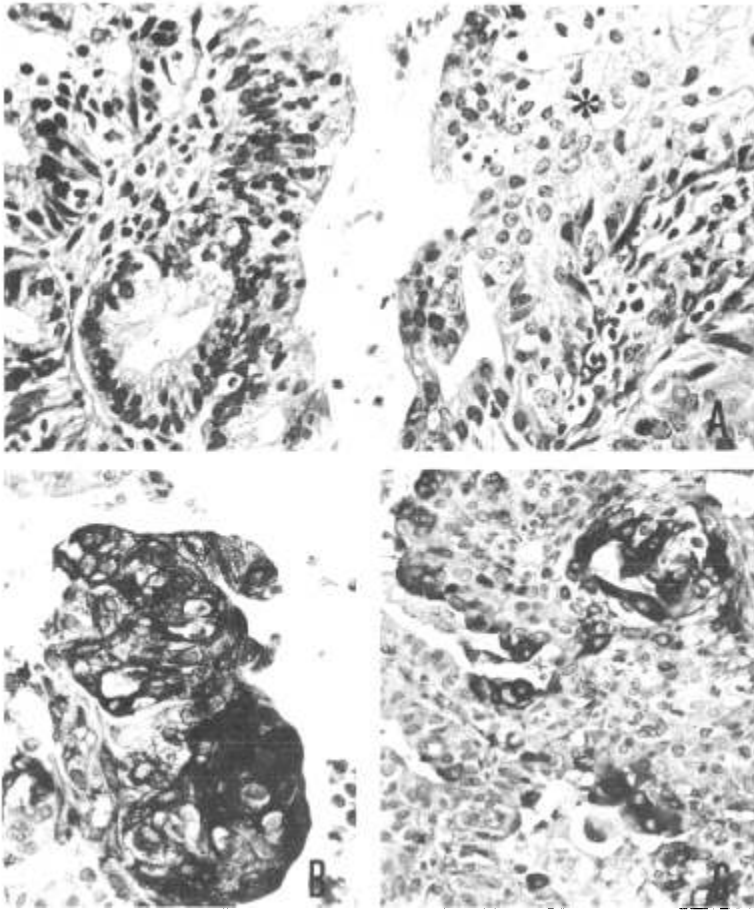


FIG. 3. A: Adenosquamous carcinoma, characterized by areas of squamous epithelium that appear moderately to well differentiated (*) admixed with glandular tissue of moderate differentiation. Squamous cells stained for keratin (B) and involucrin (C) (H&E $\times 100$; keratin $\times 200$; involucrin $\times 200$).

poorly differentiated portion. Furthermore, tumors with squamous morules within gland lumens that show more nuclear atypia than usual are considered as adenoacanthoma, whereas relatively well-differentiated squamous cells not associated with malignant glands are considered as part of an adenosquamous carcinoma if they invade the myometrium.

Recent studies have shown that the best prognostic index of an adenocarcinoma with a squamous component is the grade of the glandular component (2). If the glandular and squamous components are independently graded and correlated with survival, the patients with adenoacanthoma survive longer. In one study, when tumors were stratified by grade of the glandular component, the survival rates of women with either tumor became similar (2). In a second study, stratification by grade showed greater accord between survival rates of the two tumor types, although patients with the adenosquamous carcinoma still had a slightly worse prognosis (14). The import of these studies is that the tumors with well-

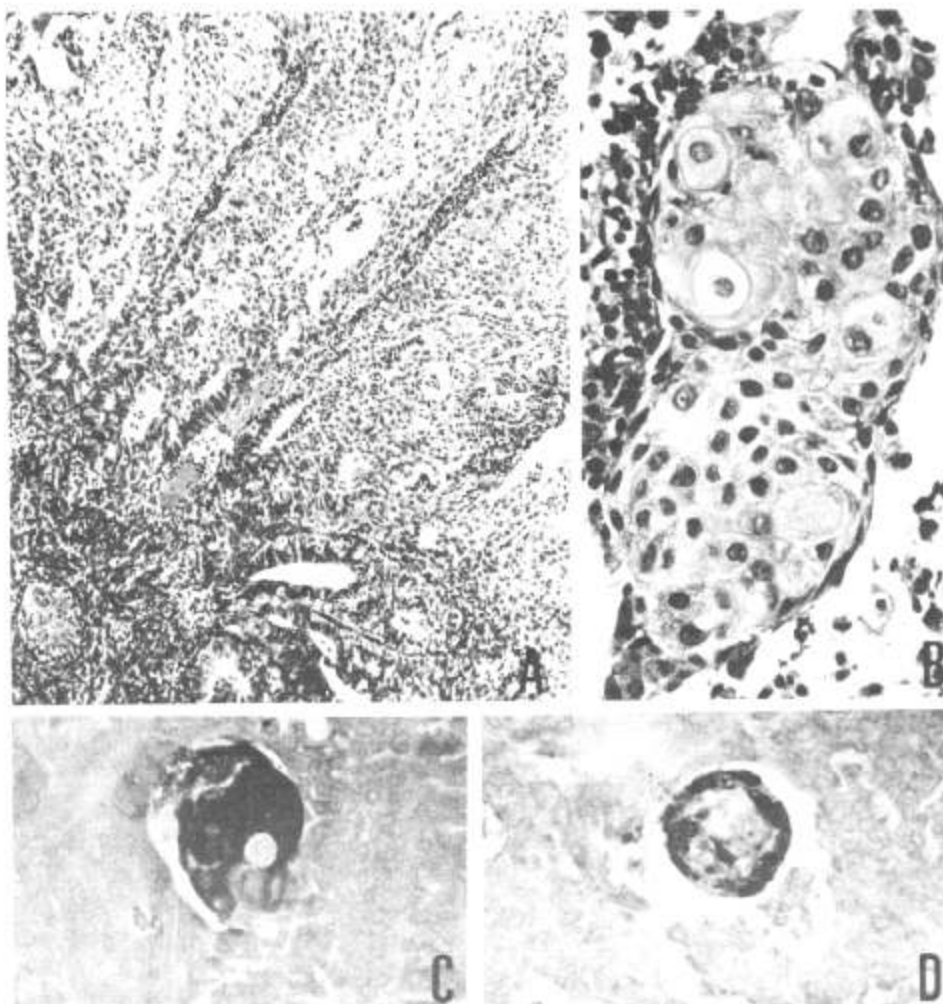


FIG. 4. Adenoacanthoma, characterized by areas of well-differentiated squamous epithelium admixed with well-differentiated glandular tissue (A). Morule (B). Squamous cells stained for keratin (C) and involucrin (D) (H&E $\times 100$, $\times 200$; keratin $\times 200$; involucrin $\times 200$).

differentiated squamous components (adenoacanthoma) are almost always found with glandular components that are Broders grade 1 or 2 or occasionally 3, but not grade 4. In contrast, the poorly differentiated squamous cells of the adenosquamous carcinoma are frequently associated with tumors that are grade 3, occasionally with glandular components that are grade 2 or 4, and virtually never with tumors that are grade 1. This suggests that in general the maturity of the squamous cell component parallels the maturity of the glandular component.

The degree of differentiation of the squamous cells appears related to the age of the patient. In several large series, women with adenoacanthoma were, on the average, 3 to 5 years younger than patients with adenosquamous carcinoma (2,10,11). If only patients under the age of 60 years were considered, the prognosis of both groups was nearly identical and in both groups survival was high (10,11).



FIG. 5. Glandular tissue in adenosquamous carcinoma which stains positively for keratin in a peripheral pattern (keratin $\times 200$).

In the present study, no qualitative differences were found in the staining reactions in the adenoacanthoma and adenosquamous carcinoma with respect to keratin and involucrin. Involucrin, a marker of suprabasal differentiation of squamous epithelium, is generally abundant in normal squamous epithelium but rare in dysplastic and malignant squamous epithelium of other organs (7). For example, in well-differentiated squamous cell carcinoma of the lung, only the squamous pearls stain positively (personal observations). In this study, well-differentiated squamous epithelium was anticipated to stain positively for involucrin, whereas rare to absent staining was expected in poorly differentiated squamous epithelium. The presence of involucrin even in the poorly differentiated tumors suggests that: (a) involucrin is not a specific sign of a highly differentiated tumor, or (b) cells that can differentiate to a degree to be recognizable as squamous, even if considered histologically malignant, are differentiated sufficiently to demonstrate involucrin.

Unlike involucrin, which to our knowledge is found only in squamous cells, as evidenced in this study, the production of keratin filaments is common to virtually all epithelial cells. While keratin is most abundant in squamous cells, it can be found to a variable degree in glandular epithelium (15). In the present study, antisera to keratin disclosed its presence in the glandular component of 71% of the adenoacanthomas and adenosquamous carcinomas. The intensity of intracytoplasmic staining for keratin appeared unrelated to the grade of the glandular component.

The results of the present study do not support the thesis that the degree of differentiation of the squamous component is a useful feature to distinguish adenoacanthoma from adenosquamous carcinoma. The intensity and frequency of staining for both keratin and involucrin were approximately the same in both the adenoacanthoma and adenosquamous carcinoma. Since determination of the de-

gree of squamous differentiation is frequently difficult based on the light microscopic characteristics of the squamous cells, and as the tumors have prognoses that are probably dependent on the grade of the glandular component, the designations adenoacanthoma and adenosquamous carcinoma should no longer be used to signify distinct clinicopathologic entities. The squamous epithelium in both tumors probably represents variable degrees of maturity dependent on the degree of differentiation of the glandular component. Perhaps the tumor should be reported as "adenocarcinoma with squamous differentiation" appended with the designated grade of the glandular component (4).

Acknowledgment: This work was supported in part by grant ES-03250 from the National Institute of Environmental Health Sciences and by the Center for Pathology Research Fund at the University of New Jersey School of Medicine and Dentistry, Newark.

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