MALIGNANT MIXED MULLERIAN TUMOURS OF THE UTERUS

- AN IMMUNOHISTOCHEMICAL STUDY

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To my husband Derck Smits, for his constant support and encouragement, for laughing and not laughing in the right places, and for tolerating the many "take-away" dinners.

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I, **Ellen Bolding**, hereby declare that the work on which this thesis is based is original (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other University.

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INTRODUCTION

Malignant Mixed Mullerian tumours (MMMTs) of the uterus account for 1-2% of all endometrial malignancies and have been a problem, in terms of both subclassification and diagnosis for many years. The entity was first described in 1864 by Virchow (92) who described a mixed form of sarcoma and carcinoma of the uterus, but the first well documented uterine carcinosarcoma was reported by Weber in 1867 (96), and again by Babl-Ruckhard in 1872 (6). Between the early 1900s and 1950s most papers on the subject consisted of single case reports with or without a review of the literature, which at that stage was still rather scanty and poorly understood. Tumours masqueraded under a variety of different names in an attempt to fit a name to the histologic pattern as well as trying to explain the histogenesis. The number of terms designated to this tumour entity became astronomical and included primary chondrosarcoma, adenoliposarcoma, sarcoma hydropicum polyposum uteri embryoides, dysontogenic tumour, mesenchymal sarcoma, carcinosarcoma, mixed tumour and malignant mixed mesodermal tumour. In 1935 McFarland (55), in a literature survey of utero-vaginal neoplasms with a list of 516 references, found 119 different names given to the entity.

In 1941 Liebow and Tennant (45) attempted to summarise the anatomical and clinical data in these mixed uterine sarcomas. The diagnosis of malignant mixed tumour was based on the presence of heterologous elements such as

striated muscle, osteoid, cartilage and osteoid. The term carcinosarcoma was reserved for those tumours with epithelioid and spindle cell elements. Whether these were true biphasic tumours or carcinomas with spindle cell dedifferentiation was uncertain.

There was much speculation as to the origin of these tumours. Many considered them to be malignant growths of metaplastic origin, while others considered the possibility that they might arise from benign tumours developing in heterotopic uterine tissue. However, because of the multiplicity of tissue types seen, most pathologists favoured the theory that the development of these tumours came from a multipotential stem cell. Another viewpoint favoured atypical connections between embryonal and mesenchymal cells from the inguinal area or misplaced ectopic rests as the primary site.

In 1954, Sternberg (87), who believed these tumours to be of Mullerian duct origin, proposed that the term malignant Mullerian tumour be used. His studies indicated that this tumour arose from specialised mesodermal tissue and that the carcinomatous components were limited by the epithelial potentialities of the Mullerian tract. Sarcomatous elements of these neoplasms were not rigidly limited in type by their Mullerian ancestry, and in common with mesenchymal cells elsewhere, retained the ability to differentiate into many mesenchymal derivations such as chondrosarcoma, fibrosarcoma, rhabdomyosarcoma and leiomyosarcoma.

Between 1950 and the mid 1970s several articles (16, 18, 39, 45, 51, 54, 72, 79, 81, 87) appeared from various large institutions describing the range of morphologic findings, documenting the clinical disease, presentation, age range, mode of spread and modalities of therapy used. The majority of patients were postmenopausal women between the ages of 45–85 with a mean age of 65 years. The commonest presentation was that of postmenopausal bleeding, followed by lower abdominal pain/discomfort and a pelvic mass. There was no significant association between this tumour and obesity, hypertension or diabetes, and the role of prior radiotherapy as a possible aetiologic factor was controversial. Generally, the prognosis was poor.

In 1966 Norris and Taylor (61) reviewed 31 cases of carcinosarcoma and addressed the problem — "Is carcinosarcoma the same entity as mixed mesenchymal tumour?" Their findings showed that the 31 patients with carcinosarcoma had an overall better prognosis than those containing mixed or heterologous elements, and recommended that the terms not be used interchangeably, but that they be regarded as separate entities because of their different behavioural patterns. In a follow up study (62) of 31 cases of heterologous mixed mesodermal tumours, they attempted to evaluate the relationship of the morphologic features to the survival of the patients. The presence of cartilaginous elements was found to be associated with a more favourable prognosis, whereas the presence of rhabdomyoblastic cells indicated a worse prognosis. They emphasised, however, that the overall prognosis of this

group was worse than carcinosarcoma. The authors also supported the view that the origin of these tumours was a multipotential stem cell which could differentiate along both carcinomatous and sarcomatous lines, and that the inherent metaplastic potential of the sarcomatous cells accounted for the wide variety of mesenchymal elements. Another clinicopathologic study of 66 cases from the Netherlands (81), evaluated after 5 years of therapy, showed the same trend as found by Norris and Taylor with their carcinosarcomas, i.e. a mean 43,7% five year survival and a mean 27% five year survival for the mixed mesodermal group.

In contrast to this, studies of a large series of patients by Chuang et al (16) and later by Williamson and Christopherson (99), found that there was no significant difference in the biological behaviour of these two groups, i.e. carcinosarcoma and mixed mesodermal tumours, and hence no difference in prognosis. These tumours differed only in their histological appearance and not in their clinical course. The issue remained unresolved.

In the following years various reputable institutions once again reported retrospective studies with follow up (22,57, 69,76,82). Little new information was added to the clinical and pathologic findings, but issues revolved around modalities of therapy, in particular the "type of surgery". Discussion centered around the use of adjuvant chemotherapy and radiotherapy. It was recognised that this was an aggressive tumour, with a propensity to both vascular and lymphatic invasion, and that an aggressive treatment programme

was warranted. Several series reported an improved survival in patients with stage I and II disease when chemotherapy was given prior to surgery and radiotherapy.

Studies to determine the most important prognostic indicator were undertaken by Barwick and Livolsi (8) who found that the most important factor was tumour extent, particularly the depth of myometrial invasion. Though their homologous group had a better 5 year survival rate than the heterologous counterpart, the true significance of heterologous elements was uncertain. Most believed that heterologous tumours were more aggressive because of a more rapid growth and, hence, deeper myometrial invasion and metastatic spread. The forty year experience of carcinosarcomas (homologous tumours) from the State of Missouri, reported by Doss et al (21), emphasised the need for accurate staging — even explorative laparotomy for definite staging to determine the optimal therapeutic modality. In 25% of cases the staging was altered by surgery, and clinical staging was thus considered inaccurate and inadequate for proper management. Accurate staging included surgical staging — total abdominal hysterectomy and bilateral salpingoophorectomy plus diaphragmatic wipes and peritoneal washings. An in depth review by Peters and Neelam (69) on the prognostic indicators in 103 stromal sarcomas, 47 homologous and 32 heterologous mesodermal tumours showed beyond any statistical doubt that the strongest factor relating to poor outcome was depth of myometrial invasion. A further adverse trend

was noted in patients with a history of previous pelvic irradiation, advancing age and an increase in uterine size. These were not considered to be statistically significant. Neither the presence of heterologous elements, nor cervical involvement was found to add any adverse prognostic factor. Irrespective of the histologic type of endometrial sarcoma, there were no long term survivors with extrauterine disease at initial presentation.

THE HISTOGENESIS

Through all these years of study, pathologists and clinicians have had differing opinions, as to both the classification of uterine mesenchymal tumours and their histogenesis. Various theories of origin have been proposed — from embryonic cell rests of the Mullerian duct; from metaplasia of the endometrial interstitial cell; from a multipotential primitive mesenchymal cell of the endometrium. Many believed that the sarcomatous cells were really carcinomatous cells in an unusual growth form. Some denied the existence of tumours such as carcinosarcomas and believed all were collision tumours. In 1959 Rubin (78) undertook to study these tumours in culture in an attempt to throw light on the vastly divergent views of many pathologists. His study using carcinosarcomas (homologous MMMTs) was the first described success at culturing these tumour cells. Important factors such as exudation, inflammation, vascularity, tumour bed and host response, thought to influence the growth pattern, were all eliminated. Tissue culture revealed two distinct cell lines with no intermediate forms or transitional areas between carcinoma and

sarcoma. These observations lent support to the belief that carcinosarcomas were a distinct entity.

In 1963 Vellios (91) delineated his histologic criteria for the diagnosis. He reported 9 cases of mixed Mullerian tumours in a series of uterine sarcomas and emphasised two points; firstly that these tumours were not as rare as initially thought, and adequate sampling was important. Secondly he reiterated the point that the histogenesis was uncertain and the discussion revolved around the various theories of that time which included: (i) derivation from embryonic rests carried along the mesonephric duct; (ii) Pfanneil's concept of uterine mucosal metaplasia giving rise to these malignancies and (iii) the most popular theory — a multipotential stem cell within the Mullerian system. However, the following questions were still unanswered: "Was this a (i) "collision tumour" with an admixture of two histogenetically distinct malignant cells, (ii) "combination tumour" with both histologic elements arising from the same stem cell, or (iii) "composition tumour" that is an endometrial carcinoma with an atypical reactive stroma?" In 1981 Ishiwata et al (34) of Japan, using in vitro cultures of carcinosarcomas succeeded in culturing two distinct and separate cell lines with specific characteristics — an adenocarcinoma line (HWUA-1, HWUA-2) and a sarcoma cell line (HWUS-1, HWUS-2, HWUS-1a). This data suggested that carcinosarcomas are combination tumours composed of 2 kinds of cells. In Rubin's study, the cultured cells died after 2 weeks, making long term cultivation unsuccessful. This study, with

the establishment of a long term cell culture line, will undoubtedly be of great use, not only in understanding the histogenesis, but also in assessment of efficacy of anti-cancer agents.

ELECTRON MICROSCOPY

The first electron microscopic (E.M.) findings were published by Silverberg in 1971 (84). The carcinomatous foci were ultrastructurally similar to pure endometrial adenocarcinomas and included findings such as an increased nuclear cytoplasmic ratio, large nucleoli, a general decrease in the number and complexity of cytoplasmic organelles, decreased desmosomes, and blunting and disorganisation of microvilli. The ultrastructural characteristics of the stromal cells were quite different in appearance to the carcinoma cells, and no transitional forms could be seen between these two. Transitional stages were seen between stromal cells and areas of other mesenchymal differentiation such as chondrocytic or fibrocytic foci. He emphasised the striking appearance of small rounded vesicles in the stromal cells which appeared to be derived from the Golgi apparatus. In addition, mitochondria were well developed and numerous, a rather unexpected finding considering that in normal endometrial stromal cells in the proliferative phase these cells were poorly developed. In the late secretory phase they were more common.

Boram et al (12) described the E.M. features of a mixed mesodermal tumour with a prominent pleomorphic rhabdomyosarcomatous component and showed a range of immature rhabdomyoblasts with barely discernible

cytoplasmic filaments, to classic pleomorphic rhabdomyoblasts with strap-like cells, tadpole-like cells and spindled cells with myofibrils and centrally located Z-band material.

Bocker and Stegner (11) in 1975 further expanded this field of ultrastructural differentiation of rhabdomyoblasts. They highlighted the fact that it might be difficult to discern the less differentiated stages of rhabdomyoblasts from primitive mesenchymal stromal cells on light microscopy. Electron microscopy was useful to determine the presence of heterologous elements. This study also paid particular attention to the types of cytoplasmic filaments found in the various stages of rhabdomyoblastic differentiation — from the undifferentiated mesenchymal cell or presumptive rhabdomyoblast to the electron microscopic "light" rhabdomyoblast with abundant intermediate filaments, to the more differentiated rhabdomyoblast with myofibrils. In addition the changes in 13 cases of homologous stromal sarcomas, 4 pure heterologous and 21 mixed heterologous tumours were documented.

This study and other E.M. studies (2,10) have shown that the stromal cell of endometrial stromal sarcomas resembles that of the normal early proliferative phase endometrial stromal cell. This cell type is also present in the group of mixed tumours and transitions to other heterologous tissues are demonstrated. A feature in all tumours was the distinct epithelial-stromal interface. Epithelial nests were also separated from the stromal cells and matrix by a continuous basal lamina, often thin and delicate, but at times

appearing as multiple layers.

An ultrastructural and immunohistochemical analysis of MMMTs by Geisinger (28) showed similar electron microscopic findings to previous studies. Cells with intermediate filaments, mostly of stromal origin, corresponded to the cells staining with anti-vimentin antibody, but focal vimentin positivity was also noted in the epithelial component of 6 out of 11 cases. Auerbach and Livolsi (4) have stated that these special techniques increased the sensitivity in detection of rhabdomyoblasts over routine H & E staining, and also added that the use of epithelial membrane antigen and cytokeratin was useful in detecting carcinoma cells and differentiating the poorly differentiated areas from sarcoma.

THE CLASSIFICATION

The pathologic classification of uterine sarcomas has been the subject of much debate. Nowadays most researchers would agree on the distinction between pure sarcomas and those with both sarcomatous and carcinomatous elements. In 1959 Ober (65) proposed a classification which used the concepts initially introduced by Fenker in 1864 (65). The uterine mesenchymal sarcomas were subdivided into pure sarcomas (i.e. composed of one cell type only) and mixed (more than one cell type); and also whether they were homologous (containing tissue elements indigenous to the uterus) or heterologous (containing tissues foreign to the normal uterus) (Table 1).

TABLE 1 A classification of uterine sarcomas — Ober 1959

1. Leiomyosarcoma
    - Arising in a leiomyoma
    - Arising diffusely in the uterine wall
2. Mesenchymal Sarcoma
    - Pure – homologous
        - Endometrial stromal sarcoma
        - Stromatous endometriosis (endolymphatic stromal myosis)†
        - Sarcoma botryoides (without heter. elements)
    - Pure – heterologous
        - Rhabdomyosarcoma
        - Chondrosarcoma
        - Osteosarcoma
        - Liposarcoma (not reported)
    - Mixed – homologous
        - Carcinosarcoma
            - Adenocarcinoma plus stromal sarcoma
            - Adenoacanthoma plus stromal sarcoma
            - Squamous carcinoma plus stromal sarcoma
            - Sarcoma botryoides plus neoplastic epithelium
    - Mixed – heterologous
        - Carcinosarcoma (plus heter. elements)
        - Mixed mesenchymal sarcoma
            - Stromal sarcoma (plus heter. elements)
            - Two or more heter. elements without mesenchymal
            - "myxomatous" stromal sarcoma
            - Sarcoma botryoides (with heter. elements)
3. Blood Vessel Sarcomas‡
    - Hemangiosarcoma (hemangioendothelioma)
    - Hemangiopericytoma†
4. Lymphomas
    - Reticulum cell sarcoma
    - Lymphosarcoma
    - Leukemic infiltration 5. Unclassified sarcoma
6. Metastatic sarcoma (not reported)

†These tumours are not uniformly malignant.
‡Lymphangiosarcoma has also been reported.
heter. = heterologous

This classification was used by some and expanded on by others. In 1966 Norris and Taylor (62) further subdivided pure endometrial stromal sarcomas into:

i)the stromal nodule

ii)low grade endometrial sarcomas

iii)high grade endometrial sarcomas

These classifications were never universally accepted, but were the most useful and histogenetically correct. However, utilisation for routine diagnostic purposes showed it was more detailed than necessary, and in 1970 Kempson and Bari (39) published a classification which they considered readily applicable to routine tissue diagnosis with good reproducibility (Table 2). This classification separated the sarcomas according to the number and types of recognisably different tissues present. Pure sarcomas contained only a single recognisable sarcomatous element, whereas mixed tumours contained more than one element. The term homologous was applied to those morphologically recognised as being of uterine origin, whereas heterologous contained elements not intrinsically of uterine origin. The malignant mixed Mullerian tumours (they prefered this term to mixed mesodermal tumour, as it reflected the histogenetic origin) contained both carcinomatous and sarcomatous el-

ements. They emphasised the need for adequate sampling together with histochemical stains to find the diagnostic areas. However, a small group would remain too poorly differentiated/undifferentiated as to defy classification, hence forcing them into group IV — the unclassified sarcomas.

TABLE 2 Classification of uterine Sarcomas — Kempson and Bari 1970

I Pure sarcomas
- A. Pure homologous
  1. Leiomyosarcoma
  2. Stromal sarcoma
  3. Endolymphatic stromal myosis
  4. Angiosarcoma
  5. Fibrosarcoma
- B. Pure heterologous
  1. Rhabdomyosarcoma (including sarcoma botryoides)
  2. Chondrosarcoma
  3. Osteosarcoma
  4. Liposarcoma

II Mixed sarcomas
- A. Mixed homologous
- B. Mixed heterologous

  Mixed heterologous sarcomas with or without homologous elements

III Malignant mixed Mullerian tumours (mixed mesodermal tumours)
- A. Malignant mixed Mullerian tumour, homologous type

  Carcinoma plus leiomyosarcoma, stromal sarcoma, or fibrosarcoma, or mixtures of these sarcomas
- B. Malignant mixed Mullerian tumour, heterologous type

  Carcinoma plus heterologous sarcoma with or without homologous sarcoma

IV Sarcoma, unclassified

V Malignant lymphoma

As an alternative, numerous institutions used the W.H.O. International classification of tumours released in 1975 (Table 3), and though it was of value to numerous clinicians, pathologists found that many subsequently defined categories, e.g. adenofibroma, adenosarcoma and endometrial stromal nodule, were not accounted for. Hence, many continued to follow the classification proposed by Kempson and Bari. In addition, recommendation was made to avoid all meaningless terminology such as sarcoma botyroides and even carcinosarcoma as the latter was often used for both homologous and heterologous tumours, and hence had lost its specificity.

Hendrickson and Kempson (33) highlighted that four combinations of histologically benign and malignant tissue components are possible.

1. Both stromal and epithelial components are malignant: malignant mixed Mullerian tumours.

2. Both stroma and epithelial components are benign: Mullerian adenofibroma.

3. The epithelium is benign and the stroma malignant: Mullerian adenosarcoma.

4. The epithelium is malignant and the stroma is benign: e.g. carcinofibroma. This category is difficult to accept as a separate entity, as all adenocarcinomas with proliferating benign stroma would fall into this group. The stroma, however, is not considered part of the usual endometrial adenocarcinoma, except in rare conditions like carcinofibroma.

Their classification, based on the above and incorporating a modification of Ober's original classification (including the benign mesenchymal tumours) is shown in Table 4. They have recommended the terms homologous and heterologous mixed malignant Mullerian tumour rather than carcinosarcoma and mixed mesodermal tumour. These terms are frequently used interchangeably and have led to confusion since it is often difficult to recall which term belongs to which tumour. Following the publication of their book (33), the classification has been used by most major academic institutions, both clinically and pathologically, and is the classification used in this study.

Earlier this year (1989) the International Society of Gynaecological Pathologists proposed a modified W.H.O. and F.I.G.O. classification (Table 5). Ironically the term carcinosarcoma has been reintroduced, qualified by the terms homologous and heterologous, and is used in preference to malignant mixed Mullerian/mesodermal tumour.

PURPOSE

It is the purpose of this study to use immunohistochemistry (i) to document the staining patterns seen in various tumours; (ii) to evaluate the use of these markers in delineating anaplastic and undifferentiated carcinomatous from sarcomatous areas, and to assess whether these stains enhance the sensitivity of detecting heterologous elements; (iii) to elucidate whether immunocytochemistry contributes any further information regarding the histogenesis of these tumours.

TABLE 3 : W.H.O. Classification of neoplasms of the uterine corpus —1975.

I Epithelial Tumours and Related Lesions
- A. Benign
  - 1. Endometrial polyp
  - 2. Endometrial hyperplasia
- B. Atypical Endometrial Hyperplasia
- C. Malignant
  - 1. Adenocarcinoma
  - 2. Clear cell (mesonephroid) adenocarcinoma
  - 3. Squamous cell carcinoma
  - 4. Adenosquamous (mucoepidermoid) carcinoma
  - 5. Undifferentiated carcinoma

II Non-epithelial Tumours
- A. Benign
  - 1. Leiomyoma (fibromyoma)
- B. Malignant
  - 1. Leiomyosarcoma
  - 2. Endometrial stromal sarcoma

III Miscellaneous Tumours
- A. Benign
  - 1. Adenomatoid tumour
- B. Malignant
  - 1. Mullerian mixed tumour
    - (a) Carcinosarcoma
    - (b) Mesodermal mixed tumour

IV Secondary Tumours

V Unclassified Tumours

VI Trophoblastic Disease
- A. Syncytial "endometritis"
- B. Hydatidiform mole
- C. Invasive hydatidiform mole (chorioadenoma destruens)
- D. Choriocarcinoma

VII Tumour-like Conditions
- A. Squamous metaplasia
- B. Adenomyosis (endometriosis interna)

TABLE 4 Classification of primary uterine mesenchymal neoplasms — Hendrickson and Kempson 1980

BENIGN NEOPLASMS

I Leiomyomas
- A. Morphologic variants
- B. Growth variants

II Endometrial stromal nodule

III Uterine tumours resembling ovarian sex cord tumours

IV Lipoma

V Hemangioma

VI Mullerian adenofibroma (papillary adenofibroma)

SARCOMAS

I Pure sarcomas
- A. Pure homologous
  1. Leiomyosarcoma
  2. High grade endometrial stromal sarcoma†
  3. Low grade endometrial stromal sarcoma (endolymphatic stromal myosis)
- B. Pure heterologous
  1. Rhabdomyosarcoma
  2. Chondrosarcoma
  3. Osteosarcoma
  4. Hemangiopericytomas

II Mixed sarcomas - homologous or heterologous

III Mixed Mullerian tumours
- A. Mullerian adenosarcoma
  1. Homologous type
  2. Heterologous type
- B. Malignant mixed Mullerian tumours
  1. Homologous type
  2. Heterologous type

IV Sarcoma, unclassified

V Malignant lymphoma

†The term "stromal sarcoma" without a qualifier refers to the high grade lesion.

TABLE 5 Uterine neoplasias : New I.S.G.P. proposed classification

I Epithelial tumours and related lesions
- A. Hyperplasias
- B. Carcinoma, and the variants thereof

II Non Epithelial Tumours
- A. Endometrial stromal tumours
  1. Stromal nodule
  2. Low grade stromal sarcoma
  3. High grade stromal sarcoma
- B. Smooth Muscle Tumours
  1. Leiomyoma and its variants
  2. Smoothe muscle tumour of uncertain malignant potential
  3. Leiomyosarcoma
- C. Mesothelial Tumours
- D. Other Non epithelial Tumours
  1. Benign soft tissue
  2. Malignant soft tissue (pure heterologous)
  3. Mixed endometrial stromal and smooth muscle type

III Mixed Epithelial – Non epithelial tumours
- A. Benign
  1. Polyp
  2. Adenofibroma
  3. Adenomyoma
- B. Malignant
  1. Adenosarcoma – homologous – heterologous
  2. Carcinosarcoma (MMMT) - homologous - heterologous
  3. Carcinofibroma

IV Miscellaneous Tumours

V Secondary Tumours

VI Unclassifiable Tumours

MATERIALS AND METHOD

All endometrial lesions diagnosed as primary malignancies from the records of the Department of Anatomical Pathology, University of Cape Town, from 1971 to 1987 inclusive were reviewed by light microscopy. Tissues had been fixed in 10% buffered formalin and were paraffin embedded. All blocks were recut at 3 μm thickness, and stained with haematoxylin and eosin. All cases had a mucicarmine and Periodic Acid Schiff stain to determine the presence of intracellular mucin. A silver stain for reticulin was used to delineate the growth pattern. In those cases where there was histologic suspicion of heterologous differentiation, further stains were done, such as Phosphotungstic acid haematoxylin to detect rhabdomyoblastic cross striations (indicative of rhabdomyoblastic differentiation) and Picrosirius Red stains for osteoid.

On the basis of these stains the tumours were classified into specific diagnostic groups as follows:

- Epithelial: adenocarcinoma, well, moderately and poorly differentiated with or without their metaplastic changes; uterine papillary serous carcinoma, mucinous carcinoma, clear cell carcinoma and undifferentiated carcinoma.

- Pure Sarcomas: endometrial stromal nodule, low and high grade stromal sarcomas,

- Mixed tumours: adenofibroma, adenosarcoma and malignant mixed Mullerian tumours (homologous and heterologous subtypes).

Immunohistochemical studies included cytokeratin, epithelial membrane antigen, desmin, vimentin, muramidase on the undifferentiated carcinomas, adenosarcomas,stromal sarcomas and MMMTs. CAM 5.2, alpha-1-antitrypsin, myoglobin, and S100 were done on a selected number of cases. Sections of endometrium from surgical hysterectomy specimens were used as normal controls and included 9 proliferative, 8 early and mid-secretory, 4 late secretory and 9 atrophic states of endometria (total n=30).

ANTIBODIES USED:

	Antibody	Clonality	Supplier
1.	Cytokeratin	Mono —	Dakopatts Denmark
2.	Epithelial membrane antigen	Mono —	"
3.	Desmin	Mono —	"
4.	Vimentin	Mono —	"
5.	S100	Poly —	Dako Santa Barbara USA
6.	Myoglobin	Poly —	"
7.	Muramidase	Poly —	Dakopatts Denmark
8.	Alpha-1-antitrypsin	Poly —	"
9.	CAM 5.2	Mono —	University College Hospital, London

The four immunohistochemical primary monoclonal antibodies — cytokeratin, epithelial membrane antigen, desmin and vimentin — were obtained commercially from Dakopatts, Glastrop, Denmark, as were the polyclonal antibodies muramidase and alpha-1-antitrypsin.

S100 and myoglobin are both polyclonal antibodies commercially available as prepacked prediluted kits from Dako (Dako Santa Barbara, CA, USA) as the DakoPapKit, system 40 for S100a protein and DakoPapKit system K520 for myoglobin. Method performed as per instructions.

CAM 5.2 (synthesised by Makin and Bobrow) was obtained from the University College Hospital London Medical School. This product is now also commercially available from Becton Dickinson.

For immunohistochemical studies, 3 μm sections were deparaffinised and rehydrated using solutions of 96% and 70% alcohol, followed by distilled water. Tissue sections were stained using the 2 step indirect conjugate peroxidase anti-peroxidase (PAP) method as follows. Sections were trypsinised using a Sigma product Porcine pancreas type II trypsin. Trypsin (0,2g) plus Calcium chloride (0,2g) was made up in 200 ml of distilled water at 37°C. The solution was left to stand for 15 mins. before use, and used between the period 15–60 mins. for optimal and most stable activity of trypsin. Trypsin times for each monoclonal antibody were: cytokeratin and EMA 20 mins., desmin 12 mins. and CAM 5.2 for 10 mins. The polyclonal antibodies, alpha-1-antitrypsin, muramidase, myoglobin and S100 were trypsinised for 12 mins. All tissues were trypsinised at 37°C. Vimentin required no prior trypsinisation.

Tissues were then inactivated by washing with cold water, followed by washing with 3% hydrogen peroxide in water for 5 mins. to block endogenous peroxidase. To enhance antigenic sensitivity and decrease background staining, tissue sections were washed in a 1:25 dilution of non immune rabbit serum for 10 mins., drained, and the primary antibody layered on. These primary antibodies were diluted in phosphate buffered saline (PBS) at pH

7.6 as follows: vimentin, CAM 5.2, cytokeratin and EMA 1:10; desmin 1:50; muramidase 1:100 and alpha-1-antitrypsin 1:200.

Slides were incubated at room temperature for 2 hours, then rinsed with PBS, the secondary linking antibody (i.e. the PAP conjugate) layered on, (1:25 dilution is made up in PBS and normal human serum to enhance chromogenicity of the substrate), incubated at room temperature for 30 mins., washed with PBS and the substrate added. The substrate used for all antibodies, except muramidase and alpha-1-antitrypsin was 3-amino-9-ethylcarbazole. After 40 mins. the reaction time was stopped by rinsing the sections with distilled water. Sections were counterstained using Mayer's haematoxylin and mounted in glycergel.

The polyclonal antibodies muramidase and alpha-1-antitrypsin were commercially obtained (Dakopatts, Glostrup, Denmark). A method similar to that used for the monoclonal antibodies was followed with some modifications. The secondary linking antibody was diluted to a 1:25 solution, and the chromogenic substrate diaminobenzidine-tetrahydrochloride was used.

Positive tissue controls included normal endometrium and skin for cytokeratin and EMA, myometrium for desmin and vimentin; pulmonary macrophages for muramidase and alpha-1-antitrypsin; and a malignant melanoma for S100. Control tissues were selected with approximately the same length of formalin fixation as the test slides and were not subjected to microwave fixation.

RESULTS

In the period under review (1971-1987) there were 461 cases originally diagnosed as primary endometrial malignancies. These were re-examined and classified on the basis of the light microscopic findings on H & E stain, together with the following special stains - Periodic acid Schiff with and without diastase, mucicarmine, Bests's carmine and reticulin. The results are tabulated below (Table 6).

TABLE 6 Distribution of total cases

	Diagnosis	No	%
1	Severe atypical hyperplasia	8	1.7
2	Adenocarcinoma	324	70.2
3	Uterine papillary serous carcinoma	18	3.9
4	Clear cell Ca	5	1.1
5	Undifferentiated Ca	24	5.2
6	Adenosarcoma	6	1.3
7	Stromal sarcoma	16	3.4
8	MMMT	58	12.5
9	Miscellaneous i.e. unclassifiable	2	0.4
	TOTAL	461	

One miscellaneous case histologically resembled a lymphoma, and the other a sarcoma. The undifferentiated carcinomas, adenosarcomas, stromal sarcomas, unclassifiable tumours and MMMTs formed the basis of further immunohistochemical study — this totalled 106 cases.

Twenty five of these 106 cases were excluded (Table 7) because either (i) the material was too degenerate and necrotic for immunohistochemistry, (ii) no tissue remained in the block, (iii) blocks were not available. This left 81 cases available for further study.

TABLE 7 Distribution of final cases

No. of cases excluded		Final No.
Undiff. Ca	6	18
AdenoSA	1	5
Stromal SA	5	11
MMMT	13	45
Unclassified	0	2
TOTAL		81

The mean age of all patients was 58,5 years ranging from 14 — 87. The mean age and age range for each diagnosis is tabulated below (Table 8).

TABLE 8 The age distribution

Diagnosis	mean age	age range
Undiff.CA	63	52 – 87
AdenoSA	46	14 – 82
Stromal SA	59	44 – 82
MMMT	66	47 – 86

All except 5 patients were postmenopausal and presented with postmenopausal bleeding and/or a pelvic mass. Two patients, aged 14 and 21, both with adenosarcomas presented with irregular menses and a pelvic mass. Of the remaining three cases, one presented with a pelvic mass and two with menorrhagia.

Routine light microscopy showed that the undifferentiated carcinomas were composed of sheets of undifferentiated cells, no evidence of glandular differentiation or mucin positivity, and a packeted pattern on reticulin stains in 50% of cases. The adenosarcomas, 2 of which showed heterologous rhabdoid elements, and stromal sarcomas showed histologic features as described in the literature. The majority (68%) of MMMTs showed a fairly even distribution of both carcinomatous and sarcomatous elements. In 12 cases the

stroma was in excess of the epithelial component, and in 7 the carcinomatous component predominated. The malignant epithelial components of MMMT varied from undifferentiated carcinoma to moderate to well differentiated adenocarcinoma (Fig. 1). Striking tubulo-glandular patterns and classic serous papillary type growth patterns were noted in 9 cases (Fig. 2). Squamous metaplasia was noted in 8 cases. Of the 45 MMMTs 26 were homologous and 19 heterologous tumours. The homologous stromal cells showed varying cytology. The majority of cells were stellate or spindled, but some were more plump and rounded (Fig. 3). Bizarre multinucleate cells with hyperchromatic nuclei were present in several homologous MMMTs, but were not arranged in a storiform pattern to justify the diagnosis of MFH (Fig. 4). In 20% of cases these cells also contained intracytoplasmic eosinophilic globules resembling effete red blood cells and were present in both homologous and heterologous tumours (Fig. 5). There was no consistent staining pattern of these globules when stained with PAS and PTAH. On H & E, 4 of the homologous MMMTs showed rhabdoid-like cells, only 2 of which were subsequently reclassified as heterologous because of strong desmin positivity (Fig. 6). There was no further evidence by PTAH and myoglobin stains to substantiate that the other 2 were of rhabdoid origin. Of the heterologous MMMTs 53% (n=10) showed rhabdomyoblastic differentiation (Fig. 7), 36% (n=7) chondrosarcomatous change (Fig. 8), 20% (n=4) showed areas resembling osteosarcoma (Fig. 9) and 20% (n=4) showed features consistent with

Malignant Fibrous Histiocytoma (Fig. 4).

Immunoperoxidase stains were recorded as being negative; focally positive and strongly positive — the latter two grouped together as positive. The tissue sections used as positive controls for the monoclonal/polyclonal antibodies were all strongly positive.

Control specimens

Thirty endometrial samples from 9 proliferative, 8 early and mid-secretory, 4 late secretory and 9 atrophic endometria were analysed. EMA, CK, desmin, vimentin and muramidase were performed on all cases, the results of which are displayed below (Table 9 and Fig. 10).

TABLE 9 Results of control endometria

		No. of positive cases				
Endometrium		EMA	CK	DES	VIM	MURAM
Proliferative (n=9)	G	8	5	0	8	0
	S	0	0	0	9	0
Secretory - early/mid (n=8)	G	6	6	0	4	0
	S	0	0	0	8	0
- late (n=4)	G	4	2	0	3	0
	S	0	0	0	4	0
Atrophic (n=9)	G	9	3	0	5	0
	S	0	0	0	9	0

G = glands; S = stroma

Those cases in which cytokeratin and/or EMA were negative or weakly positive, were subjected to further staining with CAM 5.2. All were strongly positive for this low molecular weight cytokeratin (Table 10).

TABLE 10 Results CAM 5.2 control endometria

CAM 5.2 positivity	
Endometrium	Positive
Proliferative (n=3)	3
Secretory – early/mid (n=2)	2
– late (n=2)	2
Atrophic (n=6)	6

In all cases, at least one of the epithelial markers was positive and only the epithelial elements stained positively for EMA, cytokeratin and/or CAM 5.2. EMA intensity of staining was stronger than cytokeratin in the majority of cases (Fig. 10) and similarly, CAM 5.2 was of greater intensity than cytokeratin in the cases where it was done. In all the control specimens of this study, both myometrial and vascular smooth muscle were present and there was no positive staining of these elements with CAM 5.2. The literature on CAM 5.2 states that there is cross reactivity with this low molecular weight cytokeratin and smooth muscle (13,27,64).

Vimentin showed an interesting pattern of staining with both stromal and glands elements being positive in 20 of the control cases (Fig. 10), the remaining 10 showed positivity in the stromal elements only. Both epithelial and glandular staining were present in 8 proliferative, 4 early/mid secretory, 3 late and 5 atrophic endometria. In all cases the epithelium was either equal to, or of slightly weaker staining intensity to the stromal element. No cases showed epithelial staining predominating over the stromal intensity.

All cases were negative for desmin and muramidase.

Immunoperoxidase stains for the test cases were also recorded as focally positive, strongly positive and negative (Table 11). After examination of the immunocytochemistry, some of the cases were reclassified.

TABLE 11 Results of Immunocytochemistry

Initial diagnosis	No. of cases POSITIVE				
	EMA	CK	VIMEN	DESM	MURAM
Undiff. CA (N=18)	17	15	9	0	1
Stromal SA (N=11)	2	2	10	4	0
AdenoSA (N=5)	5	4	5	2	0
MMMT (N=45)	40	34	41	11	12
Unclassifiable (N=2)	2	0	1	0	0

Those cases where cytokeratin was negative or weakly positive were subjected to immunocytochemistry with a low molecular weight cytokeratin — CAM 5.2 (Table 12).

TABLE 12 Positive cases with CAM 5.2

Diagnosis	No.
Undiff CA (n=6)	6
Stromal SA (n=11)	1
AdenoSA (n=1)	1
MMMT (n=10)	8
Unclassifiable (n=2)	1

Epithelial membrane antigen (EMA) preparations demonstrated granular membrane staining in 17 of the 18 undifferentiated carcinomas. The staining patterns were recorded as negative, weak focal positivity, and strong or diffuse positivity (i.e. in numerous cells). One case was entirely negative; 2 showed focal positivity and the remaining 15 showed strong diffuse positivity.

Two stromal sarcomas showed strong diffuse positivity. These 2 cases, together with subsequent cytokeratin and desmin results led to an alteration

of the initial diagnosis from stromal sarcoma to MMMT. The epithelial elements were not evident on H & E staining. The remaining 9 cases were negative for EMA. The adenosarcomas showed positivity in the benign glandular elements in all cases. Stromal or heterologous elements were negative.

The MMMTs showed positivity in the adenocarcinomatous (Fig. 11), tubulo-papillary, papillary serous-like and undifferentiated carcinomatous areas (Fig. 13). Isolated positive epithelial cells within the stroma, difficult to identify on H & E, were also noted to be positive. There were 5 negative cases, 7 showed focal positivity, and 33 showed strong positivity. None of the obvious stromal or heterologous elements were positive. However, in 2 cases the spindle cell elements were positive for EMA and CK, and were therefore reclassified as undifferentiated carcinoma with spindle cell change. In the miscellaneous group 1 case, morphologically resembling lymphoma, was leucocyte common antigen negative, but showed EMA and CAM 5.2 positivity and was reclassified as undifferentiated carcinoma. The second case — sarcoma NOS, showed strong EMA positivity.

Cytokeratin and CAM 5.2. Cytokeratin stains showed diffuse cytoplasmic staining of the malignant epithelial component throughout 15 of the 18 undifferentiated carcinomas, 3 being negative. Of this negative group 2 showed strong cytoplasmic staining with CAM 5.2, confirming the epithelial nature, and 1 had strong EMA positivity. In addition, 34 MMMTs showed positive staining of the adenocarcinomatous, papillary and poorly differentiated

carcinomatous elements. In most tumours the gland forming cells appeared more intensely staining than the squamous cells with CAM 5.2, though the reverse was true for cytokeratin. There was no demonstrable positivity in stromal or heterologous elements, although scattered groups and single cells of epithelial origin were detected much more easily with immunocytochemistry than with the H & E (Fig. 12 and 13). Cytokeratin was negative in 11 MMMTs, 6 of which were positive for EMA and CAM 5.2, and 4 of which were totally negative for all three epithelial markers. In all these 4 cases the tumour appeared undifferentiated and tended to have features on H & E and reticulin stain suggestive of epithelial heritage. Immunocytochemistry was unable to prove this.

The adenosarcomas showed cytokeratin positivity in the benign glandular elements of the tumour in 4 of 5 cases, the 1 negative case was strongly CAM 5.2 positive. In all 5 cases the epithelial membrane antigen was positive.

In 9 cases of stromal sarcomas the cytokeratin and CAM 5.2 were negative and 2 cases were positive for cytokeratin (1 showing strong diffuse positivity and the other weak focal positivity). The CAM 5.2 on the latter case was strongly positive.

Vimentin staining demonstrated diffuse cytoplasmic granular positivity which was recorded as focal positivity, strongly positive, and negative. Of the undifferentiated carcinomas 8 were negative, 7 showed focal positivity in scattered groups of tumour cells, and 2 were strongly positive. A to-

tal of 41 MMMTs showed positivity. The homologous stromal cells were vimentin positive in the rounded, spindled and stellate cells, though not all cells in a given neoplasm were positively stained. The staining pattern varied from diffuse intracytoplasmic granularity to dense block-like intracytoplasmic staining. Heterologous cells of rhabdomyoblastic, chondroid, MFH-like areas and osteosarcomatous differentiation also showed scattered cells to be positive. The epithelial element of MMMT showed positivity in both the well differentiated areas of adenocarcinoma and the less differentiated areas (Fig. 13). The positivity was diffuse cytoplasmic and granular and was present in 21 MMMTs. In these cases the intensity of staining was either equal to or less than the stromal staining, but never more.

A totally negative staining pattern for vimentin was noted in 4 MMMTs, 2 of these (see EMA and CK) subsequently turned out to be undifferentiated carcinomas. The other 2 clearly had a malignant stromal element, 1 with rhabdomyoblastic differentiation, but in both vimentin staining was considered to be negative. Stromal components of 5 adenosarcomas were strongly positive and 3 showed positivity in the benign glandular components as well.

Stromal sarcomas were positive in 10 instances and negative in 1 - this latter case had its diagnosis altered to MMMT on the basis of EMA positivity. Of the 2 unclassifiable cases 1 was positive and 1 was negative.

Desmin and Myoglobin showed strong cytoplasmic staining which was graded as positive or negative. No undifferentiated carcinoma was positive.

While 4 homologous MMMTs showed stromal cells with rhabdoid feature, only 2 were positive for desmin and were thus reclassified as heterologous MMMTs. The 1 homologous tumour where heterologous rhabdoid differentiation was not detected on H & E initially, showed several strongly positive desmin staining cells, adding another heterologous MMMT. Of the initially diagnosed heterologous MMMTs, 9 showed obvious rhabdomyoblastic differentiation, all staining positively with desmin, though the intensity of staining varied from cell to cell and tumour to tumour.

There was no desmin or myoglobin positivity in the remainder of the heterologous MMMTs where rhabdoid features were not present on H & E. Myoglobin was done on 11 cases, 2 of which were both desmin and myoglobin negative. In the remaining 9 cases only 3 showed definitive positivity, 6 were either negative or equivocal. Polyclonal myoglobin does not appear to be a very sensitive or specific marker for rhabdomyoblastic differentiation — considerable non specific background staining was noted in several cases and cross reactivity with both epithelial and other stromal elements, viz. chondroid, was also noted in some cases.

Muramidase. The same criteria as before were employed in interpretation of positivity and only granular cytoplasmic staining was considered positive. All except 1 undifferentiated carcinoma were negative. The exception stained positively in scattered tumour cells, but intensity was not strong. This undifferentiated carcinoma was reclassified as a homologous MMMT on the

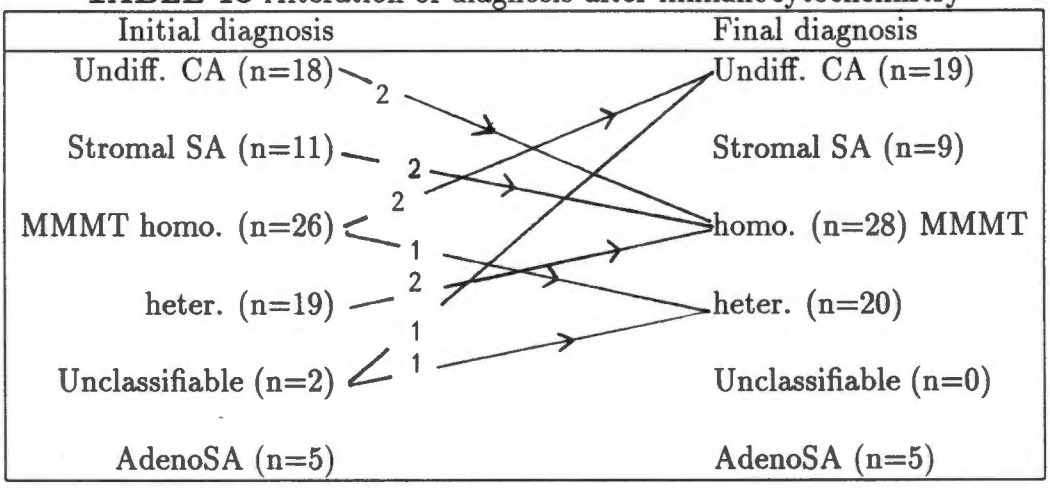
basis of immunocytochemistry. Weak positivity was present in 11 MMMTs but 1 case was strongly positive in the stromal cells, particularly the larger pleomorphic bizarre MFH-like cells. The adenosarcomas and stromal sarcomas were all negative, as well as 1 of the unclassifiable group. The second unclassifiable tumour showed patchy positivity and, together with other immunocytochemical stains, favoured a final diagnosis of MMMT.

Alpha-1-antitrypsin was performed on 9 MMMTs which showed eosinophilic intracytoplasmic globules — 6 of these were positive for alpha-1-antitrypsin as well as for muramidase. The intensity of the globule staining varied from case to case. There was poor correlation between these MMMTs which were positive for muramidase (n=11) and alpha-1-antitrypsin, as only 1 of these was positive for both.

The S100 protein stain was done on all cases which showed chondroid differentiation (n=7) and all showed strong cytoplasmic and nuclear staining of the chondrocytic cells in this area. Features suggestive of neural differentiation on H & E were noted in 1 tumour, but it was S100 negative.

As a result of immunocytochemistry 11 of the 81 cases had their initial diagnosis changed. In all, 2 undifferentiated carcinomas, 2 stromal sarcomas and 2 heterologous MMMTs became homologous MMMTs; 2 homologous MMMTs and 1 unclassifiable tumour were recategorised as undifferentiated carcinomas; and 1 homologous MMMT and 1 unclassifiable tumour were recategorised as heterologous MMMTs (Table 13).

TABLE 13 Alteration of diagnosis after immunocytochemistry



The immunocytochemistry of all the final diagnoses are displayed below in Table 14. All the undifferentiated carcinomas expressed one or more of the epithelial markers. The one case which was EMA negative did express both CK and CAM 2.5. Coexpression of vimentin was present in 37% of cases, and one epithelial tumour displayed weak muramidase positivity. This feature, though uncommon, is recognised in certain undifferentiated epithelial malignancies (89).

Two stromal sarcomas showed myoid differentiation on H & E, confirmed by strong desmin positivity in numerous tumour cells.

The heterologous elements of adenosarcomas consisted predominantly of rhabdomyoblasts and these cells were positive with desmin antibody staining. Adipose tissue and chondroid elements were also noted in one case. The spindled stromal cells were vimentin positive in all cases.

Using a panel of epithelial markers, epithelial elements were present in all MMMTs — this is mandatory for the diagnosis. Interestingly, all epithelial

cells showed EMA positivity to varying intensity. The majority (93%) had vimentin positive stromal cells, and 27% showed stromal cells with histiocytic differentiation.

TABLE 14 Immunocytochemistry of final diagnoses

	No. of positive cases				
Diagnoses	EMA	CK	DES	VIM	MURAM
Undiff. CA (n=19)	18	16	0	7	1
Stromal SA (n=9)	0	0	3	9	0
AdenoSA (n=5)	5	4	2	5	0
MMMT (n=48)	48	35	13	45	13

DISCUSSION

A diverse spectrum of histologic changes is displayed by Malignant Mixed Mullerian Tumours (MMMTs) of the uterus which are often a diagnostic challenge to the pathologist. The major differential diagnoses in homologous MMMTs where anaplastic epithelium or sarcomatous elements predominate are undifferentiated carcinoma and sarcoma respectively. Heterologous tumours are more readily identified by the presence of malignant cartilage, bone or striated muscle. Extensive sampling is mandatory to avoid misdiagnosis, but this study, as others (4,28,73), has shown that in a certain percentage (14% in this study) of cases the diagnosis cannot be made on H & E alone. Additional immunopathologic studies for identification of specific intermediate filaments (IFs) are required. These IFs are cytoskeletal components, which, once recognised by antibody staining techniques, are helpful in more accurate diagnosis and categorisation of tumours difficult to diagnose by conventional means. In normal tissues IFs seem to be tissue type specific and this cell type specific expression appears to be preserved when malignant change has taken place (even in the most poorly differentiated malignancies (73,89)). The four antibodies (cytokeratin, CAM 5.2, desmin and vimentin) used in this study show a cell type specific expression that parallels the known embryonic pathways of differentiation.

Cytokeratin (Dako Ck 1 monoclonal mouse anti-human cytokeratin) is

directed against human intermediate filaments keratin 18 and 6, but does not react significantly with keratins 1, 8 and 19 and other low molecular weight cytokeratins.

CAM 5.2 (synthesised by Makin and Bobrow, University College Hospital Medical School, London; monoclonal mouse anti-human low molecular weight cytokeratin) reacts with one group B (basic) keratin of molecular weight 50 kD, and two group A (acidic) keratins of molecular weights 43 kD and 39 kD. These are the keratin types 8, 18 and 19 respectively.

Desmin (Dako Desmin, monoclonal mouse anti-desmin) reacts with the intermediate filament protein desmin found in muscle cells by recognising an 18 kD rod piece of the molecule. It stains both striated, smooth and cardiac muscle as well as tumours of myoid origin.

Vimentin (Dako Vimentin monoclonal mouse anti-vimentin) reacts with the 57 dK intermediate filamentous protein present in cells of mesenchymal origin.

The other immunohistochemical markers include: Epithelial membrane Antigen (Dako EMA, monoclonal mouse anti-human epithelial membrane antigen) which reacts with a group of overlapping epitopes on several different molecules (related to milkfat globule protein) of molecular weight in the range 265 – 400 kD. These represent integral constituents on the surface cell membrane of epithelial cells.

Muramidase (Dako, Lysozyme [Muramidase] polyclonal rabbit anti-human

muramidase) which reacts with cytoplasmic lysozymal enzymes is present in cells of myeloid, histiocytic-monocytic lineage, and in a few types of epithelial cells. This antibody is generally used in pinpointing the histiocytic nature of a cell proliferation, whether reactive or neoplastic.

Alpha-1-antitypsin (Dakopatts, polyclonal rabbit anti-human alpha-1-antitypsin) — this antibody, though primarily used as a histiocytic marker, has been detected in a variety of epithelial cells and their tumours and also in germ cell tumours of yolk sac type.

It was the purpose of this study to (i) document the staining patterns seen in various tumours; (ii) evaluate the use of these markers in delineating anaplastic and undifferentiated carcinomatous from sarcomatous areas and assess whether these stains enhanced the sensitivity of detecting heterologous elements; (iii) elucidate whether immunocytochemistry contributed to any further information regarding the histogenesis of these tumours.

The documentation of the staining patterns has been recorded and tabulated (Table 11, 12, 14). Because some elements are difficult to detect on H & E, the reclassification following immunohistochemistry is justified and Table 14 therefore shows the definitive staining patterns of this group of neoplasms which could be used for further study or diagnostic purposes.

Unclassifiable malignancies leave clinicians in a difficult situation as regards therapeutic options for the patient and, hence, every attempt should be made to pursue these cases further. The epithelial markers were of particular

value in differentiating anaplastic undifferentiated carcinomas from sarcomas, and spindle cell squamous elements from true sarcomatous elements. Distinct epithelial markers were expressed in both unclassifiable cases — one showed focal aggregates of positivity interspersed with areas which were positive for mesenchymal markers only. The other showed a more diffuse staining pattern. Because of this, a more accurate diagnosis of undifferentiated MMMT and carcinoma respectively, was made.

All the epithelial markers proved to be of use. CAM 5.2 antibodies reacting with the low molecular weight cytokeratins, showed more intense staining than cytokeratin. In general it was also noted that EMA antibodies were more reliable than cytokeratin antibodies in the detection of epithelial elements in uterine tumours. This is in contrast to the findings in metastatic carcinomas or undifferentiated carcinomas from other sites. Due to a limited supply of the CAM 5.2 antibody, not all cases could be tested. In those tested (where CK/EMA was negative or weakly positive), the positivity was easily detectable and therefore CAM 5.2 seemed to be the most useful of these three markers in detecting carcinoma cells in this setting. As with all immunohistochemical analyses, a panel of antibodies is recommended to result in a broader specificity and greater sensitivity. The cost of the antibodies is such that many small laboratories cannot afford a panel, and the MAK-6 is now recommended as the antibody with a wide specificity and high sensitivity. This antibody is not yet available in our laboratory, and we have

no experience with its use, but Listrom and Dalton (48) state the staining patterns and false positivity are very similar to CAM 5.2.

The overall prognosis of homologous versus heterologous tumours is still controversial (14,52,69,84). This study has not incorporated the long term follow up of these cases and therefore, until more long term multicentre studies are done to accurately determine the influence of heterologous elements on prognosis, pathologists should attempt to record as accurately as possible the histologic changes. Detection of heterologous elements may be difficult and, as this study shows, one cannot rely on H & E criteria alone. Rhabdoid cells are often missed on H & E but are easily detected using anti-desmin antibodies with or without myoglobin. Detection of other heterologous elements by immunohistochemistry is not always as useful. Staining for S100 protein provided little further useful diagnostic information and was positive only in those areas where chondroblastic differentiation was seen (4). Some cartilaginous foci were also vimentin positive. Neuroectodermal tissue staining positively for S100, has been described as a heterologous element in MMMT (29).

Those areas which resembled malignant fibrous histiocytoma showed variable staining with muramidase. True histiocytic differentiation is best detected using a panel of antibodies such as muramidase, alpha-1-antitrypsin, alpha-1-antichymotrypsin, MAC 387, Leu M1 and Leu M2. Muramidase *per se* did not reveal significant histiocytic differentiation, and MFH-like areas

were only seen in 20% of cases. Scattered bizarre multinucleate hyperchromatic cells were more common, but these too, showed a very variable staining pattern with muramidase from negative to strongly positive. There was no associated tissue histiocytic inflammatory response to the tumour in any of the cases studied.

"Does the presence of MFH or muramidase positive histiocytes constitute a heterologous element?" - this question has seldom been addressed. In this study, the control endometria did not demonstrate any muramidase positive histiocytic cells, but then only a single antibody was used. Morris, Edwards et al (58) in an immunohistologic study on normal endometria showed that endometrial lymphoid tissue had many of the hallmarks of mucosal associated lymphoid tissue. Stromal histiocytes/macrophages were demonstrated (OKTM1 and HLA-DR positive) dispersed around the basal glands. These findings were only noted in adult, and not in infant uteri. The histiocytes are thus acquired with age, together with the lymphoreticular system, and are not autochthonous to the endometrium. Because of this, they should be considered as heterologous elements.

The intracytoplasmic globules often seen in these bizarre multinucleate cells were stained with PTAH, PAS with and without diastase in addition to muramidase and alpha-1-antitrypsin, but no consistent finding was noted. The most likely explanation for these globules is that they are macrolysosomes, but may indicate a degenerative cellular change.

The endometrial control specimens have shown that the epithelial markers are confined to the epithelial elements, whereas vimentin, originally thought to characterise only mesenchymal cells, was present in the glandular element in 66% of cases. Glandular positivity was seen in all phases of the endometrial menstrual cycle. Coexpression of vimentin by epithelial cells has been described (19,26,30) and hence IFs expression may vary at different stages of embryonic development. The uterus and fallopian tube develop from the Mullerian system which originates from coelomic cavity. Studies on mesothelial tissue by Czernobilsky et al (19) show that these cells may express both cytokeratin and vimentin. Studies on the mesothelium of the pleural cavity by Gosh and Gatter (30) and CJ Uys (personal communication) also show coexpression of vimentin and cytokeratin. It is therefore not surprising that these tumours, with a common ancestry, express more than one intermediate filament.

If, however, epithelial cells only express epithelial markers, and stromal cells only express mesenchymal markers, then how does this help our understanding of the histogenesis of MMMTs? If it is true that neoplastic tumour cells carry through the intermediate filament expression from their parent cell, then all cells which are negative for the epithelial markers must be of stromal or of another origin; and all cells which are positive for epithelial markers must be of epithelial origin. This then, would favour MMMTs to be of mixed lineage, i.e. of two cell types, a concept favoured in earlier times,

but which is no longer popular.

An alternative explanation is that of neometaplasia. Metaplastic carcinomas have been well described in the breast, lung, pancreas and oesophagus. Malignant cells contain the genetic material which code for all the IFs. These cells, with unstable DNA nuclear material and loss of the normal DNA/DNA and DNA/RNA regulatory mechanisms may undergo changes such that they alter their IF expression and therefore they may gain, lose, or express more than one IF. If this is true, then immunocytochemistry is of little value in elucidating the histogenesis of these tumours.

Finally, the origin of MMMTs may be from a single primitive multipotential stem cell. The "multipotential stem cell" theory has always been a convenient one as it explains both the differentiation into carcinomatous and sarcomatous elements as well as the plethora of histologic findings; yet it assumes a monoclonal proliferation. Naturally, the primitive stem cell possesses the intrinsic genetic material to code for all the cytoskeletal filaments and expression may therefore vary from one tumour cell to another. Given these restrictions, immunocytochemistry is unlikely to be of great value in determining the histogenesis.

CONCLUSION

This study has reviewed 81 cases of undifferentiated carcinoma, stromal sarcoma, adenosarcoma and Malignant Mixed Mullerian tumours. The immunohistochemical findings have been documented, and the value of immunologic

studies emphasised in accurately evaluating the precise nature of the lesion and the detection of heterologous elements. These are important both to the pathologist and to the clinician, if accurate classification and adequate clinical management are aimed for. The role of immunohistochemistry in contributing further to the histogenesis of MMMTs, has been disappointing.

ILLUSTRATIONS

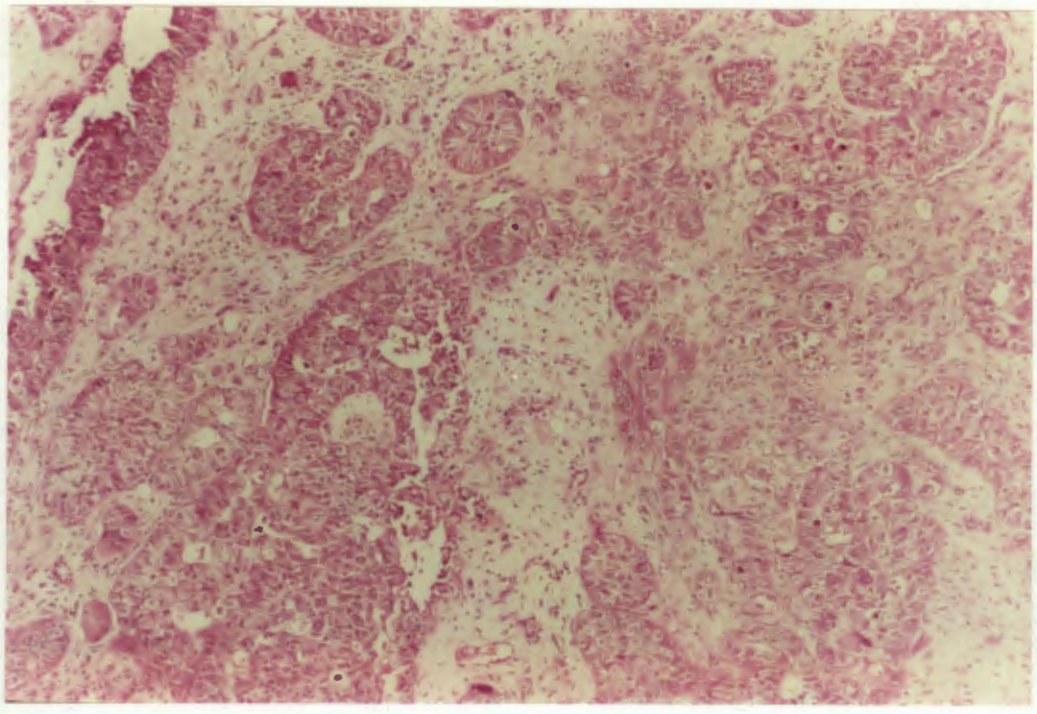
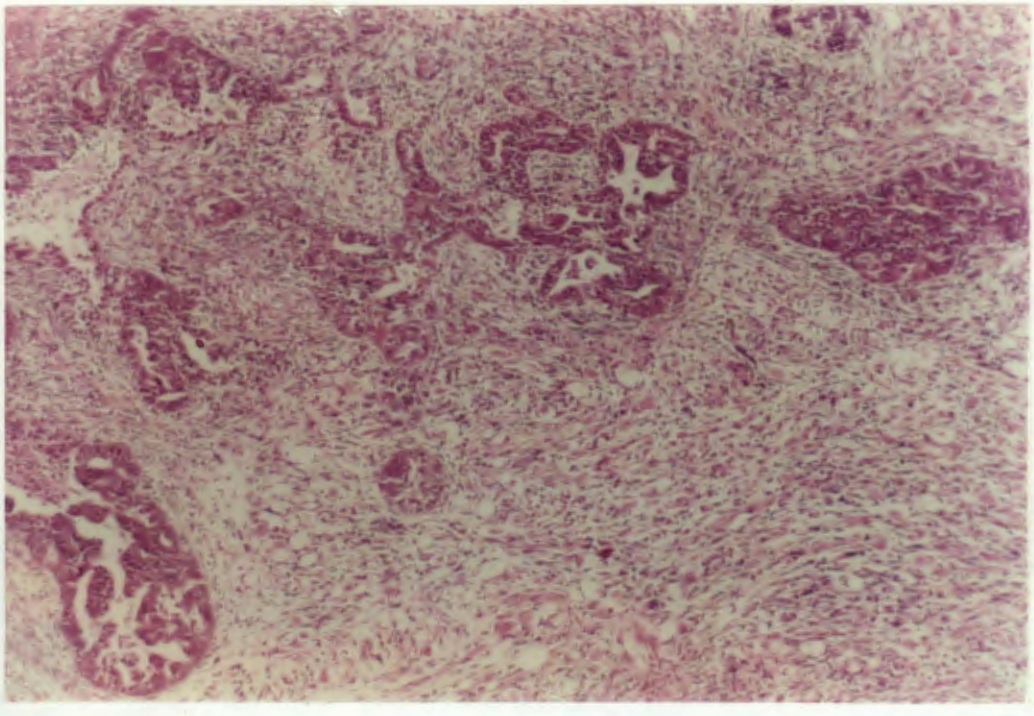


Figure 1a (above) and 1b (below) showing areas of moderately differentiated carcinoma interspersed with sarcomatous areas — the diagnosis of MMMT was made on both cases. (H & E, x 200)



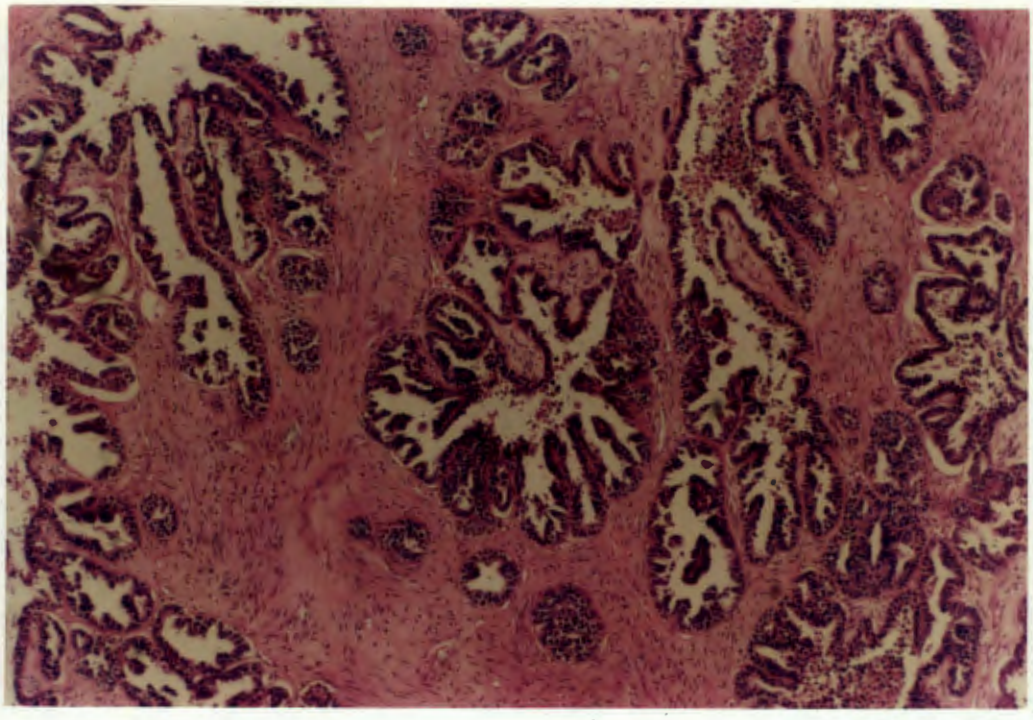


Figure 2 shows an area of serous papillary differentiation in the carcinomatous element of a MMMT. (H & E, x 150)

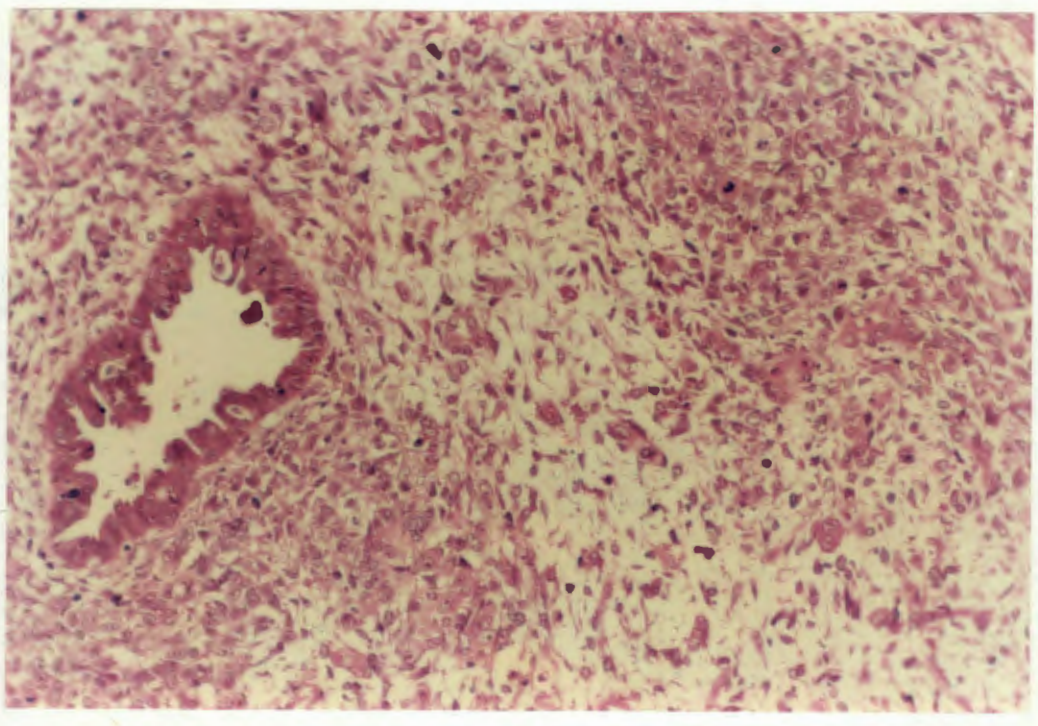


Figure 3 demonstrates the range of stromal cells in MMMT; spindled, stellate and rounded. (H & E, x 400)

Figure 4: Multinucleate and bizarre hyperchromatic cells.

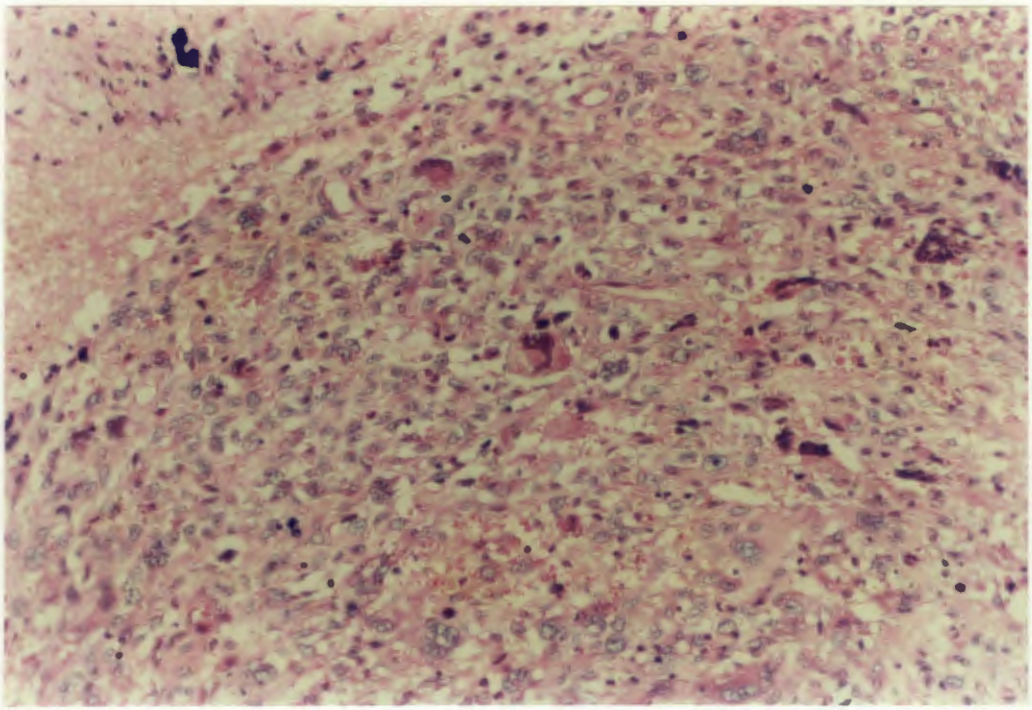


Figure 4a shows an ill-defined storiform pattern with scattered multinucleate cells — resembling MFH. (H & E, x 400)

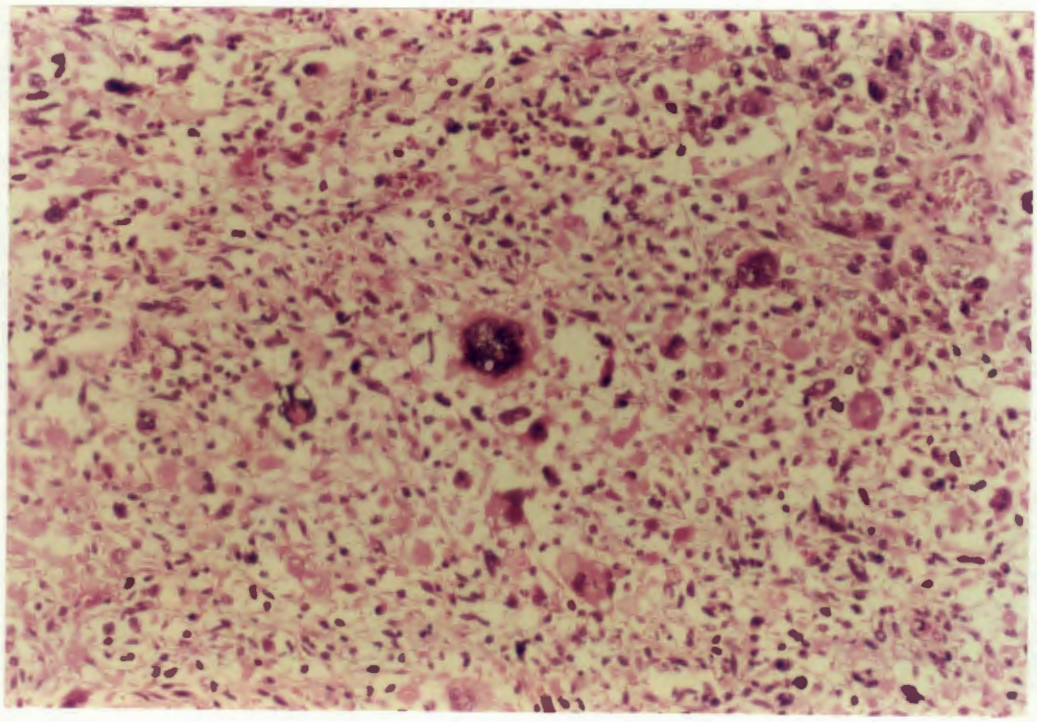


Figure 4b shows scattered hyperchromatic cells and a storiform pattern is not evident. (H & E, x 400)

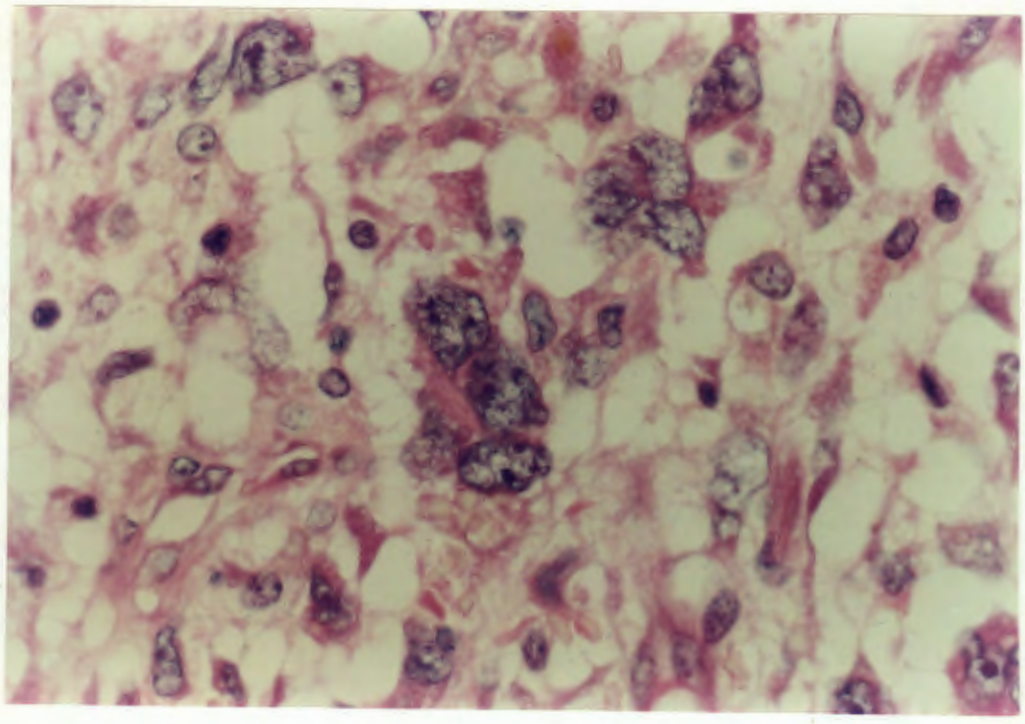


Figure 4c and 4d show bizarre multinucleate and hyperchromatic stromal cells. (H & E, x 1600)

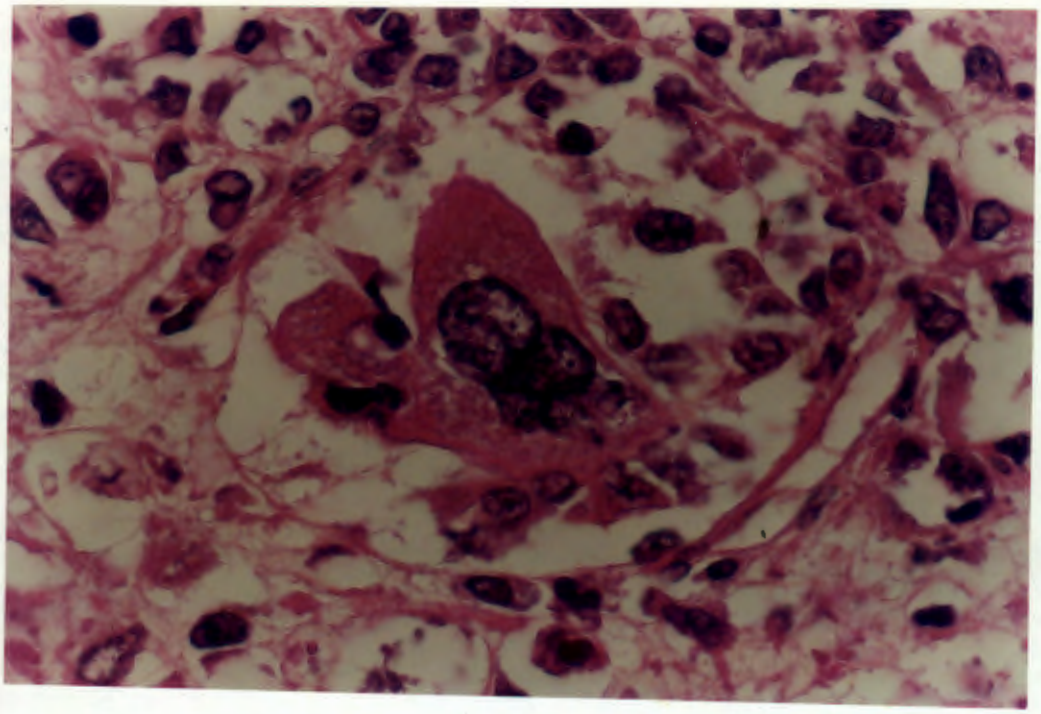


Figure 5 : Intracytoplasmic globules

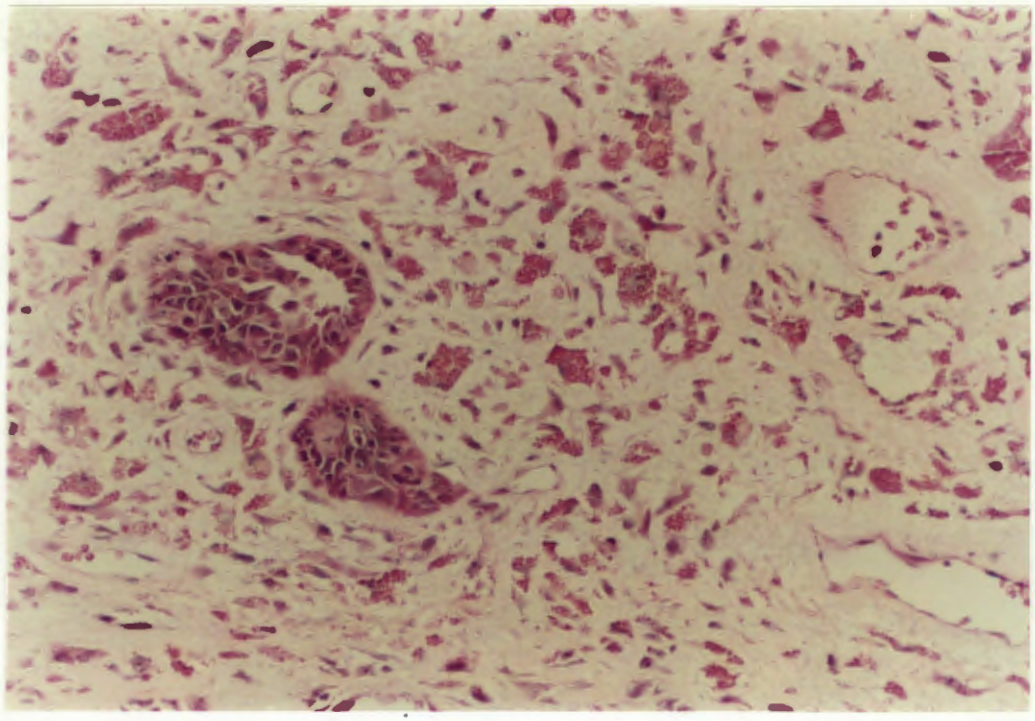


Figure 5a showing intracytoplasmic globules in the stromal cells, of varying sizes. (H & E, x 400)

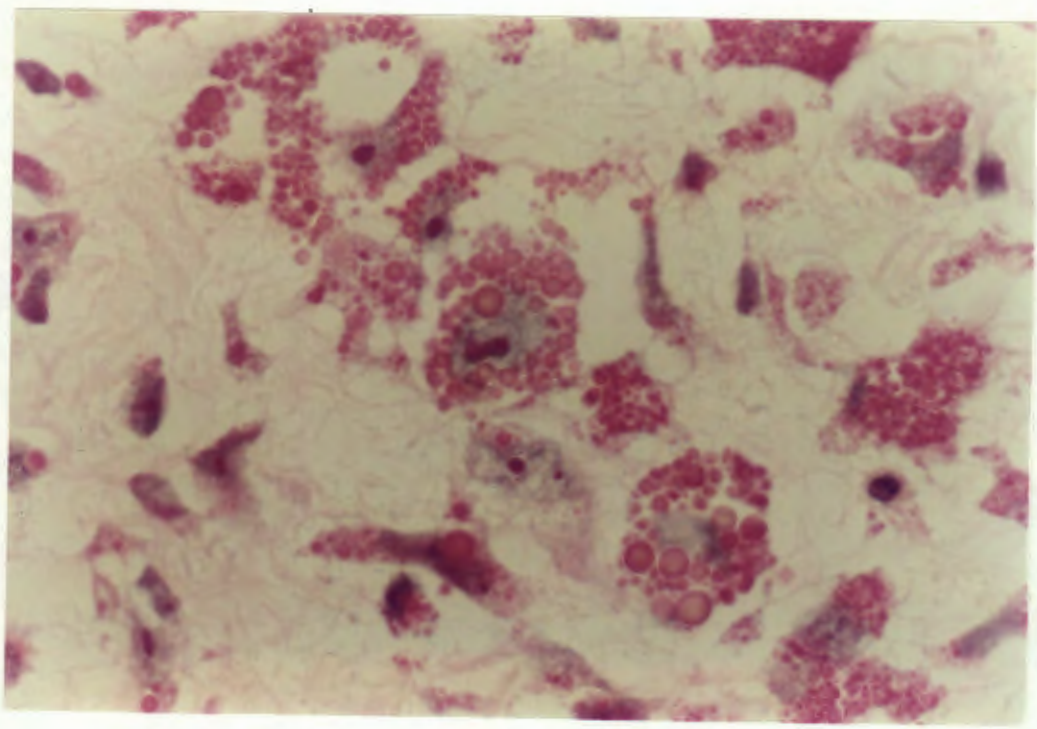


Figure 5b: shows a high power magnification. (H & E, x 1600)

Figure 6 : Rhabdoid stromal cells

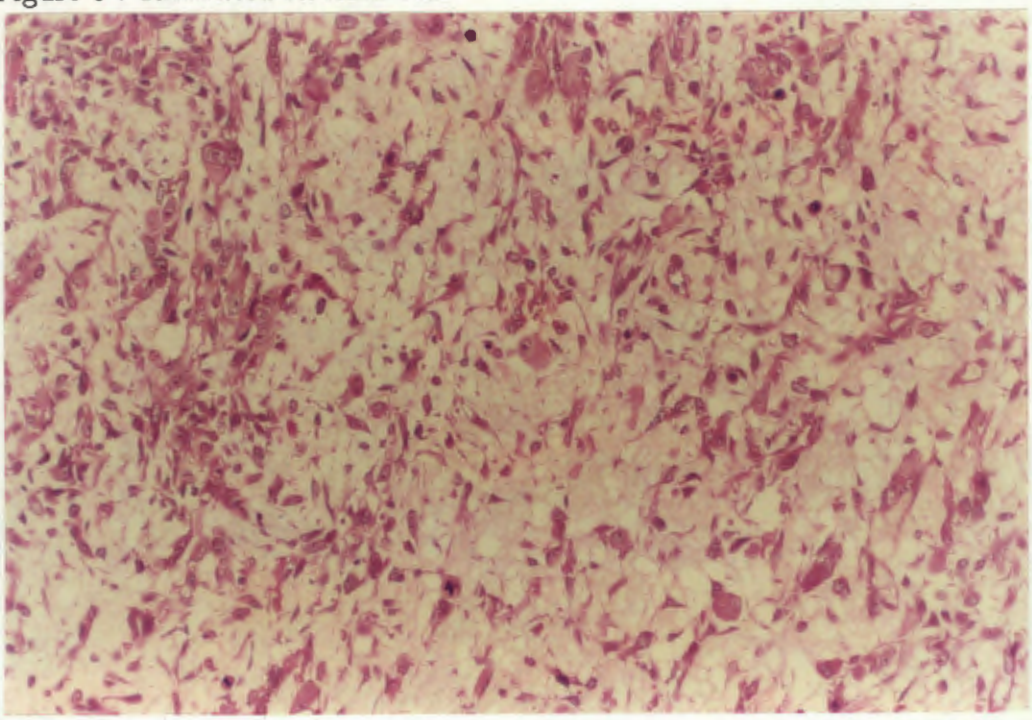


Figure 6a: showing rhabdomyoblastic differentiation of stromal cells on H & E staining (x 400).

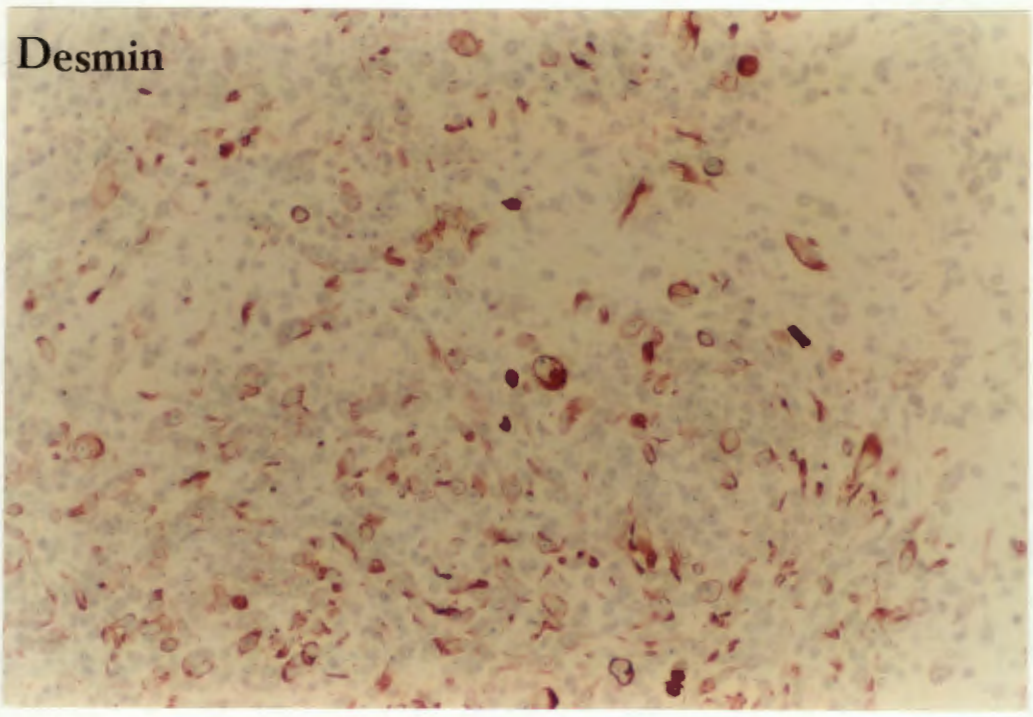


Figure 6b confirms this with strong desmin positivity in these cells. (Desmin, x 400)

Figure 7 : Rhabdomyoblasts

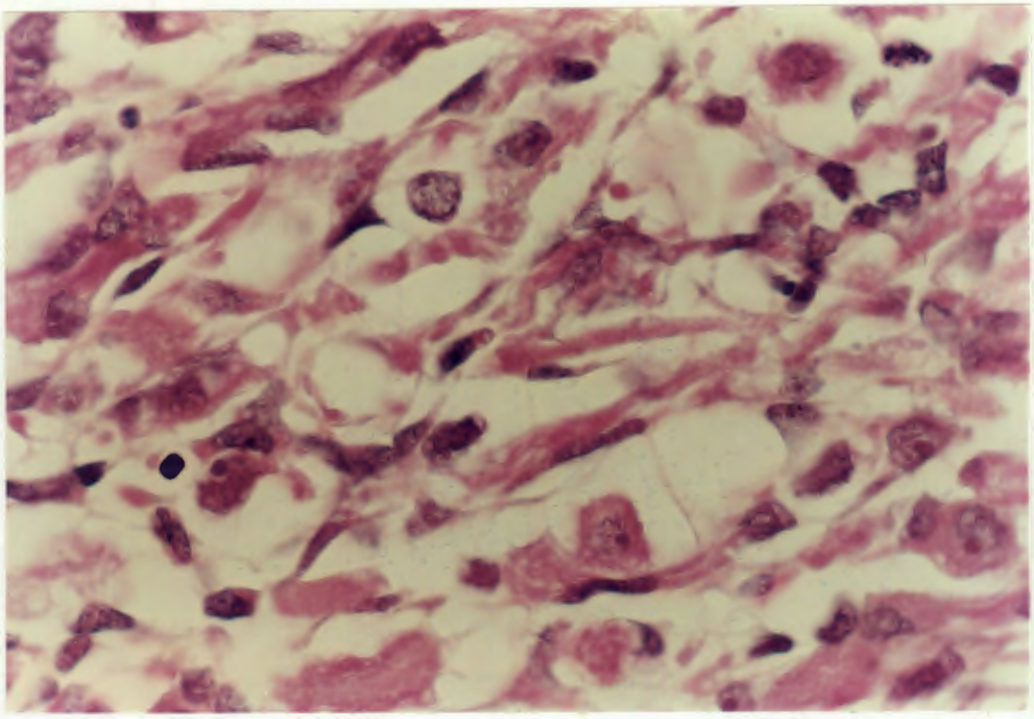
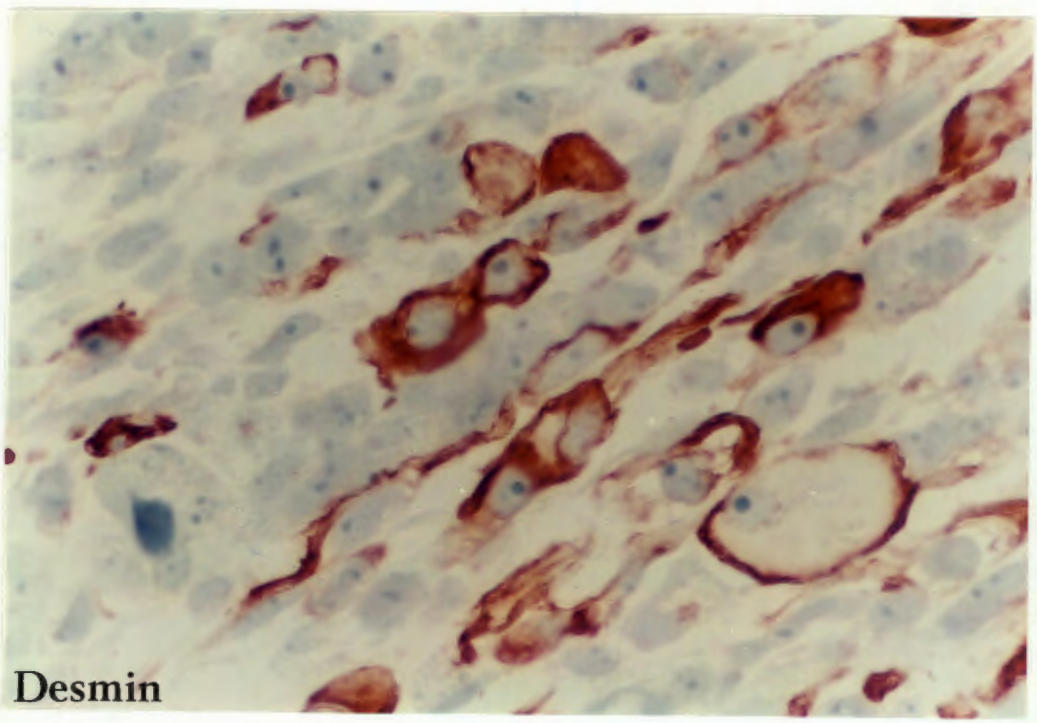


Figure 7a: (H & E, x 1600) staining spindled eosinophilic rhabdomyoblasts, which stain strongly positive with anti-desmin antibody in Figure 7b (below). (Desmin, x 1600)



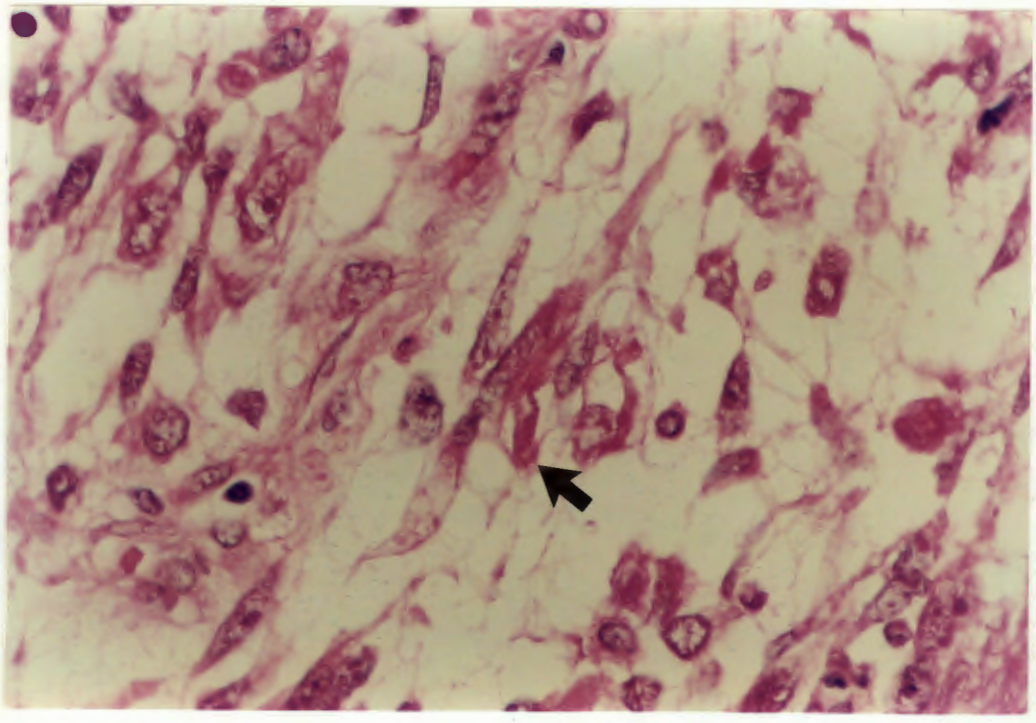


Figure 7c shows rhabdomyoblasts, the central one demonstrating cross striations. (H & E, x 1600)

Figure 8: Chrondrosarcomatous Foci.

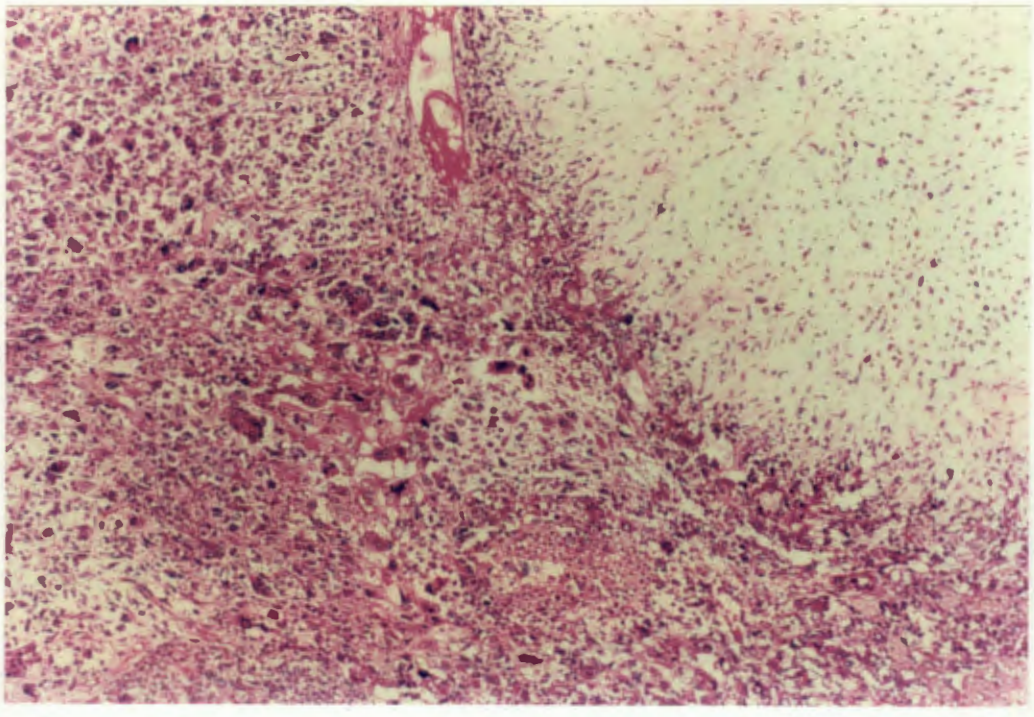
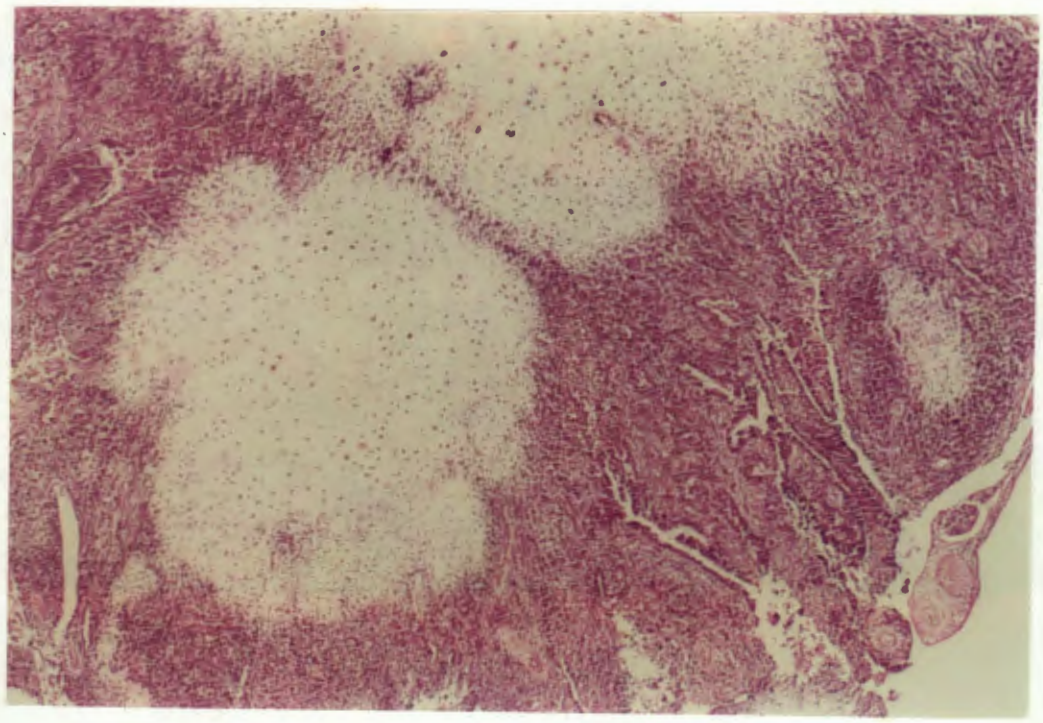


Figure 8a (H & E, x 400) and Figure 8b (H & E, x 200) showing foci of chondroid differentiation.



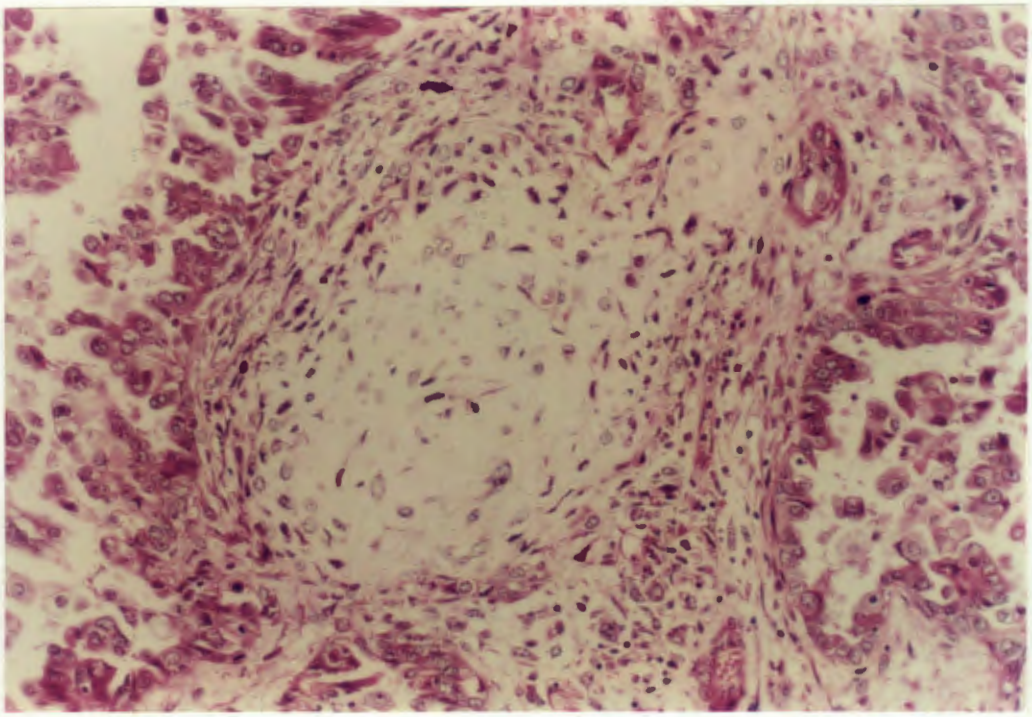


Figure 8c showing a focus of chondroid differentiation. (H & E, x 400)

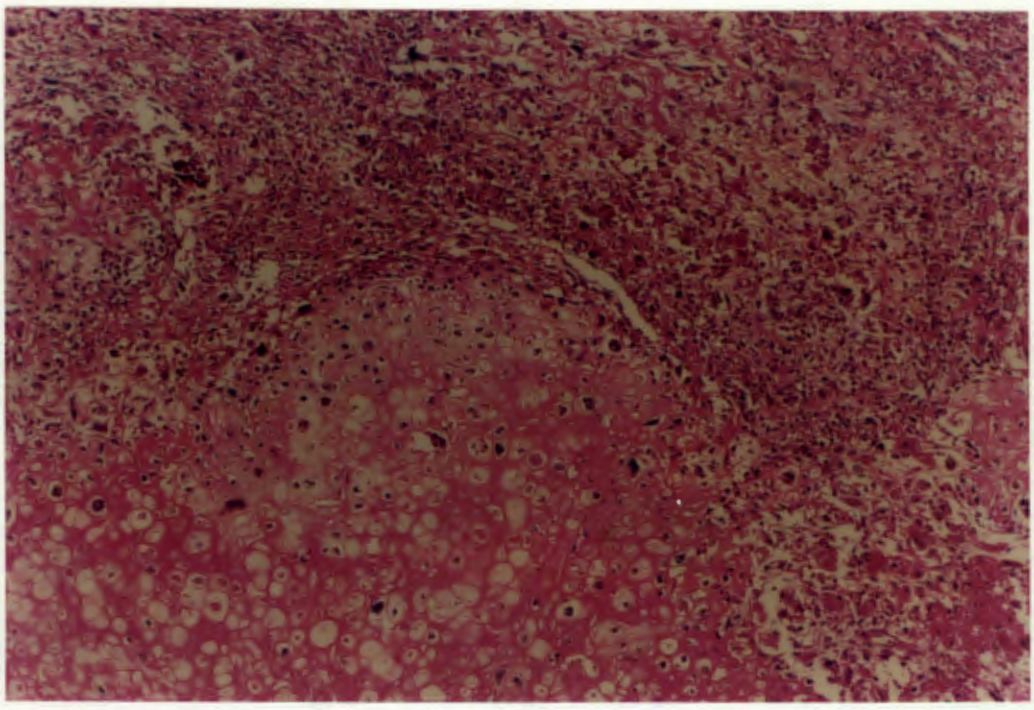


Figure 8d showing another chondrosarcomatous focus with malignant chondroid cells in lacunar spaces. (H & E, x 200)

Figure 9 : Osteosarcomatous Differentiation

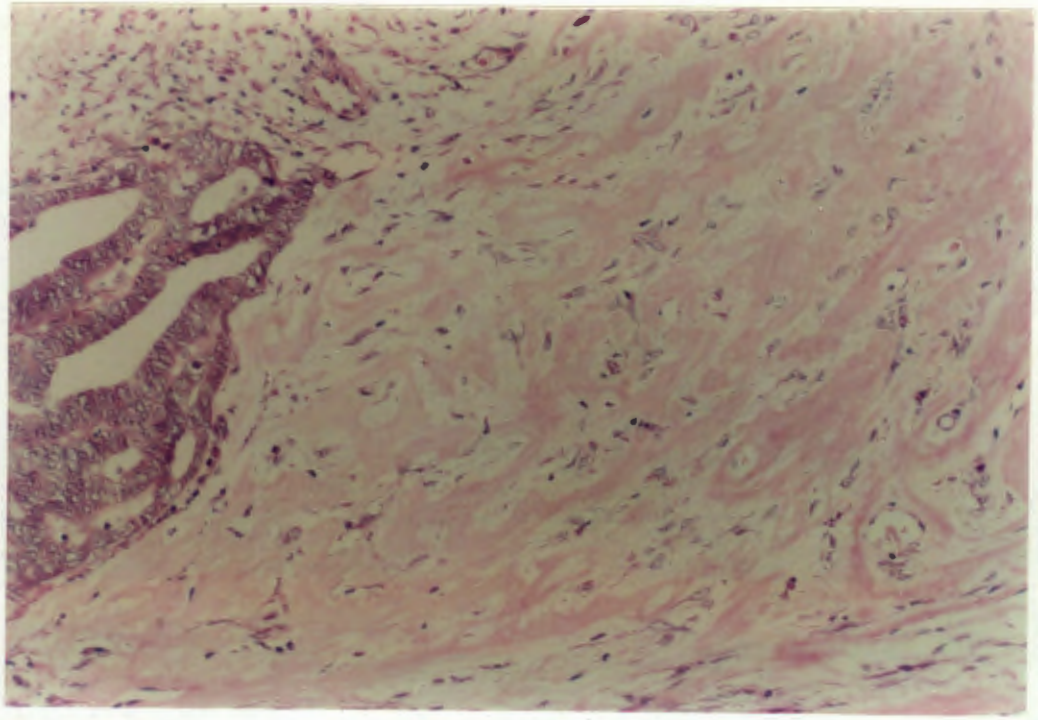


Figure 9a (above) and Figure 9b (below) showing foci of fine interlacing osteoid interspersed between malignant stromal cells. (H & E, x 400)

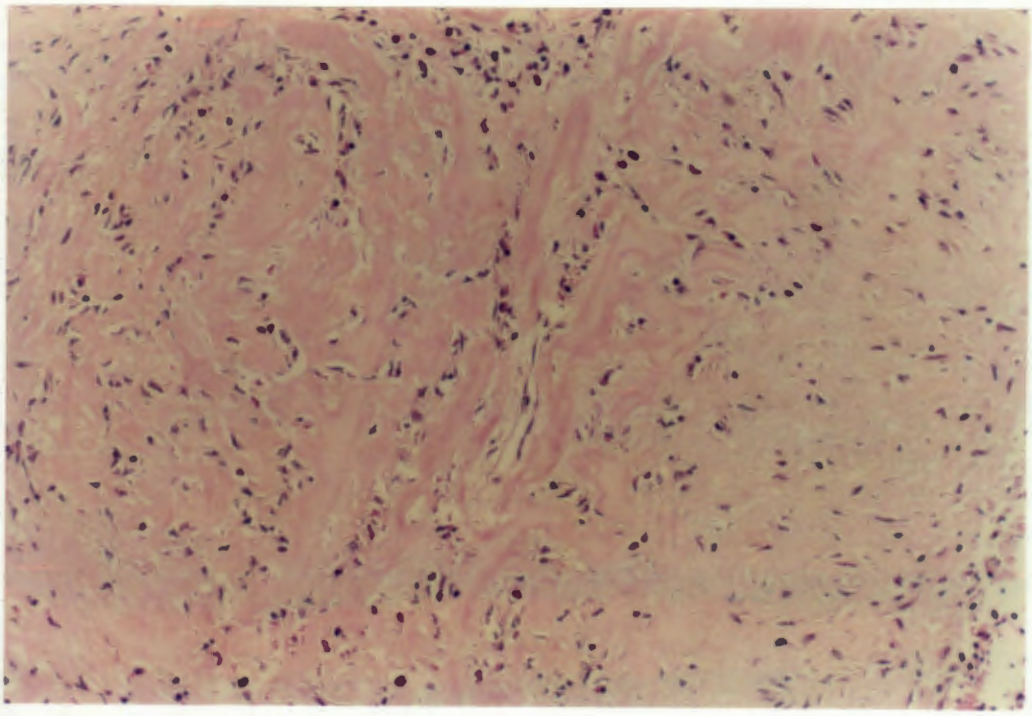


Figure 10: Control Endometrium

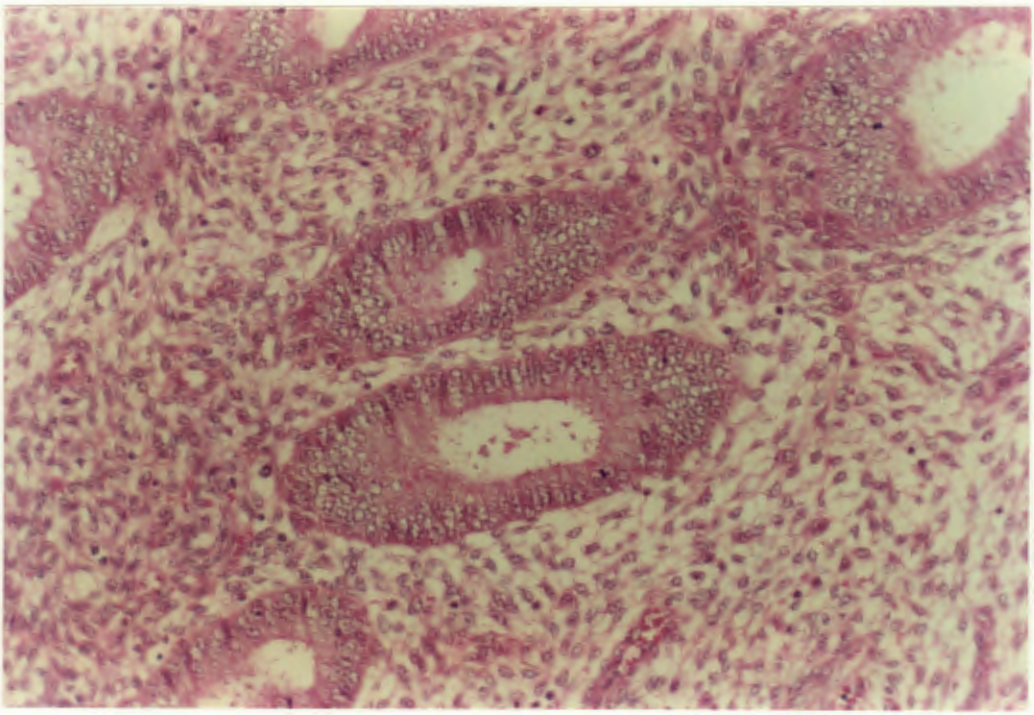


Figure 10a: Normal proliferative endometrium. (H & E, x 400)

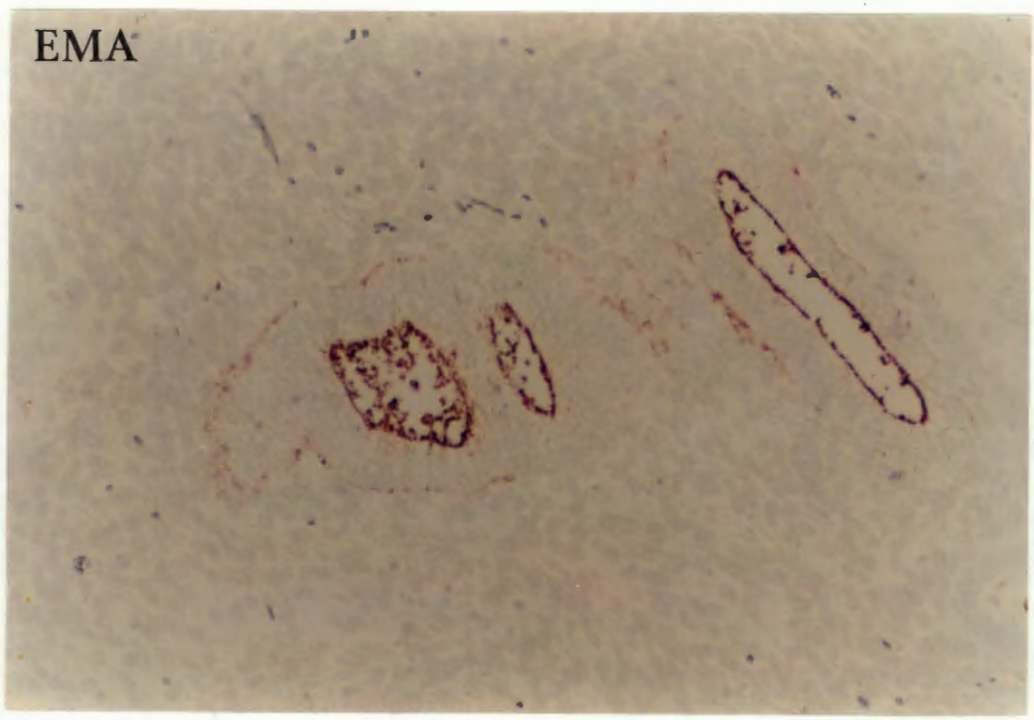


Figure 10b: The above case showing glandular staining with epithelial membrane antigen. (EMA, x 400)

Figure 10c demonstrating Cytokeratin positivity in normal endometrium. (CK, x 400)

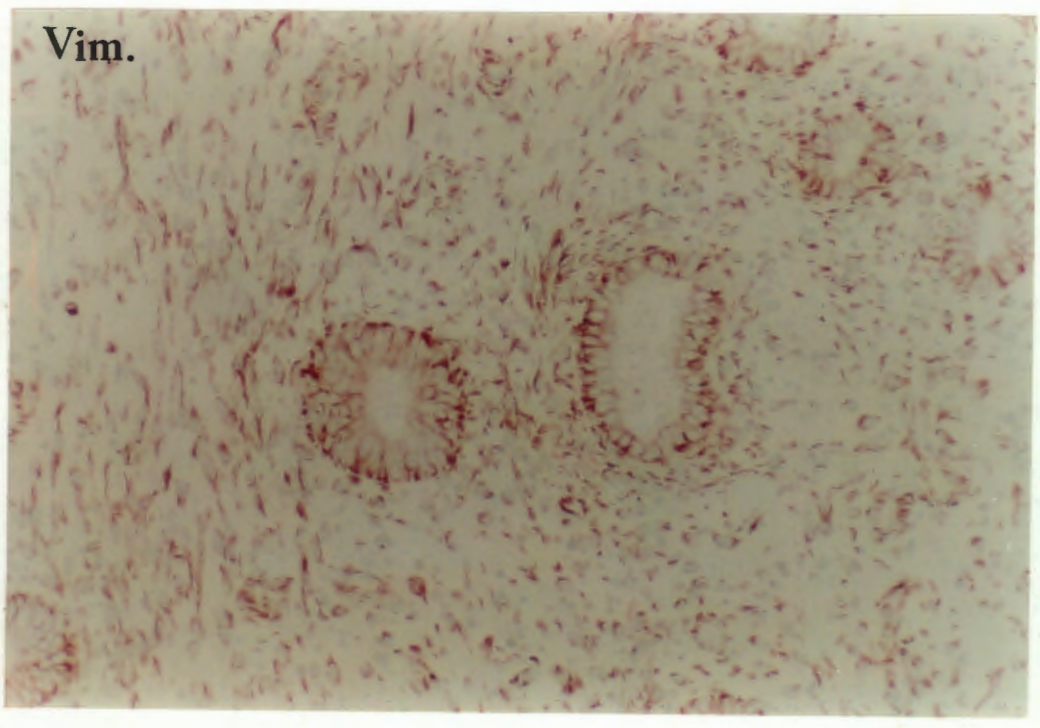


Figure 10d demonstrating Vimentin positivity in both the glands and stroma. (Vimentin, x 400)

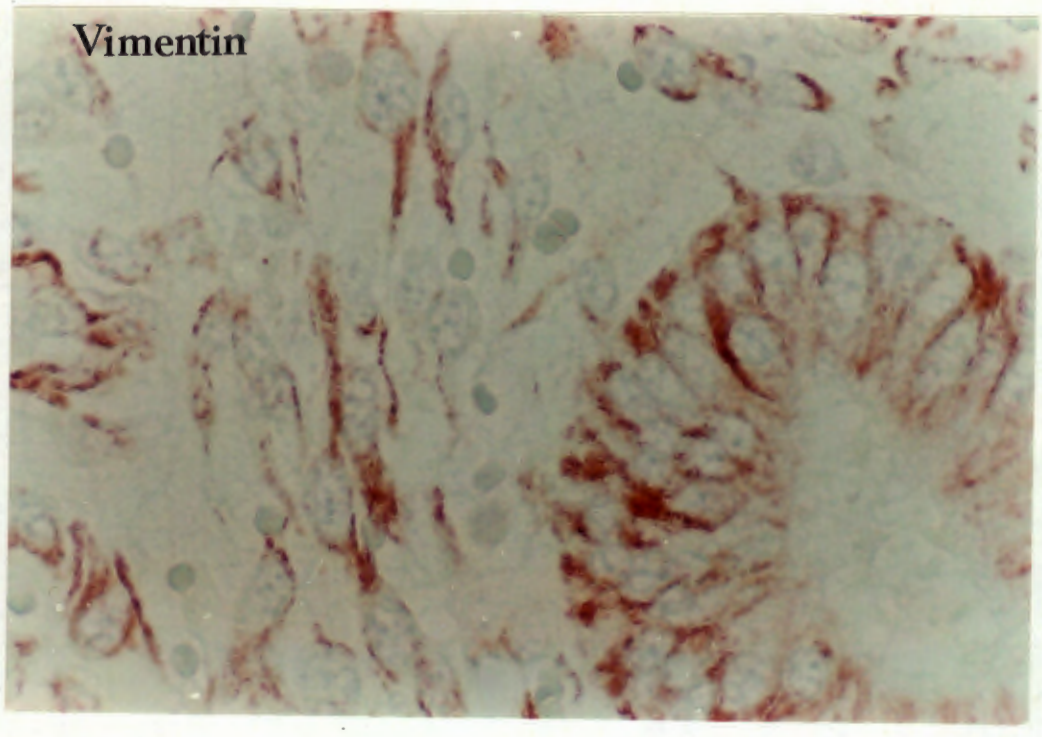


Figure 10e shows a high power magnification of vimentin positivity in both stromal and glandular elements. (Vimentin, x 1600)

Figure 11 : Homologous MMMT

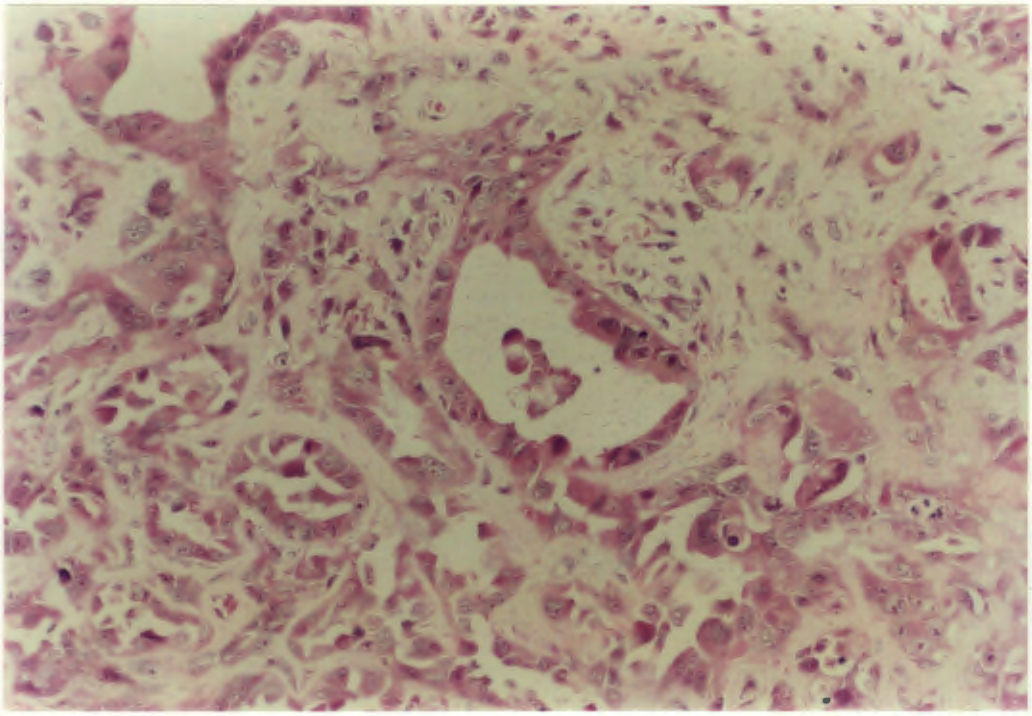


Figure 11a: Homologous MMMT showing areas of poorly differentiated adenocarcinoma. (H & E, x 400)

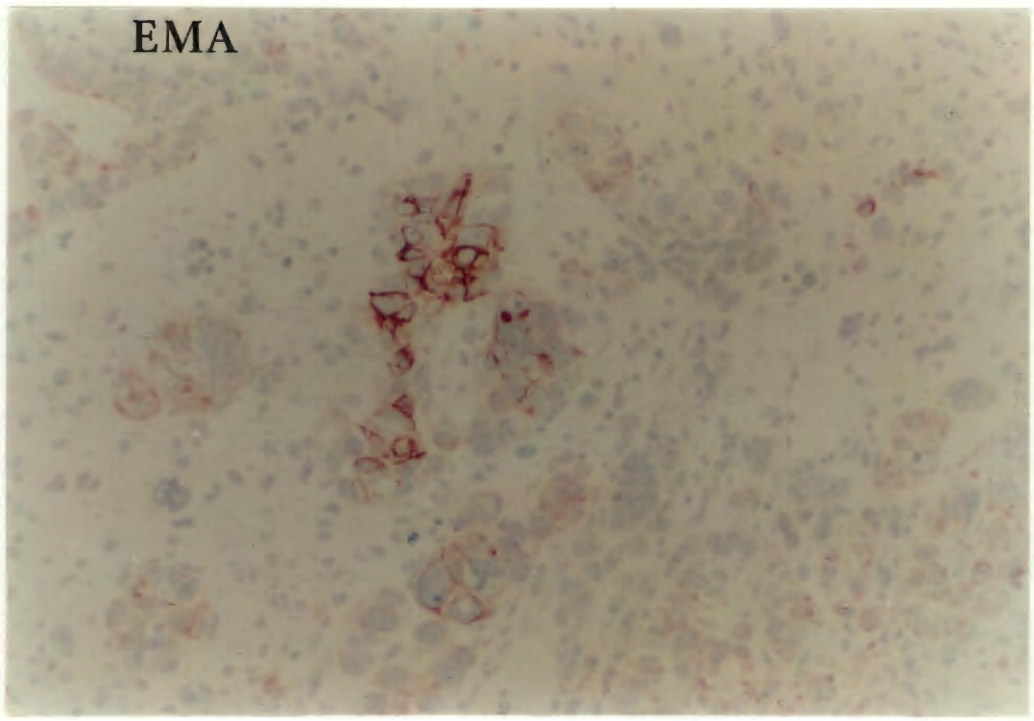


Figure 11b shows EMA positivity in some of the glandular areas. (EMA, x 400)

Figure 12 : Homologous MMMT

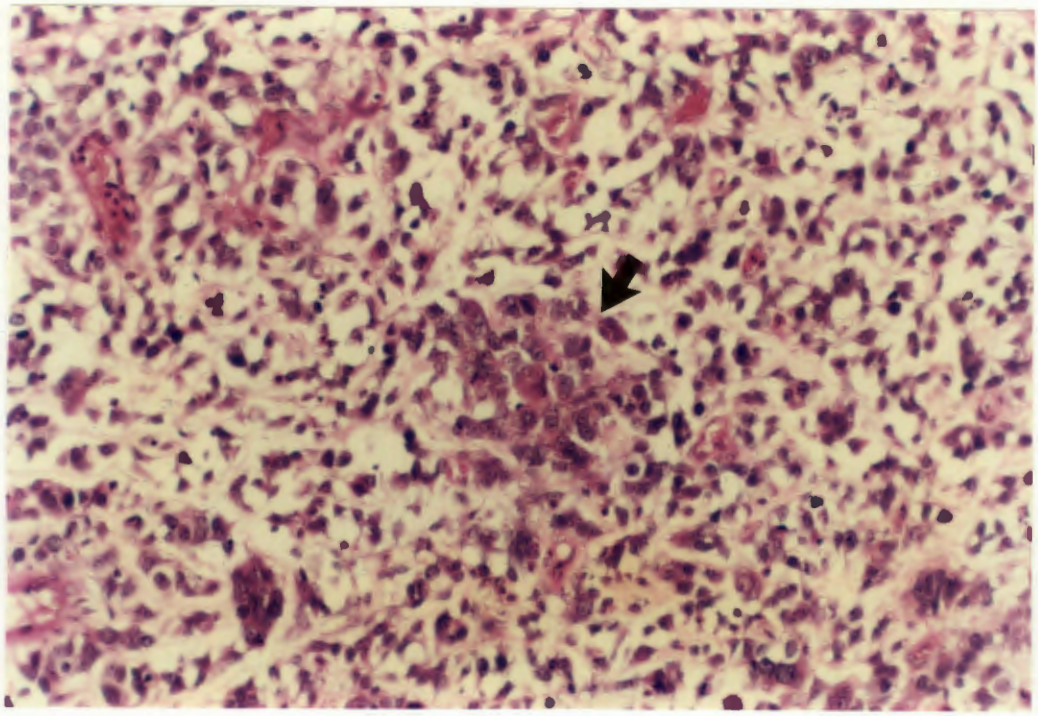


Figure 12a showing an ill-defined aggregate (arrow) of undifferentiated carcinomatous cells. (H & E, x 400)

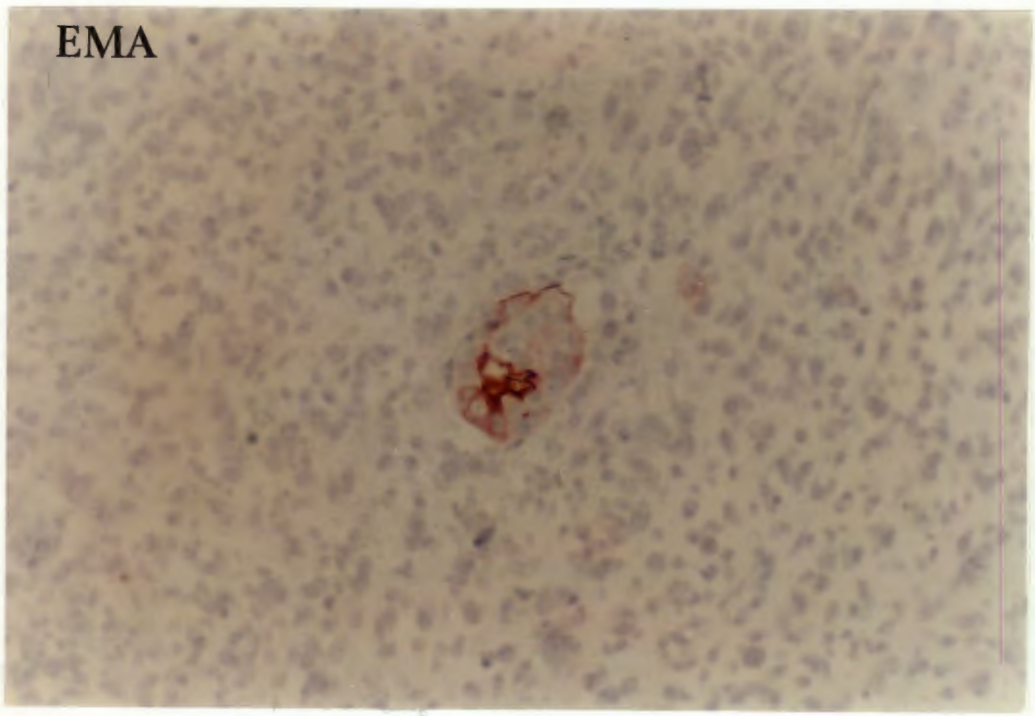


Figure 12b highlights this aggregate with strong EMA positivity. (EMA, x 400)

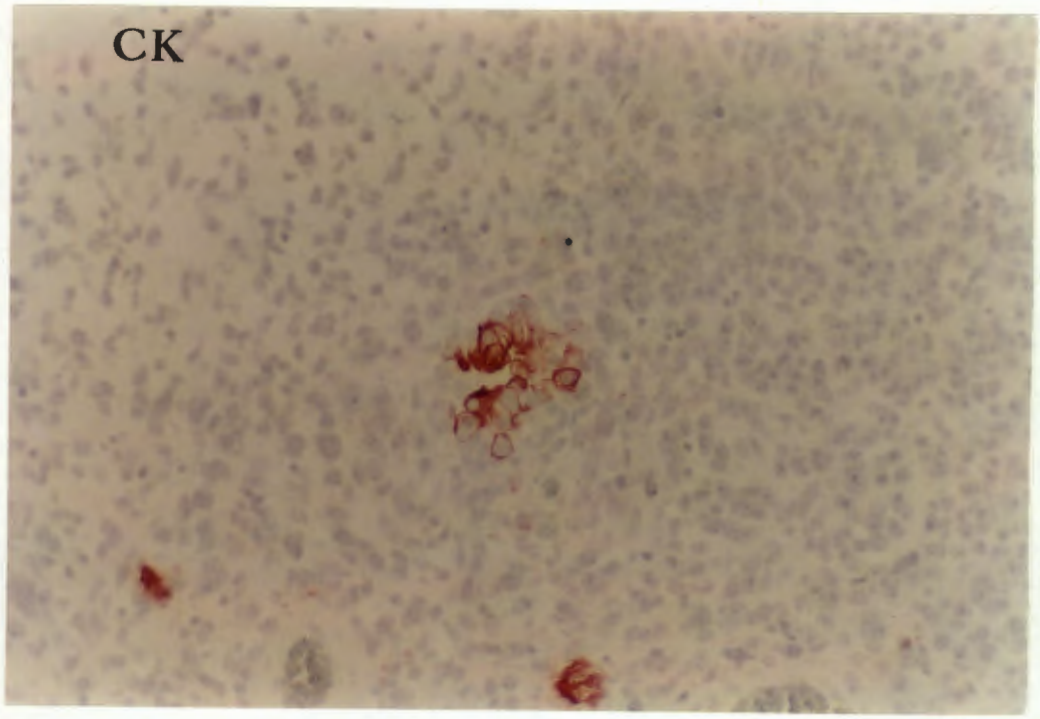


Figure 12c showing cytokeratin positivity in the carcinomatous element. (CK, x 400)

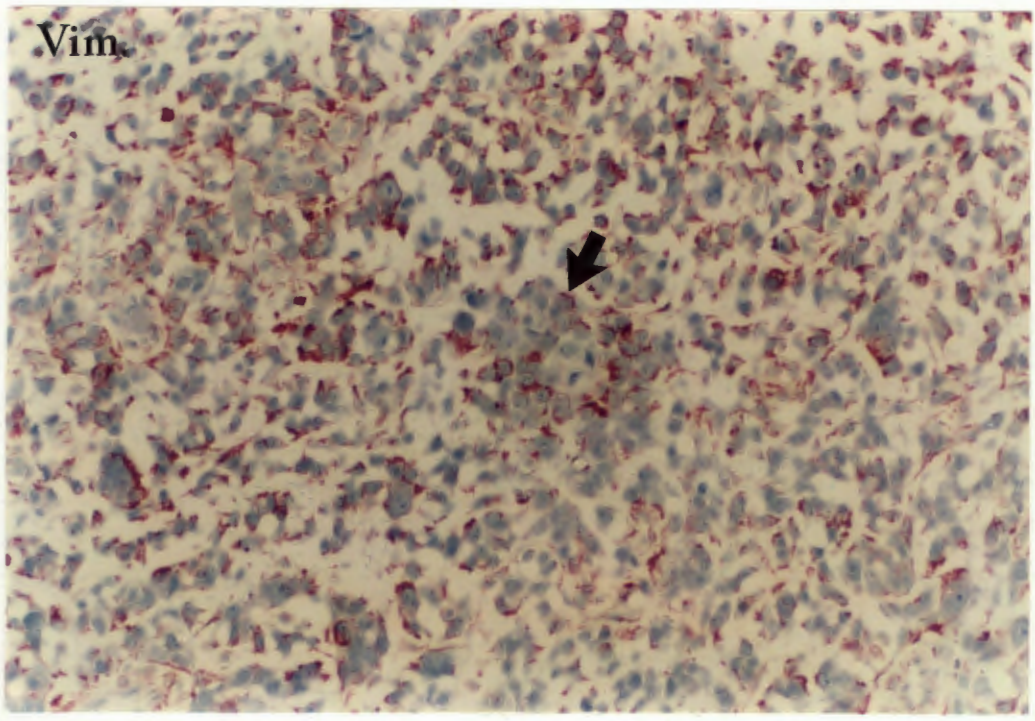


Figure 12d shows vimentin positivity in both stromal and epithelial components. (Vimentin, x 400)

Figure 13 : Case Illustration of a Typical MMMT

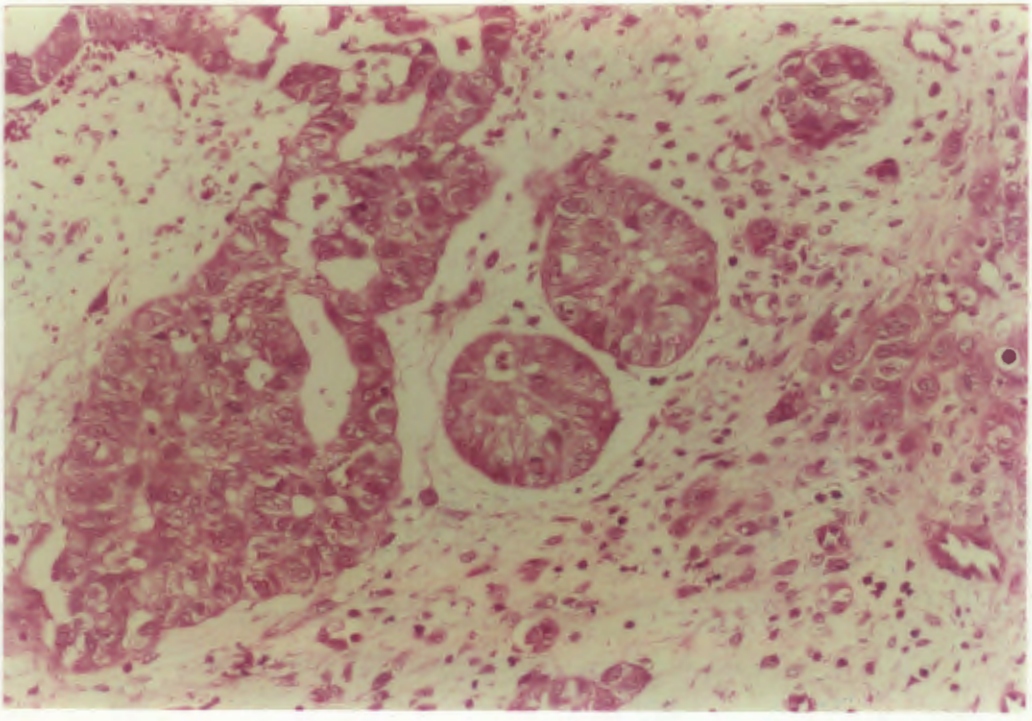


Figure 13a demonstrates a typical MMMT with plump eosinophilic stromal cells. (H & E, x 400)

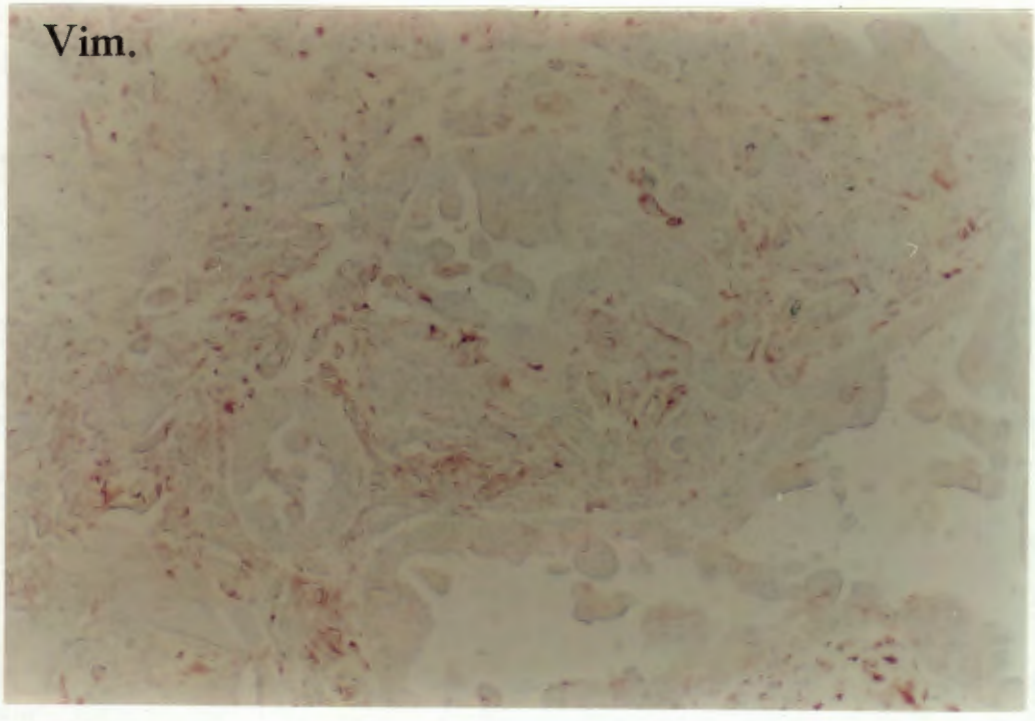


Figure 13b shows weak Vimentin positivity in the stromal cells. (Vimentin, x 400)

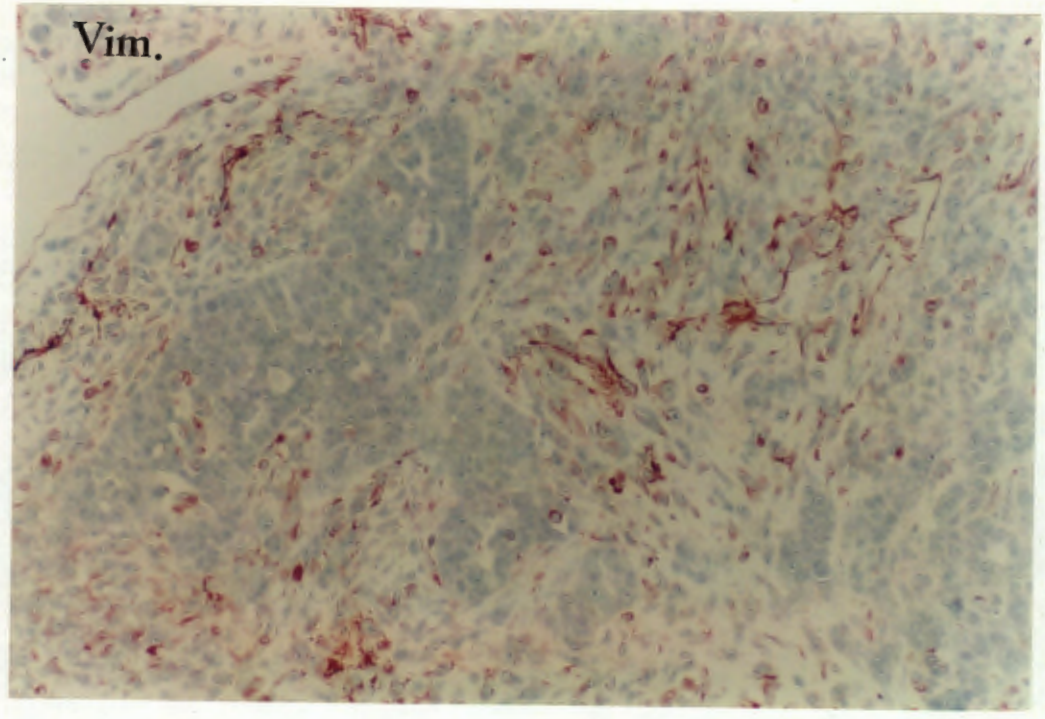


Figure 13c shows a different area of the same tumour where Vimentin staining is more pronounced. (Vimentin, x 200)

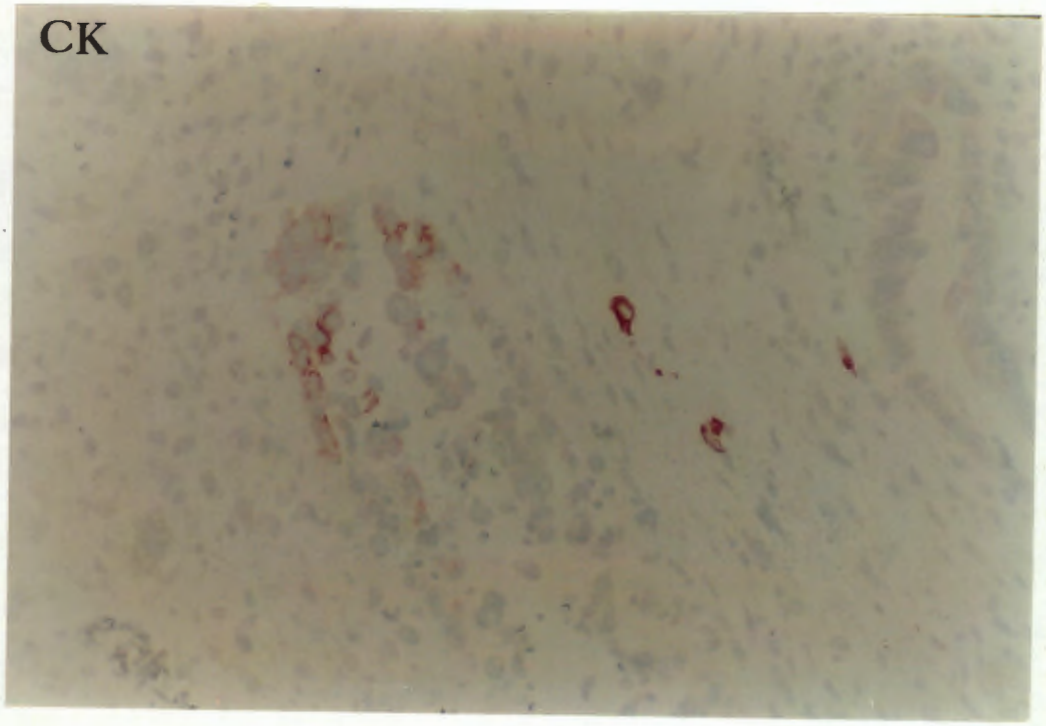


Figure 13d shows glandular elements staining positively with cytokeratin and scattered free-lying carcinoma cells. (Cytokeratin, x 400)

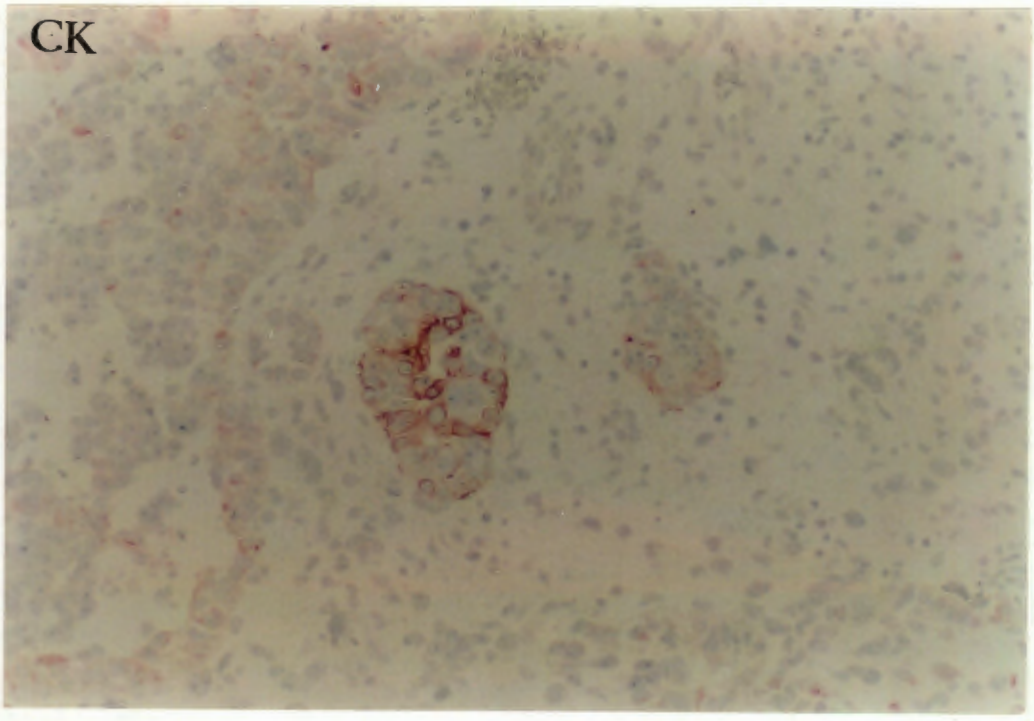


Figure 13e demonstrating Cytokeratin positivity in this tumour. (Cytokeratin, x 400)

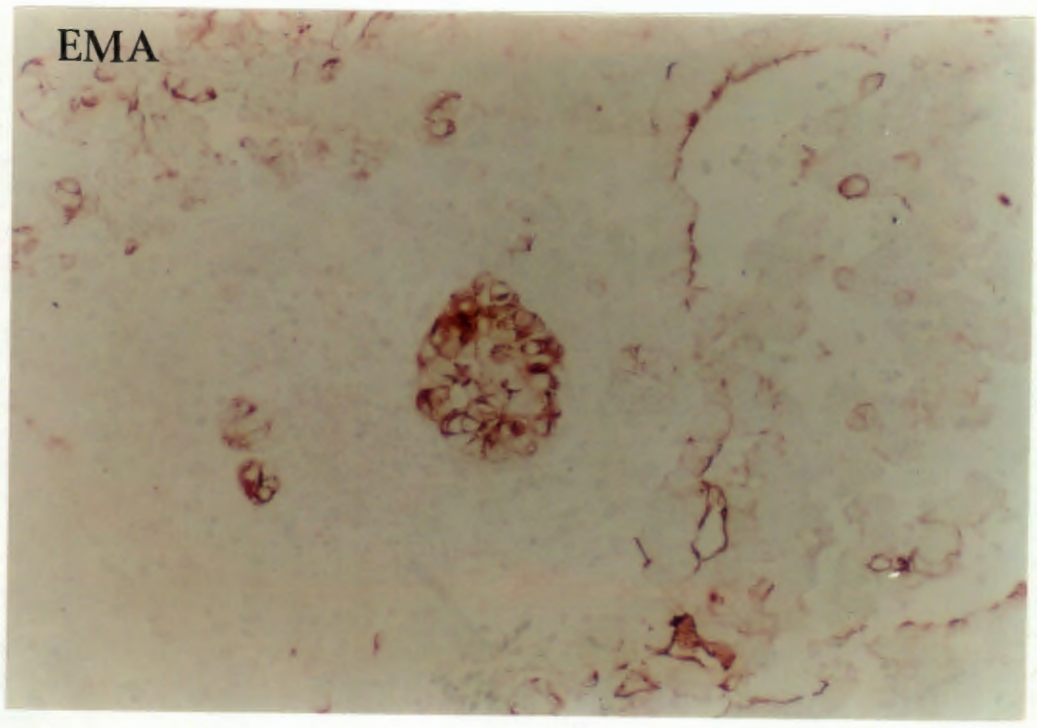


Figure 13f shows strong epithelial membrane antigen positivity in the same area of the tumour as in Fig. 13e. (EMA, x 400)

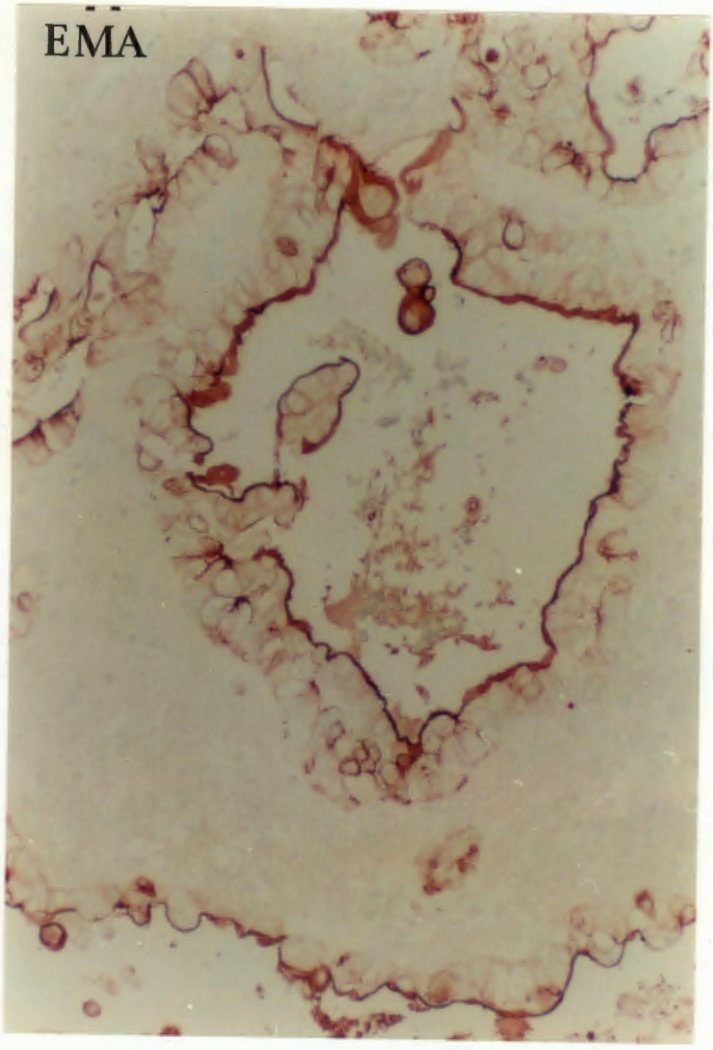


Figure 13g demonstrates the strong membrane staining in another area of the same tumour. (EMA, x 400)

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