

## Invited Review

# Epithelial-to-mesenchymal change of differentiation. From embryogenetic mechanism to pathological patterns

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**Summary.** In embryonic morphogenesis, dramatic changes from one state of differentiation to another take place, and some epithelia transform into mesenchymal cells endowed with the ability to migrate and to form connective tissue. In vitro model systems have been developed which have provided new insights into crucial aspects of this differentiation change. Triggered perhaps by either extracellular matrix or growth factors, this phenotypic conversion involves a reorganization of the cytoskeleton, and changes in both cell-cell and cell-matrix interactions.

As embryonic and adult tissues contain the same, albeit differently expressed, genetic information, one could expect, under particular circumstances, conversion to mesenchyme from epithelium to occur in adult tissues too. Indeed, there is evidence that this change really occurs in human diseases: some tissue reactions to injury; the process of tumour invasion and metastasis; and the development of carcinosarcomas, are all pathological conditions in which an epithelial conversion into mesenchyme probably plays a role. Here, recent observations on embryonic and in vitro epithelial-mesenchymal conversion are reviewed, and these data are compared with findings from some pathological situations. Many similarities emerge which further strengthen the belief that this change in differentiation is involved in the pathogenesis, and underlies the pathological pattern of some diseases.

**Key words:** Differentiation, Epithelial-mesenchymal conversion, Tumour invasion, Carcinosarcoma, Tissue repair

### Introduction

The large number of specialized functions of the adult organism is accomplished by differentiated cells which

form the various tissues and organs. It is known that early embryonic cells are endowed with a great differentiation potential and are able to give rise to many different tissues. This potentiality is soon lost in the subsequent stages of development, and cells become limited to a unique pathway of differentiation, resulting in tissue-specific phenotypes which remain stable in adult life. Nevertheless, the state of differentiation of some cells may undergo some change under particular conditions, indicating that a substantial phenotypic plasticity is also retained in adult specialized cells (Di Berardino et al., 1984; Okada, 1986; Blau, 1992). Although epithelial and mesenchymal cells segregate early in embryonic life and are destined to form distinct tissues, during morphogenesis a series of peculiar interconversions between these basic tissues occurs. The purpose of this article is to review recent observations concerning these phenotype changes, focusing on the conversion of epithelium to mesenchyme. The reverse process conversion of mesenchyme to epithelium, has been documented in embryonic development (Eklom, 1989), in experimental studies (Darmon et al., 1984; McNeil et al., 1990), and tumours (Guarino, 1993a; Guarino and Christensen, 1994), and will not be further re-examined here. In the following discussion the possible role of epithelial-mesenchymal conversion in some pathological conditions, including reactive and neoplastic situations, will be investigated in the light of data available from embryology and from in vitro studies.

### The epithelial and mesenchymal phenotypes and embryonic epithelium-to-mesenchyme conversion

All mesenchymal cells of the body are derived from embryonic epithelia (Hay, 1984, 1989, 1990). Indeed, conversion of epithelium into mesenchyme represents an important morphogenetic mechanism, as it allows dissociation and migration of cells far from an epithelial sheet to form a new tissue in distant sites (Hay, 1990). An epithelium is a sheet of closely-linked cells that

exhibit apical-basal polarity and reside on top of a basement membrane. The lateral surfaces of adjacent epithelial cells are linked by cell junctions and contain cell adhesion molecules typical of epithelia. A typical mesenchymal cell, on the other hand, has an usually elongated shape, exhibits front end-back end polarity and produces interstitial matrix components. These cells interact with extracellular matrix (ECM) through receptors distributed on the whole cell surface, and do not form intercellular junctions. In keeping with their capability to migrate, they are often endowed with locomotory appendages such as filopodia and pseudopodia. Moreover, whereas epithelia are typically rich in cytokeratin intermediate filaments, mesenchymal cells have a vimentin-based cytoskeleton (Hay, 1989, 1990). Therefore, when an embryonic epithelium transforms into mesenchyme (Fig. 1), a very profound change takes place. As a matter of fact, this process occurs in a highly-controlled fashion only in predetermined epithelia, at specific times of embryonic development. Formation of primary mesenchyme, neural crest cells, sclerotome mesenchyme, and endocardial cushions are well studied examples of embryonic epithelial-mesenchymal conversion (Hay, 1984, 1989,

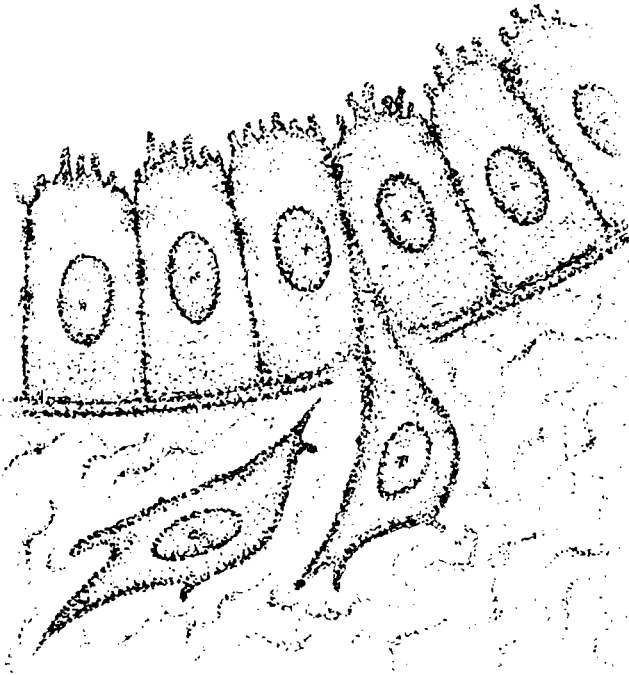
1990). Regression of male Müllerian ducts and fusion of palatal shelves, also seem to involve this mechanism (see Hay, 1984, 1989, 1990, and refs. therein).

### Mechanisms of epithelial-mesenchymal conversion

The changes observed during conversion of epithelium to mesenchyme occur in a coordinated way, probably resulting from activation of a specific genetic program (Hay, 1989, 1990). What triggers this complex mechanism is not yet clear, but some evidence emphasizes the importance of ECM components (Hay, 1984, 1989, 1990). ECM stores and regulates activity of many growth factors (Flaumenhaft and Rifkin, 1991), and it is known that both ECM and growth factors influence cell differentiation. The first morphological change probably occurs at the epithelial basal surface, where filopodial processes appear (Hay, 1990). Basement membrane is either already absent, or disrupts in the early stages of the conversion (Hay, 1984, 1989, 1990). In the next step, filopodia extend into the interstitial matrix, the cell elongates, and finally it detaches from the parent epithelium. The newly-formed mesenchymal cell has an elongated shape and shows a front end-back end polarity (Fig. 1).

This profound morphological transformation likewise involves biochemical and functional changes in the cytoskeleton, cell-cell adhesion, and cell-matrix interactions (Fig. 2). Studies on both embryonic and *in vitro* epithelial-mesenchymal conversion indicate that, on the onset of this change, synthesis of the mesenchymal filament vimentin is started, whereas cytokeratin disappears. Indeed, a vimentin cytoskeleton seems to be important for cell elongation and subsequent migration (Greenburg and Hay, 1988; Hay, 1989, 1990).

## CONVERSION TO MESENCHYME FROM EPITHELIUM IN THE EMBRYO

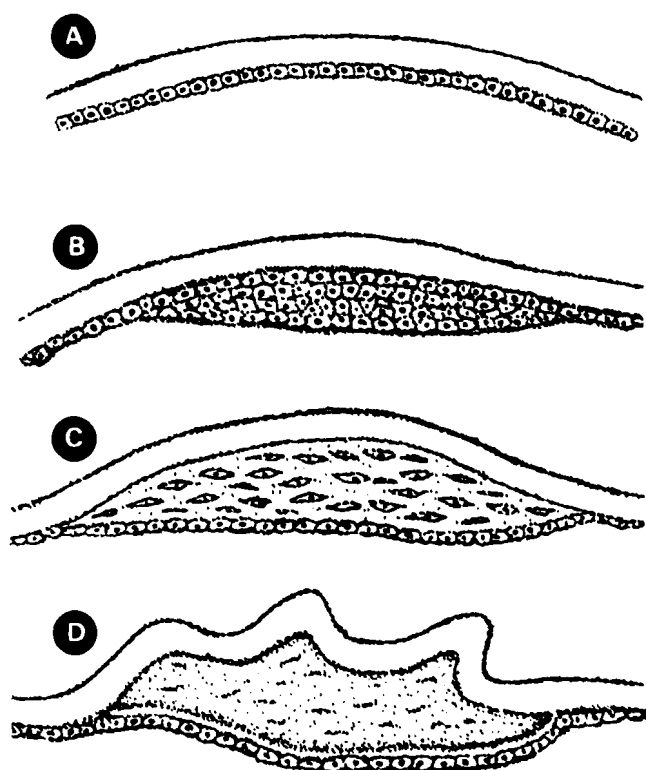


**Fig. 1.** Drawing depicting the morphological changes occurring when an embryonic epithelium transforms into mesenchyme. The transforming cell becomes elongated, extends into the underlying ECM through a basement membrane interruption and, finally, leaves the parent epithelium as a mesenchymal cell. Modified from Hay (1984); by permission of J. Wiley & Sons, Inc.

### PHENOTYPE CHANGES IN EPITHELIAL-MESENCHYMAL CONVERSION

- INTERMEDIATE FILAMENTS
  - Appearance of vimentin
  - Loss of cytokeratin
- CELL-TO-CELL ADHESION
  - Loss of intercellular junctions
  - Changes in cell adhesion molecules
- EXTRACELLULAR MATRIX
  - Loss of basement membranes
  - Synthesis of mesenchymal matrix
- CELL MORPHOLOGY AND FUNCTION
  - Loss of cell cohesiveness
  - Cell shape and polarity changes
  - Gain of motility

**Fig. 2.** A series of biochemical and functional changes underlies epithelial-mesenchymal conversion, the final result being a morphological transformation. The main changes occur in the cytoskeleton, intercellular adhesive interactions, and cell-matrix relationship.



**Fig. 3.** Schematic representation of steps occurring in the formation of anterior capsular cataract. After an injury, epithelial lens cells (A) migrate and proliferate in the damaged area (B) and transform into fibroblastic cells which elaborate a fibrous matrix (C and D). Redrawn from Font and Brownstein (1974); by permission of the American Journal of Ophthalmology. Copyright by the Ophthalmic Publishing Company.

The desmosomal plaque components desmoplakins, desmoglein and plakoglobin (Thiery et al., 1988; Boyer et al., 1989a,b; Zuk et al., 1989; Boukamp et al., 1992; Boukamp and Fusenig, 1993), and the epithelial cell adhesion molecule E-cadherin (also known as uvomorulin or L-CAM) (Thiery et al., 1988; Tucker et al., 1990; Boukamp and Fusenig, 1993), are lost or undergo redistribution. Therefore, epithelial intercellular junctions disrupt, resulting in reduced cell-cell adhesion, which, in turn, favours cell detachment and emigration from the parent epithelium. In order to move into the interstitial matrix, new ECM receptors must appear on the converting cells, and mesenchymal-type ECM components may be produced by these cells (Greenburg and Hay, 1986, 1988; Hay, 1990; Boukamp and Fusenig, 1993). So, synthesis of the basement membrane components laminin and type IV collagen is replaced by that of fibronectin and type I collagen (Hay, 1989).

#### In vitro models and experimental studies

In a series of experiments, Greenburg and Hay observed that various types of epithelia transform into mesenchymal cells when suspended in type I collagen gel. More surprisingly, not only embryonic epithelia but also highly differentiated adult epithelia undergo this extraordinary change (Greenburg and Hay, 1982, 1986, 1988). Similarly, Thiery's group found that NBT-II rat bladder carcinoma cells undergo an epithelial-mesenchymal conversion when exposed to a serum substitute, to type I collagen (Boyer et al., 1989a,b, 1990; Tucker et al., 1990), or to some growth factors, such as acidic fibroblastic growth factor and transforming growth factor  $\alpha$  (Gavrilovic et al., 1990; Vallés et al., 1990; Tucker et al., 1991).



**Fig. 4.** When exposed to interstitial-type collagen, cultured retinal pigment epithelial cells acquire a fibroblast-like morphology.  $\times 1,395$ . Reproduced from Vidaurri-Leal et al. (1984); by copyright permission of the American Medical Association.

Treatment of an immortalized human keratinocyte line with the hypomethylating agent 5-aza-2'-deoxycytidine results in loss of epithelial characteristics and gain of a mesenchymal phenotype, including appearance of ECM receptors and differentiation markers typical for mesenchymal cells (Boukamp et al., 1992; Boukamp and Fusenig, 1993).

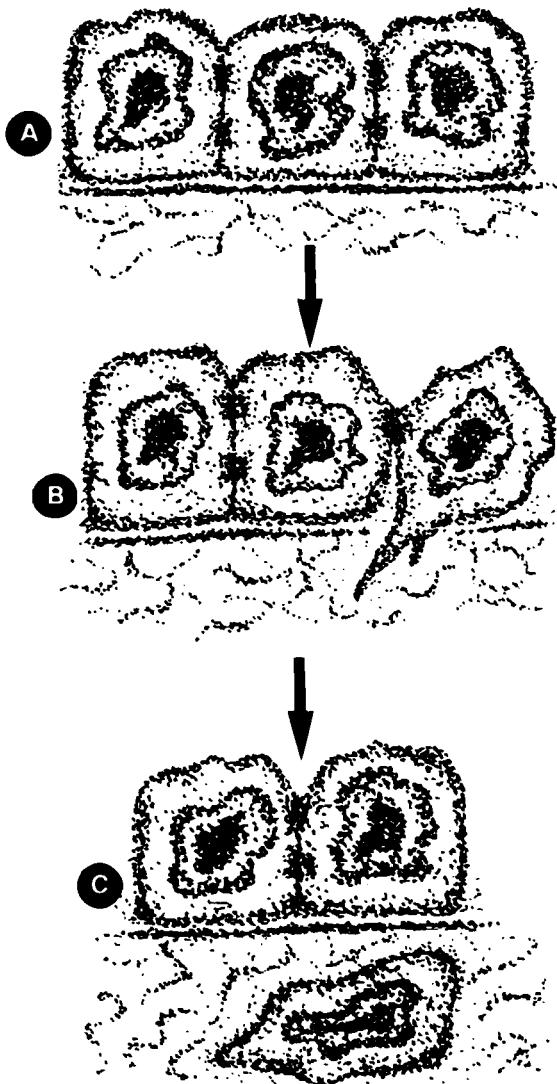
Mammary epithelial tumours induced in rats by nitrosomethylurea or dimethylbenz(a)anthracene, may give rise to clonal cell lines with fibroblast-like features

(Dulbecco et al., 1981), or to mesenchymal cells with skeletal muscle differentiation (Rudland et al., 1984).

S115, a cell line derived from mouse mammary carcinoma, undergoes conversion to a mesenchymal phenotype in presence of testosterone, and this change is related to loss of syndecan (Leppa et al., 1991, 1992), a cell surface proteoglycan binding both ECM molecules and growth factors.

These studies definitely demonstrate that the gene program for mesenchymal differentiation is retained in adult epithelial cells, and provide strong evidence that extracellular stimuli may profoundly alter gene expression and differentiation state.

## CANCER CELL INVASION



**Fig. 5.** Drawing illustrating the transition from in situ to invasive carcinoma. Non-invasive tumour cells are cohesive and rest on a continuous basement membrane (A). Focal disruption of the basement membrane in area of invasion occurs (B). At this site tumour cells detach from the neighbouring cells, produce pseudopodial extensions (B), and migrate into the underlying stroma (C). Modified from Boyer et al. (1990); by permission of Expansion Scientifique Française.

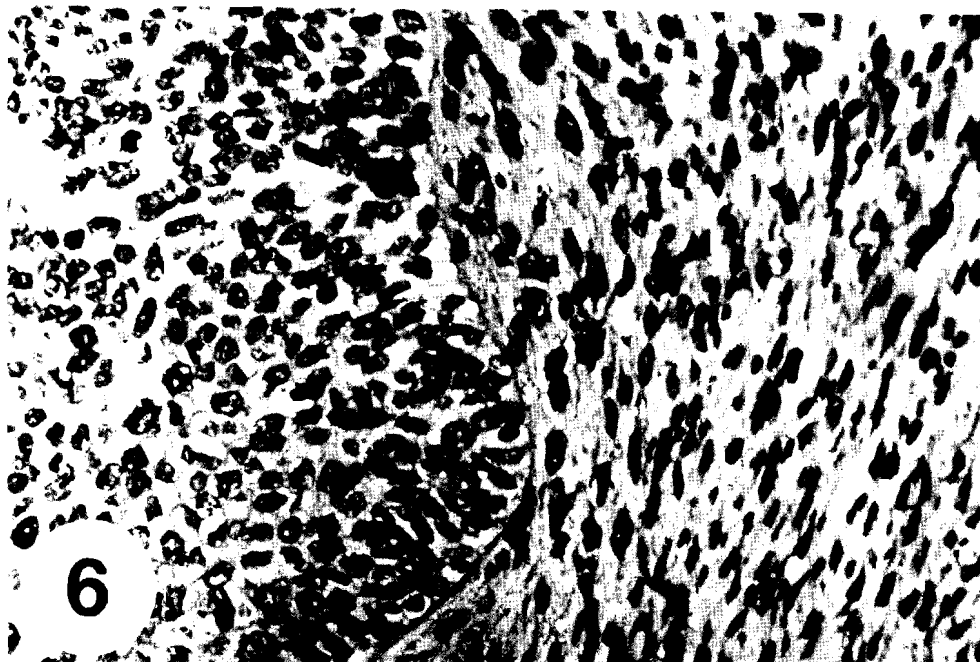
### Tissue repair changes

In some reparative processes a tissue lesion develops by a series of changes that seem to involve the conversion of an epithelium into mesenchymal cells. This peculiar change presumably represents some means by which the injured tissue, in order to replace the damaged area, recruits fibroblastic cells that migrate in it, and produce a connective tissue matrix.

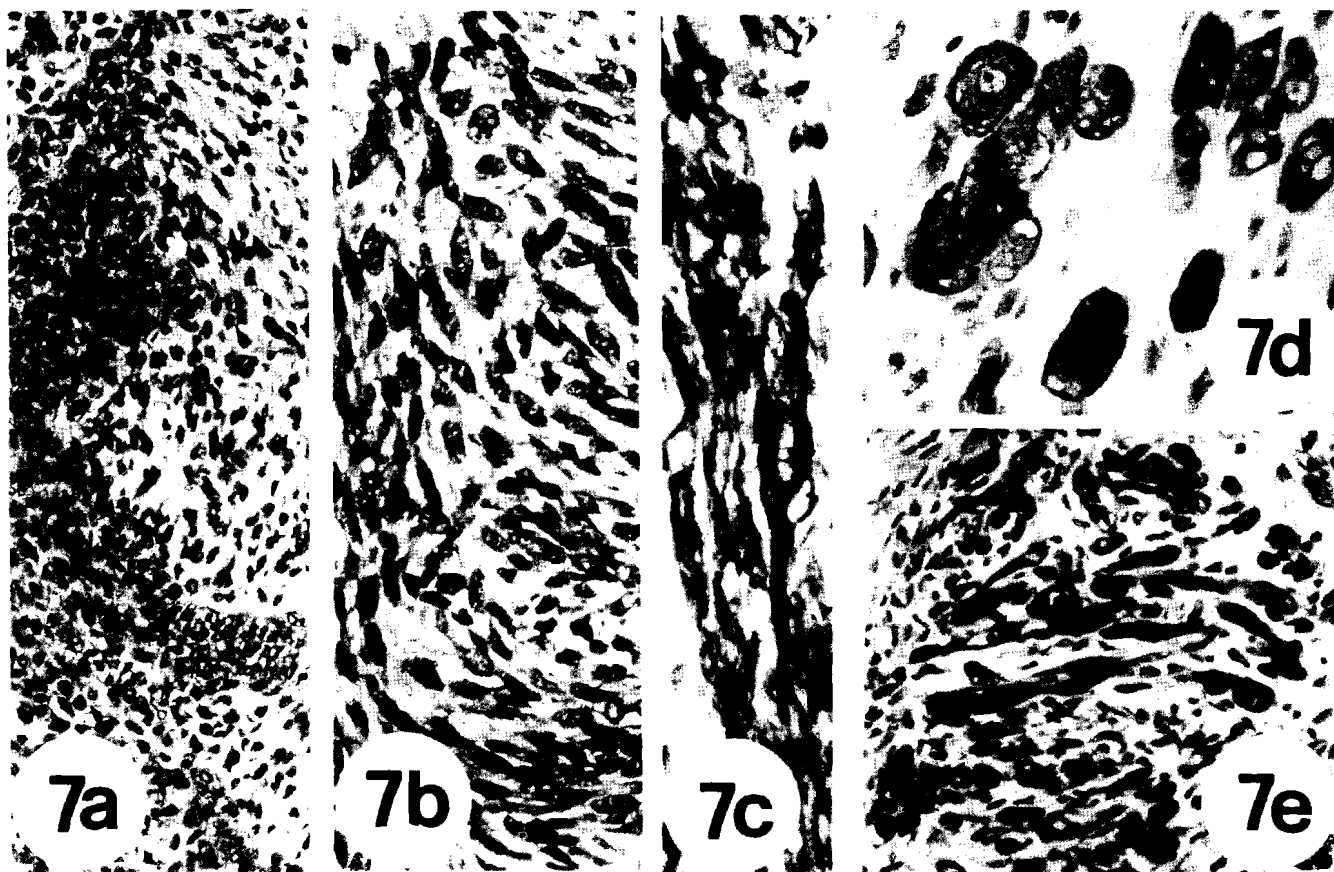
An unusual type of cataract, named anterior capsular cataract, consists of a fibrous plaque which forms in the lens subcapsular zone. In this condition, lens epithelial cells respond to injury by migrating, proliferating, and transforming into fibroblast-like cells which lay down a fibrous matrix (Henkind and Prose, 1967; Font and Brownstein, 1974) (Fig. 3). These cells closely resemble myofibroblasts (Novotny and Pau, 1984; Schmitt-Gräff et al., 1990), which are the cellular elements usually involved in repair phenomena.

Proliferative vitreoretinopathy is a severe complication of retinal detachment in which retinal pigment epithelial cells migrate into the vitreous cavity, and undergo a conversion to fibroblast-like cells producing a collagenous matrix. The final result is the formation of fibrous membranes that contract and cause the retina to fold (Mandelcorn et al., 1975; Machemer et al., 1978; Machemer, 1988). The converted cells have many characteristics of myofibroblasts, and this could account for the contraction of the membranes (Machemer et al., 1978). Vitreous collagen (Vidaurre-Leal et al., 1984) (Fig. 4), fibrin (Vidaurre-Leal and Glaser, 1984), and some factors produced by macrophages (Kirchhof et al., 1988; Martini et al., 1991), all seem to be involved in the transformation of the retinal pigment epithelial cells into fibroblast-like cells. In severe penetrating trauma of the eye, haemorrhage, membrane formation, and traction retinal detachment may occur. If prominent shrinkage and disorganization of ocular tissues take place (a condition known as phthisis bulbi), fibrous or osseous tissue may form which is believed to derive directly from retinal pigment epithelium (Kurz and Zimmerman, 1962; Frayer, 1966; Albert and Dryja, 1989).

Finally, the connective tissue production occurring in crescents of human glomerulonephritis has been related



**Fig. 6.** Typical histological appearance of carcinosarcoma comprising both carcinomatous (left) and sarcomatous (right) tissue. The latter consists of pleomorphic spindle cells with unspecific mesenchymal appearance. x 250



**Fig. 7.** Oesophageal tumour comprised of squamous cell carcinoma (a) showing transition to spindle cell sarcoma (a, b) and rhabdomyosarcoma (c, d, e). A specific mesenchymal differentiation toward striated muscle phenotype has taken place in the sarcomatous component, as tumour cells show cytoplasmic cross striation and are reactive to muscle actin (c) desmin (d), and myoglobin (e). a and e, x 160; b, x 250; c and d x 400. For further details see Guarino et al., 1993a.

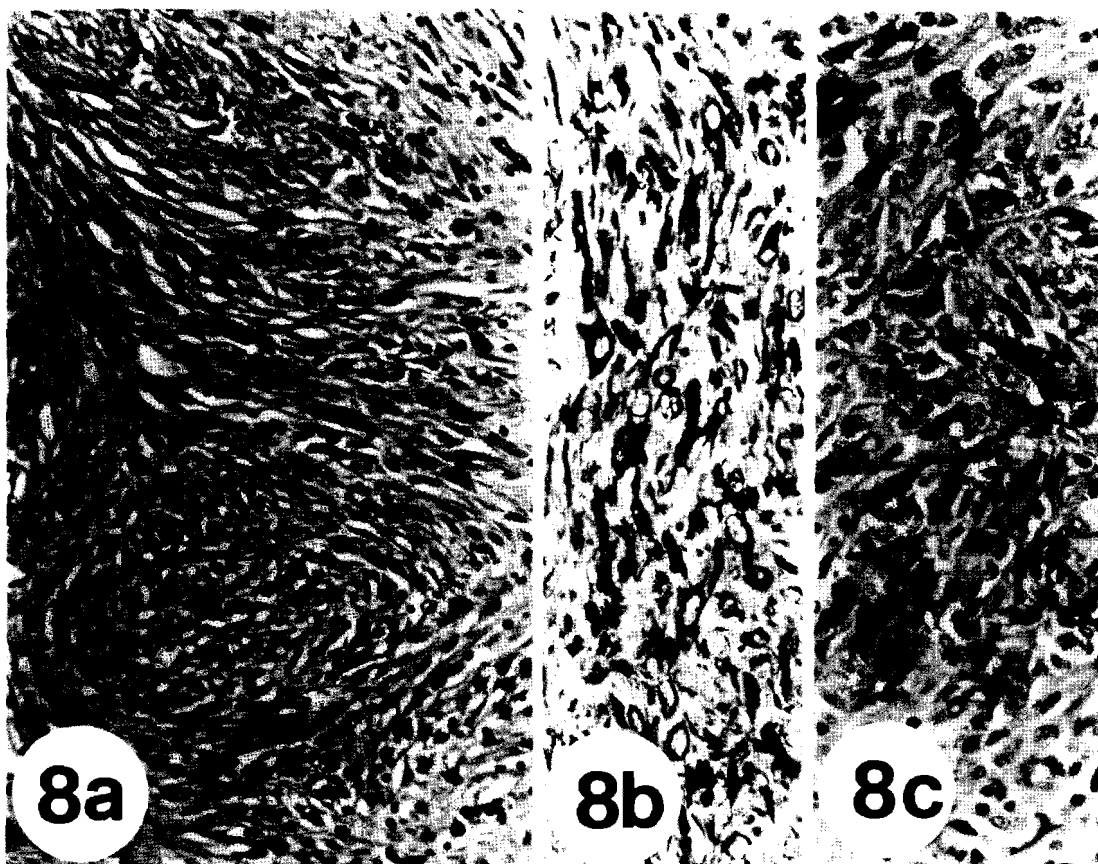
by some authors to transformation of epithelial glomerular cells into fibroblast-like cells, responsible for collagen production in the fibrous stage of this disease (Jones, 1968; Morita et al., 1973).

### Tumour invasion and metastasis

Invasion by neoplastic cells is a highly complex multistage process that involves cell-cell adhesion, cell-matrix interactions, and cell motility (Liotta et al., 1983; Mareel et al., 1990; Starkey, 1990; Van Roy and Mareel, 1992; Aznavoorian et al., 1993). In order to invade and metastasize, tumour cells must escape from the primary tumour, and acquire a migratory behaviour. Carcinomas, the most frequent form of human malignancy, begin as collections of cohesive malignant epithelial cells circumscribed by a basement membrane, which separate them from the surrounding stroma. The transition from this in situ stage to invasive carcinoma implies a weakening of intercellular adhesion and disruption of the basement membrane, so enabling tumour cells to detach and migrate into the underlying stroma (Liotta et al., 1983; Mareel et al., 1990; Starkey, 1990; Van Roy and Mareel, 1992; Aznavoorian et al., 1993) (Fig. 5). During this process, stationary epithelial cells, that face their basement membrane and that are linked by cell junctions and cell adhesion molecules, must undergo a very drastic

change to become motile cells which interact with the interstitial matrix of the stroma. It is easy to see that these changes are somewhat similar to the ones occurring in embryonic epithelial-to-mesenchymal conversion, and it has been suggested that, in order to invade and metastasize, epithelial carcinomatous cells transiently acquire a mesenchymal-like phenotype, recapitulating this embryonic process (Thiery et al., 1988; Boyer et al., 1990).

Both disruption of cell junctions (Gabbert, 1985) and loss of E-cadherin (Takeichi, 1991; Behrens, 1993) take place during tumour invasion, accounting for decreased cell cohesiveness commonly observed at the invasion front (Gabbert, 1985). Basement membrane loss and ECM degradation could have key roles in the changes occurring during tumour invasion. In normal conditions, epithelial cells rest on a continuous basement membrane, and interaction of the basal surface with this matrix is essential for the maintenance of the epithelial phenotype (Gumbiner, 1990). Indeed, in vitro studies have shown that epithelial basal surface becomes disorganized and produces cytoplasmic extensions when deprived of this extracellular scaffoldings (Sugrue and Hay, 1981). In addition, the loss of basement membrane occurring in tumour invasion could also expose tumour cells to type I collagen of the underlying stroma. Both basement membrane loss and contact with interstitial collagen



**Fig. 8.** On histological examination, this thyroid tumour is entirely composed of atypical spindle cells (a), which are strongly reactive for vimentin (b), and could suggest a sarcoma. However, many tumour cells are positively stained for cytokeratin (c), favouring a diagnosis of sarcomatoid carcinoma. a, x 160; b, c x 250

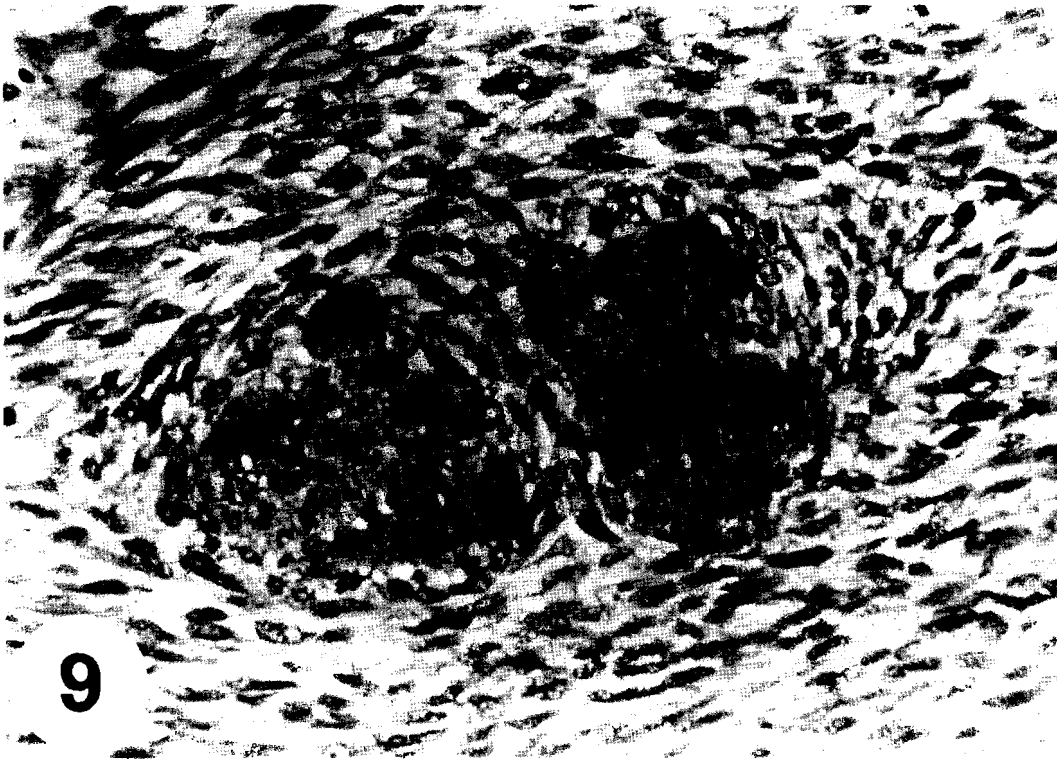


Fig. 9. Section of carcinosarcoma showing evident morphological transition of carcinomatous nests (centre) to surrounding sarcomatous spindle cells. This feature strongly suggests that a conversion of epithelial to mesenchymal tissue is taking place. x 250

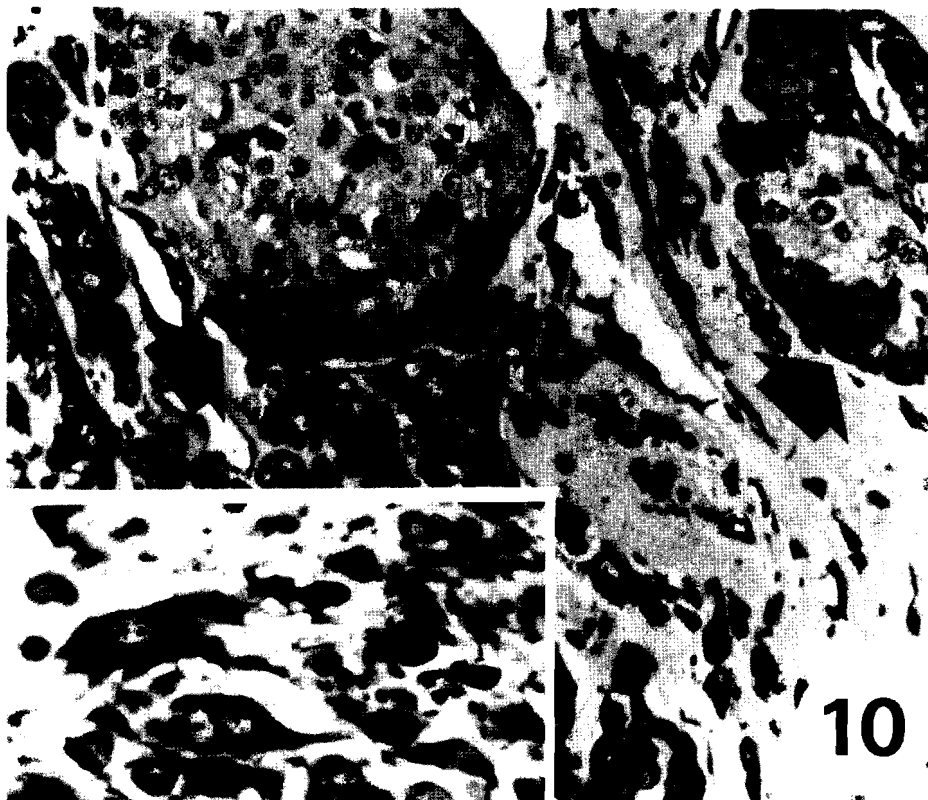


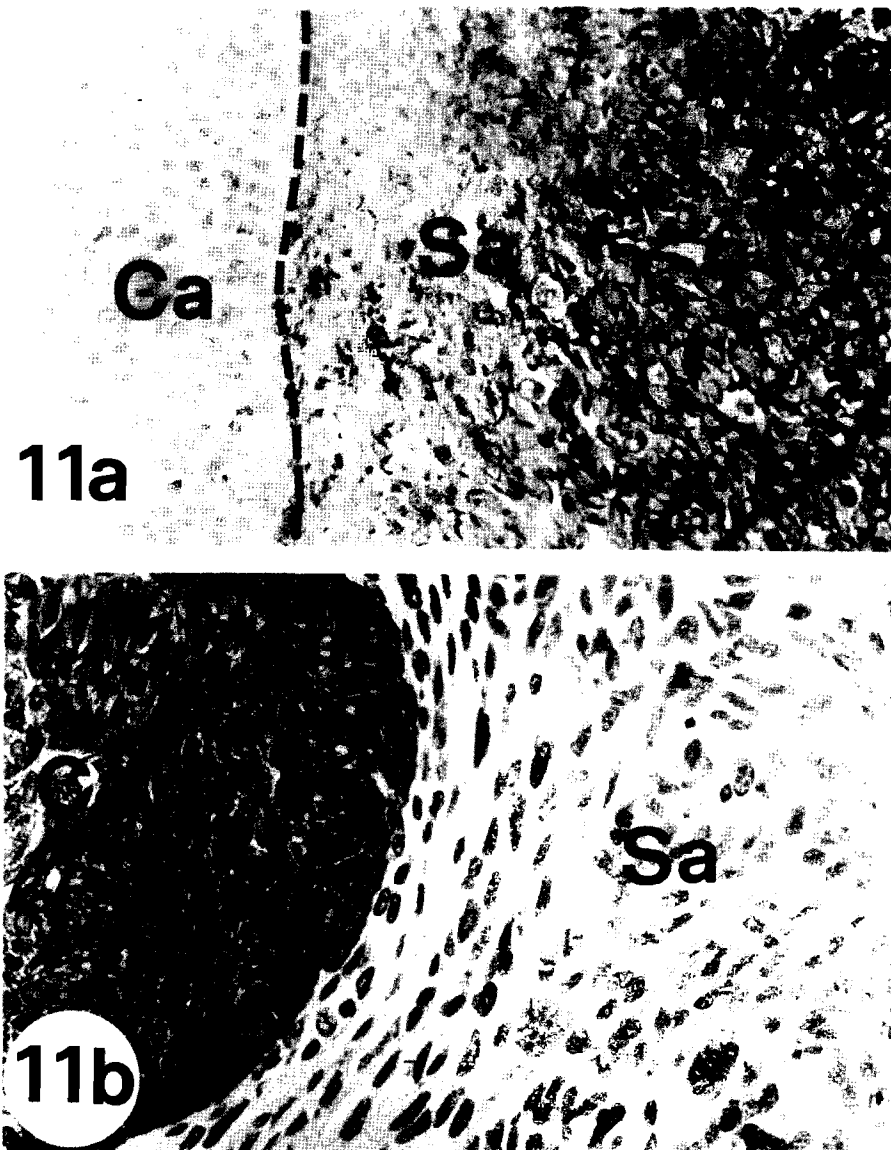
Fig. 10. Carcinosarcoma stained for vimentin showing two carcinomatous nests (top) surrounded by vimentin-positive, malignant-appearing (see the inset) mesenchymal cells. Many carcinomatous cells, mostly in the basal layer, are also reactive (arrows), suggesting that they are gaining some mesenchymal feature. x 160, inset x 400

could result in a shift toward a mesenchyme-like phenotype, favouring tumour invasion (Starkey, 1990). Moreover, ECM degradation at tumour invasion front could result in local release of ECM-bound growth factors and motility factors (Flaumenhaft and Rifkin, 1991; Van Roy and Mareel, 1992) which, in turn, might affect the phenotype of tumour cells.

Motility factors are recently discovered cytokines that promote cell dissociation and locomotion in an autocrine or paracrine fashion. Autocrine motility factor is produced by a variety of tumour cells and induces protrusion of pseudopodia that contain receptors for ECM components (Guirguis et al., 1987). Scatter factor is a fibroblast-derived paracrine factor, to which carcinoma cells respond by disrupting cell junctions and assuming a fibroblastic, motile phenotype (Weidner et al., 1990). It is of interest that this factor also seems to

be involved in the formation of the primitive streak mesenchyme from the ectoderm (Stern et al., 1990).

An active cell motility clearly involves a participation of the cytoskeleton, and, as a matter of fact, changes in its components occur in tumour invasion (Zimmermann and Keller, 1987). The mesenchyme-specific filament vimentin has been detected in tumour cells at the invasion front of some carcinomas (Schaafsma et al., 1993), and expression of this intermediate filament may indicate a more aggressive behaviour in some epithelial malignancies (Raymond and Leong, 1989), suggesting that this cytoskeletal element could be involved in tumour invasion. Likewise, vimentin is a marker of invasive phenotype in some breast carcinoma cell lines (Sommers et al., 1991, 1992; Thompson et al., 1992) and, interestingly, these cell lines do not express E-cadherin, and have a fibroblastoid morphology



**Fig. 11.** Some carcinosarcomas show a clearcut difference in the immunostaining of the two components, vimentin labelling only the sarcomatous tissue (Sa), (a) and cyokeratin only the carcinoma (Ca) (b). Absence of vimentin in the carcinoma, and of cyokeratin in the sarcomatous tissue might indicate that an abrupt shift toward the mesenchymal phenotype has taken place in carcinoma cells, a, x 160; b, x 250



(Sommers et al., 1991, 1992). These findings seem to suggest that tumour invasion shares at least some features with embryonic epithelial-mesenchymal conversion, and this observation would be in keeping with the notion that no new behaviour appears in the course of tumour development, and that the mechanisms of tumour growth and invasion are likely to be similar to the ones used by embryonic cells (Nicolson, 1987; Aréchaga, 1993; Papaioannou, 1993).

#### Carcinosarcoma (sarcomatoid carcinoma)

Although the overwhelming majority of human neoplasms are made up of a single cell type, a group of distinctive tumours are composed of both carcinomatous and sarcomatous elements. According to the different criteria used to define them, they have been called by a variety of names, including carcinosarcoma and sarcomatoid carcinoma. These neoplasms are relatively

more frequent in the upper aerodigestive tract (Sherwin et al., 1963; Zarbo et al., 1986; Weidner, 1987), skin (Battifora, 1976; Harris, 1982), and breast (Harris, 1982; Meis et al., 1987), but they may occur in practically any anatomical site. Histologically, typical carcinosarcomas are composed of either in situ or invading carcinoma, and of a generally predominant sarcomatous component, which in most cases has an unspecified spindle cell appearance (Fig. 6), but may sometimes show a specific mesenchymal differentiation (Fig. 7). In some tumours, the carcinomatous portion may be so inconspicuous, as to be undetectable on routine histological examination (Zarbo et al., 1986) (Fig. 8). Many theories have been proposed for the histogenesis of these unusual neoplasms (for a review see Weidner, 1987), but evidence is accumulating which suggests that the sarcomatous component originates from conversion of carcinoma cells to sarcoma (Battifora, 1976; Gould and Battifora, 1976; Addis and Corrin, 1985; Guarino et al., 1993a).

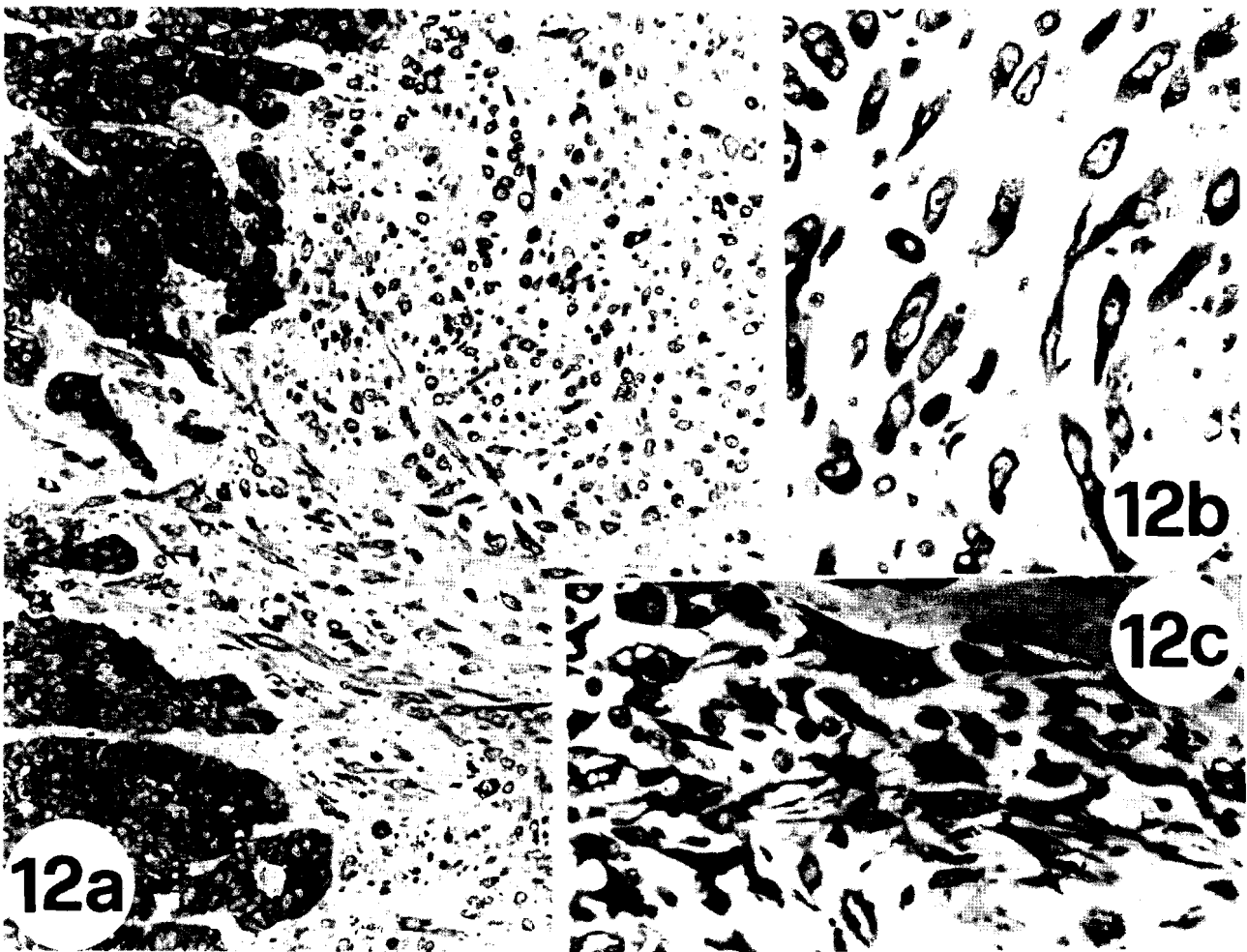
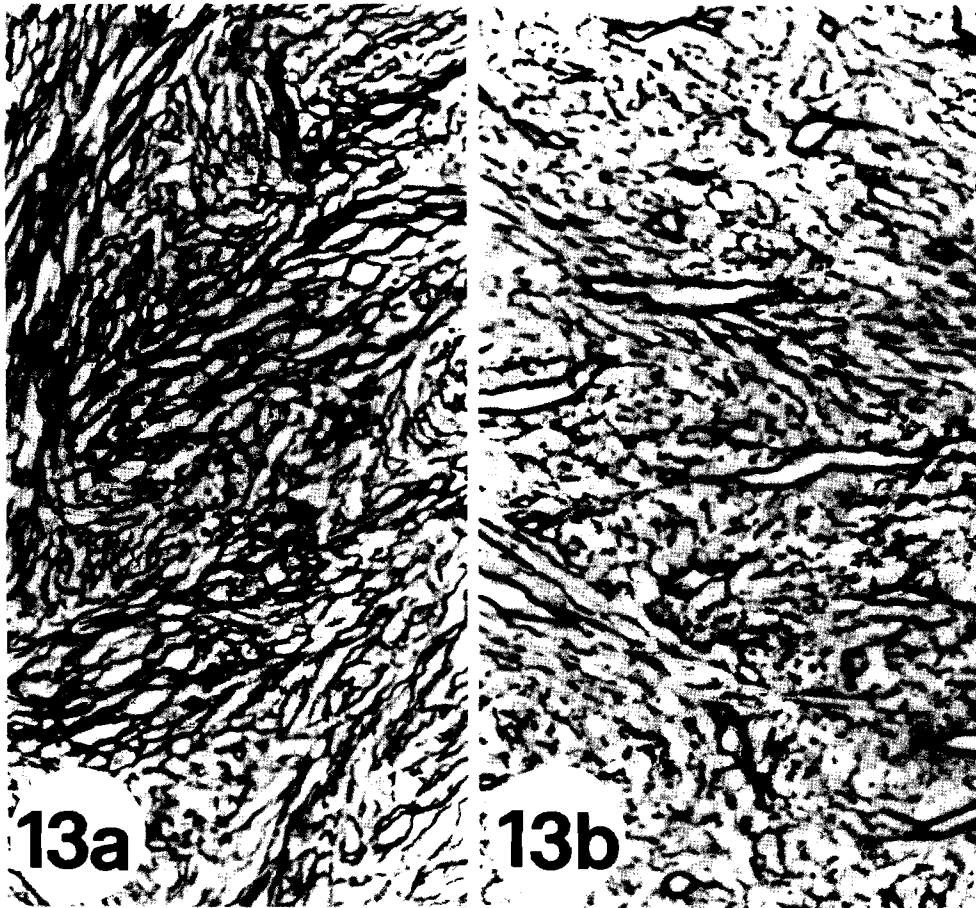
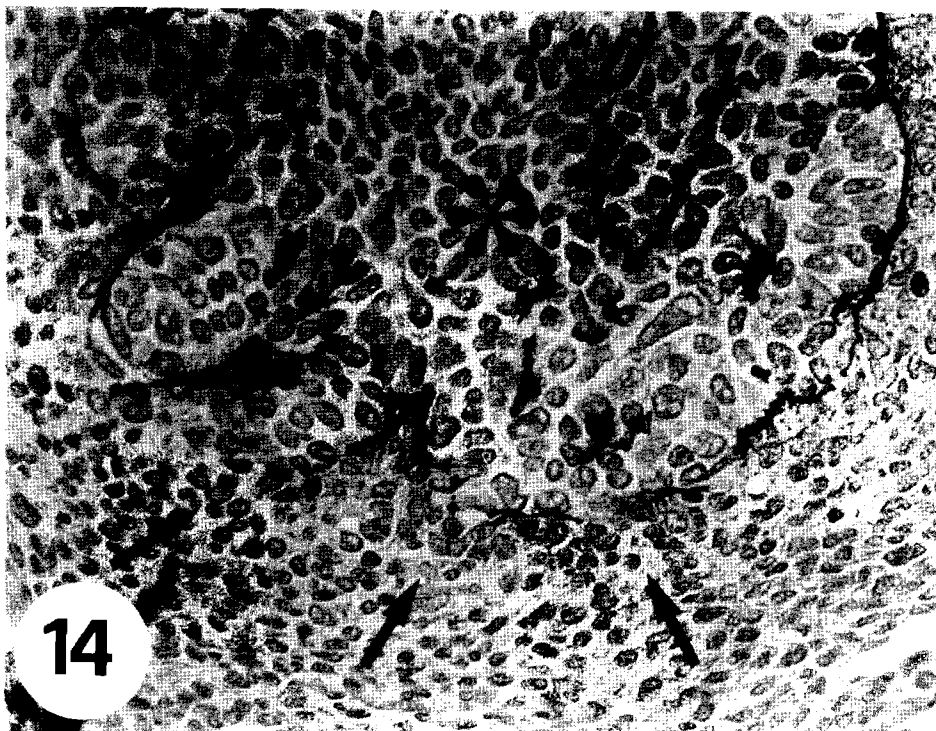


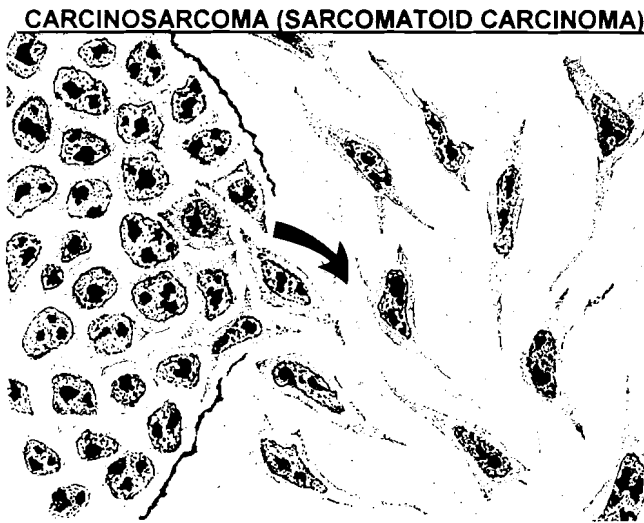
Fig. 12. Carcinosarcoma consisting of carcinoma strongly reactive for cytokeratin (a left), and spindle-shaped sarcomatous cells (a right, b, c). In spite of their mesenchymal morphology and reactivity for vimentin (c), the spindle cells label for cytokeratin (a right, b). Persistence of cytokeratin in cultured cells which have undergone epithelial-mesenchymal conversion has been related to incomplete conversion (Boyer et al., 1989b; Zuk et al., 1989) or to slow turnover of this intermediate filament (Greenburg and Hay, 1988). a, x 160; b and c x 250



**Fig. 13.** The sarcomatous component of this carcinosarcoma is strongly reactive to type III interstitial collagen (a), but also shows some pericellular immunoreactivity for the basement membrane-specific type IV collagen (b). A strong labelling of blood vessel basement membrane for type IV collagen is also seen (b). a and b x 160



**Fig. 14.** Section of carcinosarcoma stained with antibodies to laminin showing a carcinomatous island (asterisk) surrounded by an immunoreactive basement membrane. At some sites defects in the basement membrane profile occur and carcinoma blends with the sarcomatous tissue (arrows). x 250



**Fig. 15.** Drawing summarizing the postulated changes occurring in the development of carcinosarcoma. Carcinoma cells (left) are surrounded by a basement membrane, express cytokeratin and are highly cohesive. At some sites, basement membrane disrupts, cells elongate, lose their cohesion with neighbouring cells, and convert (arrow) to vimentin-positive sarcomatous spindle cells, which elaborate a mesenchymal matrix (right). These cells may or may not continue to express cytokeratin and basement membrane components, or may further progress along a specific lineage of mesenchymal differentiation. Reprinted with permission, from Guarino et al. (1993c).

This view is supported by many histological, ultrastructural and immunohistochemical data. A common finding in carcinosarcomas is to observe a morphological transition between carcinomatous and sarcomatous tissues (Sherwin et al., 1963; Battifora, 1976; Harris, 1982; Guarino et al., 1993a) (Fig. 9). In these areas a loss of cell-cell cohesion in the epithelial basal layer occurs (Sherwin et al., 1963), and a «dropping-off» of spindle shaped tumour cells into the sarcomatous component may be observed (Sherwin et al., 1963; Battifora, 1976; Gould and Battifora, 1976). By electron microscopy, the sarcomatous tissue often displays characteristics that are not typical for true mesenchymal cells, such as well-formed desmosomes and tonofibrils (Battifora, 1976; Gould and Battifora, 1976; Gould et al., 1981; Harris, 1982), indicating an epithelial rather than a mesenchymal origin for this tissue component. Although normal cells (Knapp et al., 1989; Markl, 1991) and tumours (Brown et al., 1987; Weiss et al., 1988; Guarino, 1993a,b) of mesenchymal nature may occasionally express cytokeratin, this intermediate filament is usually absent in sarcomatous cells. It has been found that, in addition to extensive expression of vimentin (Figs. 10, 11a), as it should be expected in mesenchymal tumour cells, the sarcomatous component of carcinosarcoma very frequently expresses cytokeratin, too (Fig. 12), further supporting its epithelial derivation (Addis and Corrin, 1985; Zarbo et al., 1986; Guarino et al., 1993b). It is of interest that *in vitro* studies have shown, in a similar way, a persistence

of cytokeratin in cells that have undergone an epithelial-mesenchymal conversion (Greenburg and Hay, 1988; Boyer et al., 1989a,b; Zuk et al., 1989). Immunostaining for ECM components in carcinosarcoma shows that the epithelial component produces a basement membrane matrix immunoreactive to laminin and type IV collagen, whereas sarcomatous cells make a mesenchymal-type matrix rich in fibronectin and interstitial collagens (Guarino et al., 1993b) (Fig. 13a). However, in keeping with their presumptive epithelial origin, some production of basement membrane components also occurs among the sarcomatous cells (Guarino et al., 1993b) (Fig. 13b), and this observation closely parallels the ultrastructural finding of both fibrillar collagen and basement membrane material in these tumours (Gould and Battifora, 1976; Gould et al., 1981). Moreover, immunolabelling for laminin or type IV collagen often shows disruptions of the basement membrane pattern at the interface between carcinomatous and sarcomatous tissue, which is in keeping with the hypothesis that an epithelial-to-mesenchymal conversion is taking place there (Guarino et al., 1993a,c) (Fig. 14). These data seem to suggest that a spectrum of changes, finally resulting in a sarcomatous appearance, may take place in carcinomatous cells. Changes can range from acquisition of vimentin and of a spindled cell shape (Fig. 10), through loss of polar organization of basement membrane and production of mesenchymal matrix components (Fig. 13a,b), to loss of cytokeratin (Fig. 11b) and acquisition of a specific mesenchymal pathway of differentiation (Fig. 7). Therefore, carcinosarcomas could develop by progressive phenotypic change of carcinoma cells that undergo a partial to complete epithelial-mesenchymal conversion (Gould and Battifora, 1976; Harris, 1982; Addis and Corrin, 1985; Guarino et al., 1993a,b) (Fig. 15). According to the degree of this change and to the amount of cells involved in this phenotypic conversion, different morphological and immunohistochemical features will result.

## Conclusion

Change in state of differentiation is a fundamental process in embryonic tissue morphogenesis, but evidence is growing that this mechanism operates in some conditions of adult life too. Embryonic conversion of epithelium to mesenchyme results in formation of a tissue with new characteristics, as newly-formed mesenchymal cells are suited for both migration and production of interstitial matrix material. Since several pathological conditions involve either cell migration or connective tissue production, it is not surprising that cellular mechanisms similar to embryonic epithelial-mesenchymal conversion are used in these circumstances. In order to replace a damaged tissue, both cell migration and formation of a matrix scaffolding are required, and it is possible that in some situations tissue repair occurs by a process of epithelial-mesenchymal conversion meeting both these requirements. Invasion of

carcinoma cells into the underlying stroma implies both cell detachment and active cell locomotion. In order to accomplish this, it is likely that carcinoma cells activate part of the genetic program for conversion to mesenchyme, which results in some transitory acquisition of the mesenchymal phenotype. In carcinosarcomas a more stable and extensive activation of the mesenchymal gene program could take place in carcinoma cells, resulting in cell populations with increasing degree of mesenchymal differentiation. A better knowledge of the molecular mechanisms governing epithelial-mesenchymal conversion could further clarify pathogenetic aspects of these conditions, providing the bases for a more effective control.

*Acknowledgements.* I am indebted to G. Brambilla, G. Ornaghi and G. Tadini for their technical assistance, and to C. Dorigo for help during the preparation of the manuscript.

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