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Immunohistochemical observations of keratins, involucrin, and epithelial membrane antigen in urinary bladder carcinomas from patients infected with *Schistosoma haematobium*

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Summary. Squamous cell carcinomas of the urinary bladder and the epithelial lesions associated with infection by *Schistosoma haematobium* were histopathologically and immunohistochemically described for keratin proteins (TK, 41-65 kDa; KL1, 55–57 kDa; PKK1, 40, 45 and 52.5 kDa), involucrin, and epithelial membrane antigen (EMA). Normal urothelial epithelium was positive for all keratins, and showed absent or slight reactions for involucrin and EMA in superficial umbrella cells. The intestinal type of epithelium was composed of columnar cells and small basal cells; TK was positive in the basal cells, KL1 staining was positive in the columnar cells, whereas PKK1 was negative or slight in the columnar cells. Involucrin was confined to columnar cells. Squamous metaplastic epithelium showed a rather regional keratin distribution: TK was distributed in all layers, KL1 decorated upper spinous and granular layers, but PKK1 did not bind, and involucrin staining existed only in upper spinous and granular cells. Keratin expression in squamous cell carcinomas indicated heterogeneity and its stainability was dependent on the degree of keratinization: The G 1 type revealed strong reaction, the G 2 type showed a similar distribution pattern, but the staining intensity was less, and the G3 type showed irregular staining with decreased intensity. Involucrin staining was limited to keratinized cells of carcinoma as was that for EMA.

Key words: *Schistosoma haematobium* – Urinary bladder carcinoma – Keratin proteins – Involucrin – Epithelial membrane antigen

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Introduction

A high incidence of urinary bladder carcinoma associated with infection by *Schistosoma haematobium* has been reported (Bilharz 1852; Goebel 1905). Investigations from Egypt have shown that wide spread infection with *Schistosoma haematobium* occurs in human beings (Higashi and Aboul-Enein 1981), and tryptophan metabolites are commonly found at elevated levels in the urine in such cases (Khalafallah and Abul-Fadl 1964). Histopathological studies have also recorded the histological sequence leading to urothelial carcinoma: thickening and proliferation of the urothelial epithelium followed by squamous metaplasia, which frequently leads to squamous cell carcinoma in man (Dimmette et al. 1956; Hashem 1961; Higginson and Oettle 1962; Koss 1975; El-Bolkainy et al. 1981 a, b; Koss 1985). However, papillary tumours more frequently occur in non-human primates (Kuntz et al. 1972; Brown et al. 1976; Cheever et al. 1976).

By immunohistochemical study, normal epithelial cells or epithelium-derived tumours are characteristically positive for keratin proteins. Human keratin polypeptide are usually classified into 19 types by the electrophoresis (Moll et al. 1982). In the epidermis of the skin and epithelium of oral mucosa, keratin proteins have a particular pattern of zonal or regular distribution (Löenig et al. 1980, 1982; Woodcock-Mitchell et al. 1982; Nelson and Sun 1983; Sun et al. 1983; Viac et al. 1983; Nakai and Mori 1986). Keratin distribution in squamous cell carcinomas has already been reported to show irregularities, whereas a rather zonal or regular distribution is observed in benign tumours (Viac et al.

Table 1. Antibodies used in present study

Antibodies	Immunogen	Dilution	Incubation time	Source
TK (41–65 kDa)	stratum corneum of the sole of the human foot	1:50	1 h	Dakopatts (Copenhagen, Denmark)
KL1 (55–57 kDa)	human keratinized squamous epithelium	1:50	1 h	Immunotech (Marseilles, France)
PKK1 (40, 45 and 52.5 kDa)	Pig kidney epithelium cell line	1:80	1 h	Labsystem (Helsinki, Finland)
Involucrin immuno kit	a protein component of the cross-linked envelope synthesized by mature cells of human stratified squamous epithelia		1 h	Biomedical Technologies, (Cambridge, USA)
EMA	human milk fat globules	1:50	1 h	Dakopatts (Copenhagen, Denmark)

1982; Thomas et al. 1984; Mori et al. 1985; Nakai and Mori 1986). Involucrin is a marker of terminal keratinization of keratinocytes and also occurs in keratinized regions of epidermoid carcinomas (Warhol et al. 1982; Said et al. 1984; Walts et al. 1985; Sumitomo et al. 1986; Itoiz et al. 1986). Epithelial membrane antigen (EMA) from human milk fat globules has been used to detect surface membrane of glandular epithelia (Gusterson et al. 1982; Cordell et al. 1985; Heyderman et al. 1985; Pinkus and Kurtin 1985; Zotter et al. 1985; Pinkus et al. 1986; Tatemoto et al. 1987a) and recently it has been detected in squamous cell carcinoma cells although it does not appear in normal squamous epithelial cells (Cordell et al. 1985; Pinkus and Kurtin 1985; Zotter et al. 1985; Pinkus et al. 1986; Tatemoto et al. 1987b).

The present study describes the distribution profiles of keratins as visualized by polyclonal (Total Keratin; TK: 41–65 kDa) and monoclonal antibodies (KL1: 55–57 kDa, PKK1: 40, 45 and 52.5 kDa), involucrin and EMA in normal and several type of proliferative cells, and in varying grades of urinary bladder squamous cell carcinomas in Egypt. These patterns are compared with those of squamous cell carcinomas of other origins and more specific cellular markers for assessment of keratinization in bilharzial bladder cancer are examined.

Materials and methods

Materials. A total of 20 cases of urinary bladder carcinoma were examined by immunohistochemical methods. All the materials were from total cystectomy cases obtained at the National Cancer Institute, Cairo, Egypt, and diagnosed as squamous cell carcinoma with infection of *Schistosoma haematobium*. The specimens were fixed in 10% formalin solution for 12 h and serial paraffin sections at 4 μm were made and the lesions revaluated in the Department of Pathology, Nagoya City University School of Medicine. They were immunohistochemically examined in the Asahi University School of Dentistry.

The indirect immunoperoxidase method was used. Deparaffinized sections were treated with 0.3% H_2O_2 methanol solution for 20 min to inactivate endogenous peroxidase and rinsed well. In the case of PKK1 staining, trypsin (Nakarai Chem. Co. Ltd; Kyoto, Japan) pretreatment (30 min at 37° C with 100 ml of 0.01 M phosphate-buffered saline (PBS, pH 7.6) containing 0.1 g each of trypsin and $CaCl_2$) was carried out prior to inactivation of the endogenous peroxidase. The sections were treated as follows; **KL1, PKK1 and EMA:** normal rabbit serum (1:20, Wheaton, USA) and **TK, involucrin:** normal goat serum (1:20, Biomedical Technologies, Cambridge, USA) were used with incubation for 30 min, then blotted dry with filter paper, and incubation with antibodies (see Table 1). After rinsing in PBS was carried out 3 times, **KL1, PKK1 and EMA:** HRP-labelled rabbit anti-mouse immuno-globulins (1:20, Dakopatts, Copenhagen, Denmark), **TK:** HRP-labelled rabbit anti-goat immunoglobulins F(ab)′ (1:20, Jimro, Takasaki, Japan), and **involucrin:** HRP-labelled rabbit anti-goat immunoglobulins (1:20, Biomedical Technologies, Cambridge, USA) were applied for 30 min. They were rinsed in PBS well, and immersed for 5 min in 0.05 M Tris buffer (pH 7.6) containing 0.005% DAB and 0.03% H_2O_2.

Results

Of the 20 patients with proven bladder carcinoma, 18 were men and 2 were women. They ranged in age from 23 years to 75 years and average age was 49.5 years for men and 47.5 years for women. Histological grading of bladder carcinomas was G 1 in 6 cases, G 2 in 6 and G 3 in 8. Calcified schistosome eggs were observed in the cancerous tissue of all cases. Squamous cell carcinomas in human bladder associated with *Schistosoma haematobium* infection usually show no or slight keratinization. In Egypt, G 1 squamous cell carcinoma (well-keratinized type) was rarely seen. Most specimens were in the category of G 2 or G 3 squamous cell carcinomas with an intermediate or low degree of differentiation.

Normal human urothelial transitional epitheli-

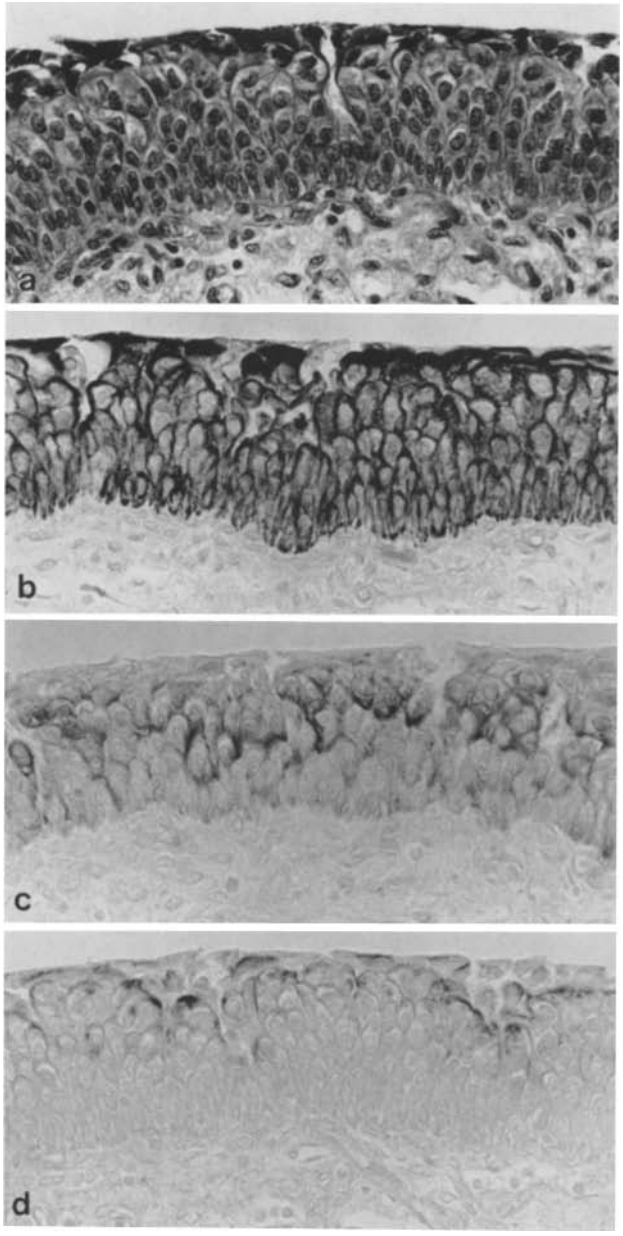

Fig. 1a–d. Normal transitional epithelium (×160). **a** H&E staining. There are 5–7 cellular layers; the superficial cells of urothelial epithelium compose of umbrella cells, intermediate layer cells show plumped columnar cells, and basal layer cells are columnar or high columnar shape. **b** KL1 staining. Apical zones of umbrella cells show strong staining to KL1. **c** Involucrin staining. Umbrella cells are almost negative. Intermediate layer cells of urothelium display slight-to-moderate positive reaction. **d** EMA staining. Some of umbrella cells and intermediate layer cells show slight positive reaction

um is composed of a layer of 5 to 7 cells (Fig. 1a), and gives positive staining for keratin proteins (TK, KL1, and PKK1) and weak staining for involucrin and EMA. Superficial umbrella cells were

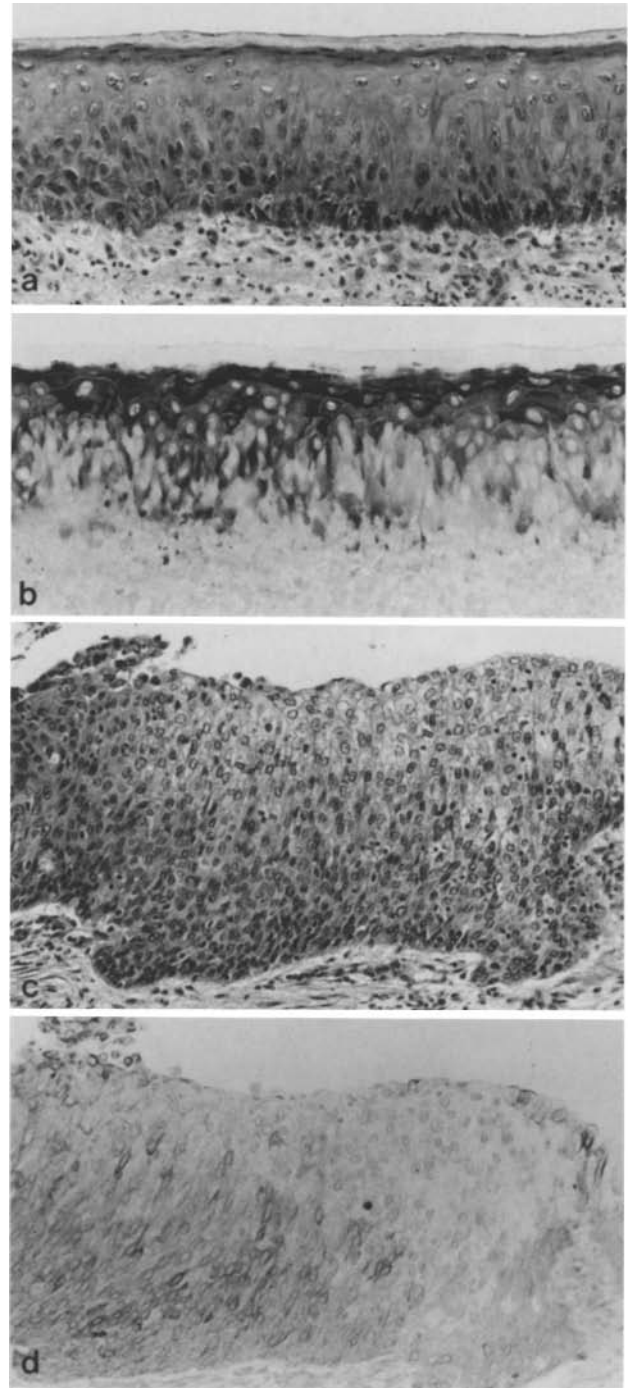

Fig. 2a–d. Squamous metaplastic (Fig. 2a and 2b) (×160) or hyperplastic trasitional epithelium (Fig. 2c and 2d) (×80). **a** H&E staining. The degree of keratinization is slight, and granular cell layer is not prominent. Intermediate layer cells show columnar in shape, and basal layer cells high columnar. **b** Involucrin staining. Strong reaction is occurred from intermediate layer cells to beneath hornified layer cells (*upper spinous cells*). **c** H&E staining. **d** KL1 staining. KL1 keratin staining is drastically reduced in hyperplastic epithelium compared to that in normal epithelium (cf. **b**)

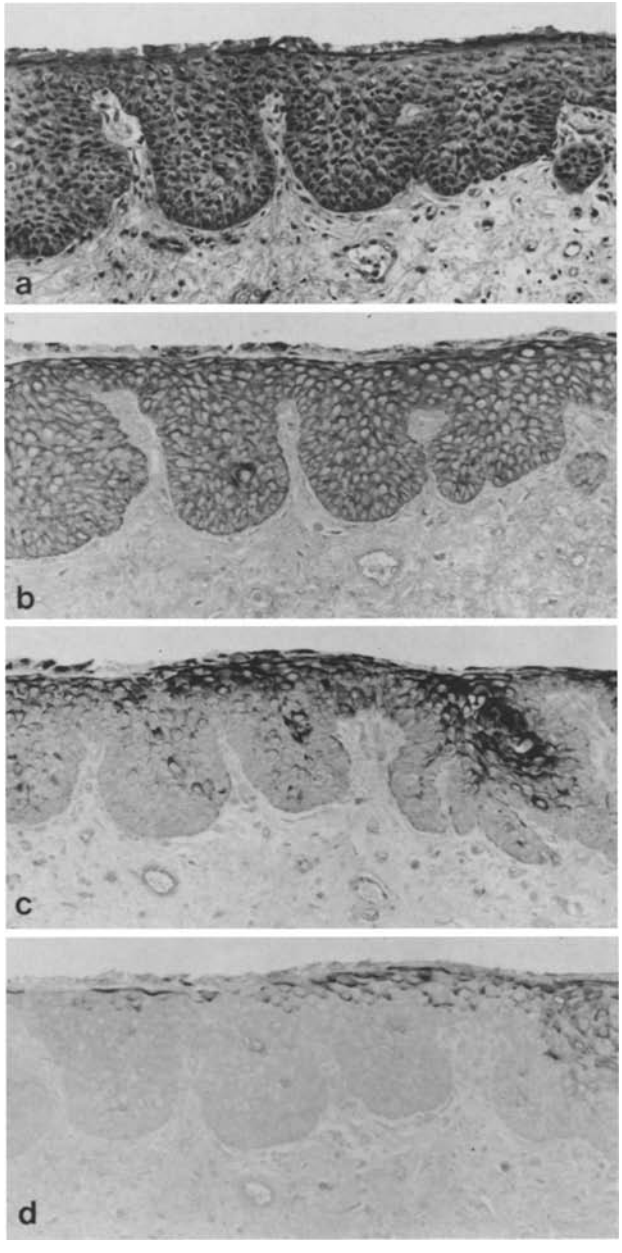

Fig. 3a–d. Basal hyperplasia of squamous metaplastic transitional epithelium. (×80). **a** H&E staining. Basal layer cells proliferate into connective tissue as down growth pattern. Superficial zone of the epithelium shows keratin-like materials. **b** TK staining. All the epithelial layer cells indicate moderate positive. **c** KL1 staining. KL1 staining is particulally strong in upper spinous cells, while it is absent in basal layer cells. **d** Involucrin staining. Involucrin reaction is existed narrow layer upper cells, while basal, parabasal and lower spinous layer cells are devoid of involucrin staining

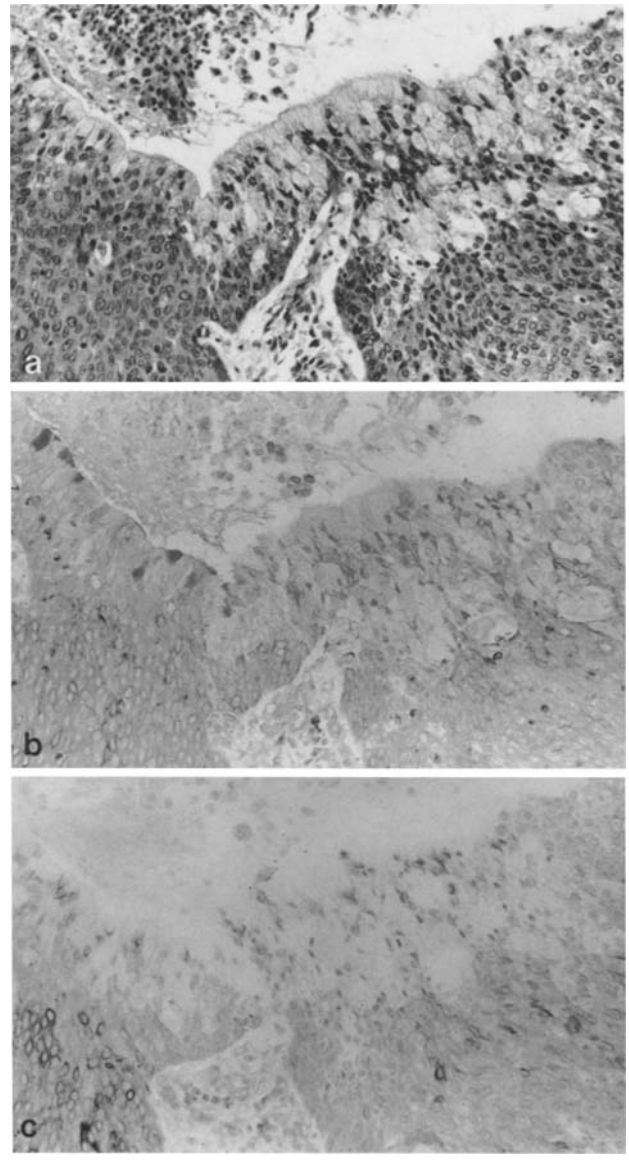

Fig. 4a–c. Intestinal type of urothelium combined with hyperplasia (×80). **a** H&E staining. There are marked intestinal metaplasia in hyperplastic transitional epithelium. Intestinal epithelial cells compose of gobulet-like mucinous cells. **b** TK staining. Intestinal metaplastic cells display not strong TK reaction as well as in hyperplastic transitional epithelium, however, moderate TK reaction occures in the several cells of intestinal epithelium. **c** KL1 staining. Most of epithelium are lacking to KL1 reaction

characterized by an intense reaction with all the anti-keratin antibodies. Intermediate and basal layer cells were strongly positive with TK, moderate-to-strong positive with KL1 (Fig. 1b), and slightly with PKK1. Involucrin staining was only positive in the intermediate layer just beneath the superficial umbrella cells, whereas absent or slight from in other cells (Fig. 1c). EMA staining was similar to that of involucrin reaction (Fig. 1d).

Metaplastic epithelium showed absent or slight keratinization with parakeratosis (Fig. 2a, c). This epithelium generally showed no signs of extension into connective tissue stroma, having flat rete pegs.

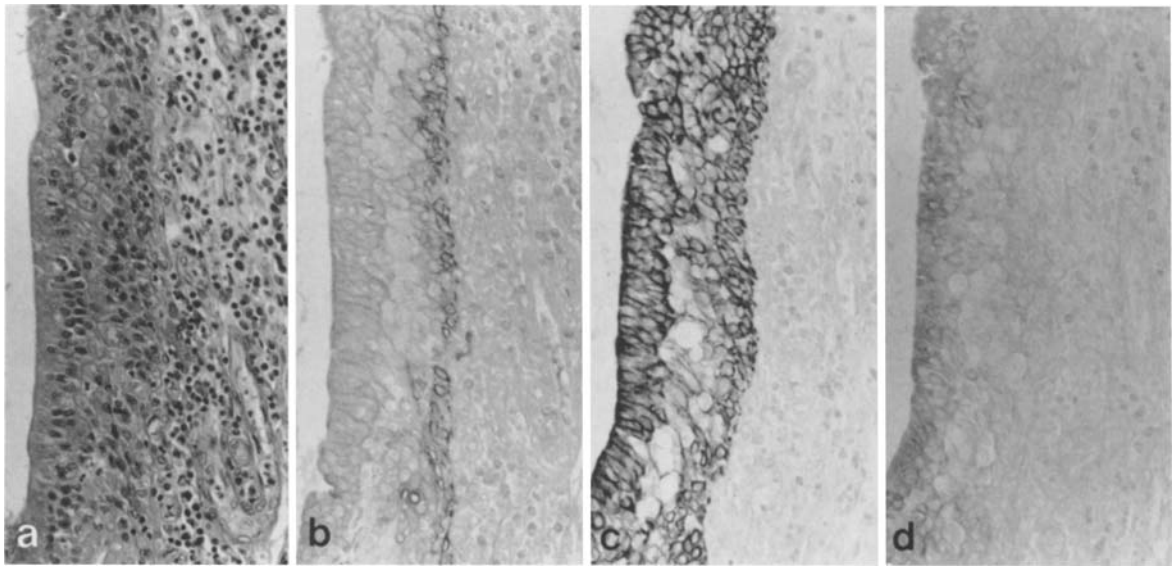

Fig. 5a–d. Glandular metaplastic epithelium (×100). **a** H&E staining. The epithelium is roughly divided into 3 layers; superficial columnar cells, vacuolated intermediate layer cells and compacted basal cells. Intermediate layer cells are occasionally absent in many instances. **b** TK staining. Basal layer cells show moderate-to-strong reaction. **c** KL1 staining. All the epithelial layers show marked staining to KL1. **d** EMA staining. Superficial columnar cells only existed EMA staining

Squamous metaplastic epithelium showed regular stratification from the basal layer to the upper strata; however the cellular shapes did not resemble keratinocytic differentiation in the skin epidermis. Squamous epithelium indicated a positive TK reaction of moderate level in the basal to upper cells, strong KL1 staining in upper layer cells, and negative staining in basal layer cells. No PKK1 staining was found in any cell layers. Involucrin reaction was markedly restricted to upper layer cells (Fig. 2b) and some cells of the upper layer showed very slight or even absent EMA reaction. In hyperplastic epithelium without keratinization (Fig. 2c), these distribution patterns had tendency to be decreased in intensity (Fig. 2d).

In 6 of 20 cases, basal cell hyperplasia or proliferation was found in the neoplastic squamous epithelium (Fig. 3a). Such a histological change was the initial alteration in the premalignant stage of bladder squamous cell carcinoma. Keratin, involucrin and EMA expression was essentially the same as found in squamous metaplastic epithelium (Fig. 3b–d).

Intestinal type epithelium originated from the mucin-producing glandular tissue (Fig. 4a). The metaplastic epithelium showed high columnar cells at the superficial zone, compacted cells at the basal region and occasionally cuboidal or polyhedral cells were found between two cellular zones (Figs. 5a, 6a, and 7a). Vacuolization was usually found in columnar cells (Fig. 6a). Keratin distribution varied throughout the epithelial strata. Columnar cells displayed slight TK staining, strong KL1 staining, and slight-to-negative PKK1 staining. In contrast, basal cells showed moderate-to-strong staining with TK and negative staining with KL1 and PKK1 (Figs. 4b, c, 5b, c, 6b, c, 7b). Involucrin reaction was particularly strong in some limited cells located among columnar cells or superficial cuboidal cells of glandular structures, which could not be discriminated by routine histology (Figs. 7c, e, f). EMA staining was confined to columnar cells (Figs. 5d and 7d).

Squamous cell carcinoma may arise from squamous metaplastic epithelium or from intestinal type of epithelium with squamous metaplasia. The basal layer cells of squamous metaplastic epithelium proliferated with downgrowth into connective tissue and muscle zone following papillary projection. Invasion results in frank keratinized squamous cell carcinoma (Fig. 8a). The intestinal type of epithelium underwent partial squamous metaplasia, transforming into non-keratinized squamous cell carcinoma. Histologically, these lesions could be divided into large and small cell types. The large cell type showed rather good stratification extending from the peripheral cell layer corresponding to basal cells, to the center of the focus corresponding to upper spinous parakeratinized cells (Fig. 9a, 10a). The small cell type was usually undifferentiated, arranged in small nests and strands without stratification (Fig. 11a, c). Occa-

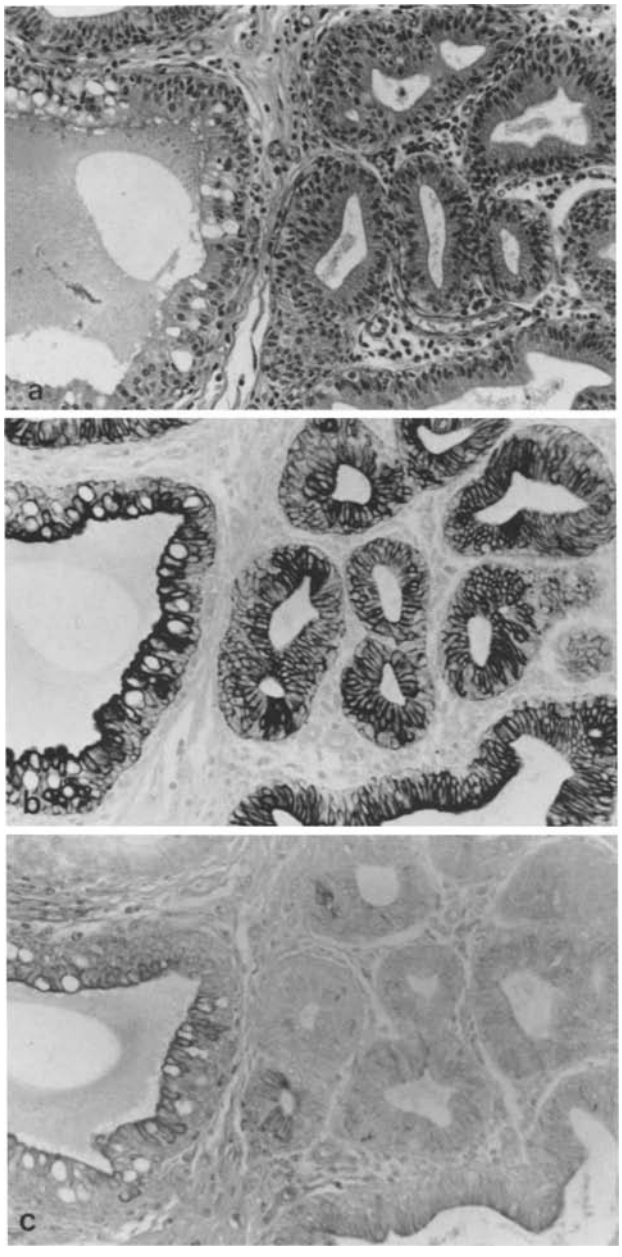

Fig. 6a–c. Multiple proliferation of glandular metaplastic epithelium (× 80). **a** H&E staining. Cystic spaced epithelium shows vacuolization and small tubular structures consist of well developed high columnar cells and basal cells. **b** KL1 staining. High columnar cells indicate strong staining to KL1 keratin, and basal cells less reactive to KL1 staining. **c** PKK1 staining. There are generally weak response to PKK1 staining in all the epithelial components, however some area of columnar epithelial cells display a little intense staining

sionally structures with a gland-like appearance were seen (Fig. 11e). KL1 staining in the transformed areas from mucous columnar cells to undifferentiated squamous cell carcinoma was characteristically strong, whereas PKK1 was slight. Some columnar cells were strongly positive for involucrin in these areas, although no evidence for keratinization was seen by routine histology (Fig. 11d).

Early squamous cell carcinomas showed moderate-to-strong TK staining in whole neoplastic epithelium and slight or negative in peripheral transformed layers. Strong KL1 staining was seen in well-differentiated squamous carcinoma (G 1) cells (Fig. 8b). No PKK1 staining was observed in any tumour cells. Involucrin staining was restricted to spinous tumour cells and squamous metaplastic epithelium (Fig. 8c). EMA reaction was confined to upper squamous cells which had differentiated toward keratinization. Peripheral tumour cells with papillary projections showed neither keratin, nor involucrin and EMA. Squamous cell carcinomas of the G 2 type showed a comparatively similar distribution of keratin, involucrin, and EMA as seen in the G 1 carcinomas. Irrespective of the low grade of keratinization in this tumour focus, KL1 and involucrin reactions were limited to central squamous tumour cells, but some reactions were seen in the peripheral tumour cells (Fig. 9b, c, 10b, c) and PKK1 staining was absent to the tumour focus. EMA staining indicated a high level in central tumour cells (Fig. 9d, 10d). In the G 3 squamous cell carcinomas, the small cell type, the distribution profiles of keratin proteins were irregular (for KL1 especially) and PKK1 keratins, involucrin and EMA were lacking in these tumour cells (Fig. 9b–d, 11b, d, f). These results are given in Table 2.

Discussion

Since the first description of bladder carcinoma associated with an infection of *Schistosoma haematobium* (Bilharz 1852), many aetiological factors have been discussed. Histological comparisons of these and other urinary bladder carcinomas have been made by many investigators (Goebel 1905; Moore and Meleney 1954; Friedman and Ash 1959; Hashem 1961; Gillman and Prates 1962; Higginson and Oettle 1962; Mustacoli and Shimkin 1966; Higashi and Aboul-Enein 1981; El-Bolkainy et al. 1981a, b). Recently, Cheever et al. (1976) reported the Capuchin monkey infected with *Schistosoma haematobium* as an animal model of human bilharzial bladder carcinoma and they monitored the urinary levels of several tryptophan metabolites as carcinogens in experimental carcinoma (Brown et al. 1976). Quantitative and qualitative estimations of urinary tryptophan metabolites have already been made in man (Khalafallah and Abul-Fadl 1964; Brown et al. 1976) and a

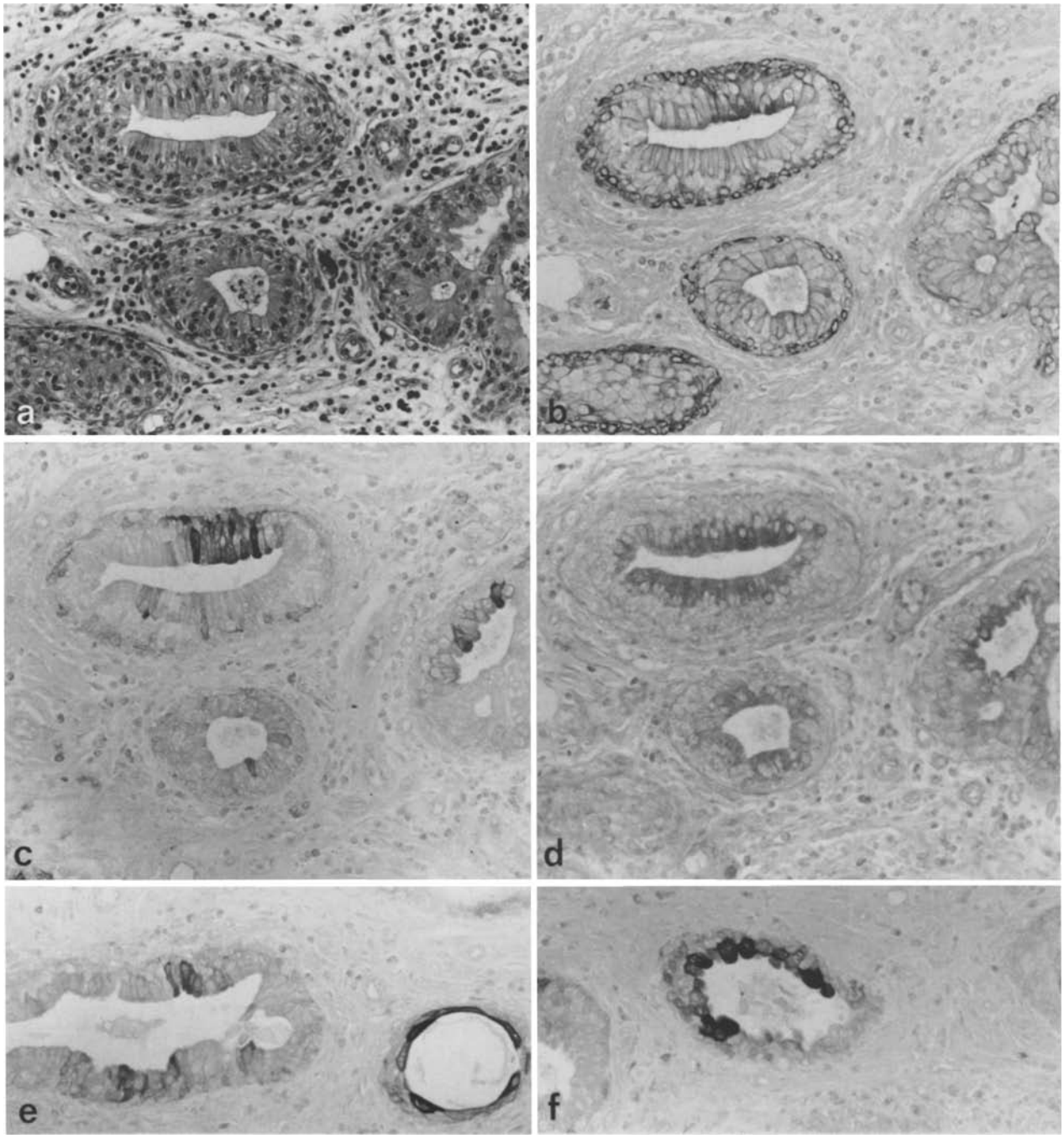

Fig. 7a–f. Glandular metaplastic epithelium (×100). **a** H&E staining. Glandular epithelia consist of the columnar cells in luminal surface. Polyhedral cells in intermediate layer and small round cells in basal side. **b** TK staining. Strong TK staining is limited to basal cells, and moderate-to-slight staining in surface columnar cells. **c** Involucrin staining. Almost epithelial cells are lacked to involucrin staining, whereas some epithelial cells located at superficial and intermediate layer cells are strongly reactive. **d** EMA staining. Luminal surface cells indicated slight positive reaction. **e** and **f** Involucrin staining. Some variant expressions of involucrin in glandular epithelium. Strongly positive cells scattered and located in the superficial layer of the tubule

causal relationship between urinary schistosomiasis and human bladder carcinoma may be indicated by the elevated levels of urinary tryptophan metabolites in infected patients when compared with non-infected controls.

Experimentally induced bilharzial carcinomas in the Capuchin monkey are of the papillary type (Kuntz et al. 1972; Brown et al. 1976; Cheever et al. 1976), whereas schistosomal carcinomas in human beings are frequently squamous cell type.

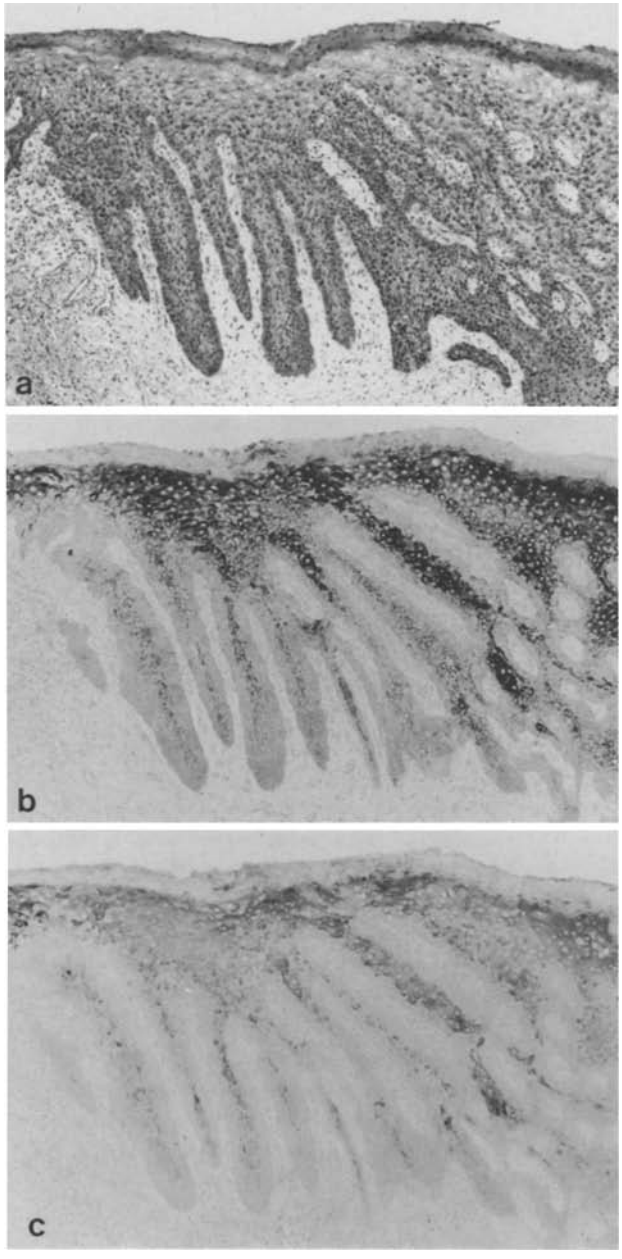

Fig. 8a–c. Early squamous cell carcinoma (×32). **a** H&E staining. There are numerous epithelial projections into subepithelial connective tissue and muscle zone. The neoplastic growth shows endophytic type of proliferation. **b** KL1 staining. Peripheral tumour cells corresponding to the basal layer cells are devoid of KL1 keratin staining, whereas upper spinous tumour cells strong positive. **c** Involucrin staining. Upper spinous tumour cells show positive reaction

However, bilharzial squamous cell carcinoma are not generally remarkable in terms of their tendency towards keratinisation in contrast with carcinoma of the skin and oral cavity, and the mechanism of squamous metaplasia showed some especially interesting features here.

Table 2. Keratin, involucrin (Invo) and epithelial membrane antigen (EMA) in variants of urothelial epithelium and squamous cell carcinoma

	TK	KL1	PKK1	Invo	EMA
Normal transitional epi.					
Umbrella cells	+3–4	+3–4	+3–4	0–+1	0–+1
Intermediate layer cells	+3	+2–3	+1–2	+2	+1
Basal layer cells	+3	+2–3	+2	0	0
Squamous metaplastic epi. (& Basal hyperplastic epi.)					
Upper layer cells	+2–3	+3	0	+3–4	0–+1
Intermediate cells	+2–3	+1–2	0	+1–3	0
Basal layer cells	+2–3	0	0	0	0
Intestinal type of epi. (& Glandular metaplastic epi.)					
Columnar (or cuboidal) cells	+1	+3–4	0–+2	0 or +3	+1
Basal layer cells	+2–3	0	0	0	0
Squamous cell carcinoma					
G 1 & G 2	+2–3	+3–4	0	+2–3	+3–4
G 3	+2	+1 or +3	0	0	0

epi.: Epithelium
0: negative; +1: slight; +2: moderate; +3: strong; +4: strongest

In the present study, immunohistochemical evaluation of keratins, involucrin, and EMA has been made in bilharzial bladder carcinomas which usually have non-keratinized squamous cells; and a comparison of staining levels of these markers among variants of squamous metaplastic epithelia and carcinomas was also made. Keratin expression in squamous cell epithelium has been well documented as being zonal in distribution (Löenig et al. 1980, 1982; Woodcock-Mitchell et al. 1982; Nelson and Sun 1983; Sun et al. 1983; Viac et al. 1983; Nakai and Mori 1986); however, the pattern in transitional cell epithelium had not been previously described in detail and no report has been previously made concerning the histochemical properties of these cells. Recently it has been reported that keratin decoration in the umbrella cells of transitional epithelium was one of their most important features and the biological significance of these umbrella cells among the superficial cells may be as a protection from the toxic materials present in urine (Asamoto et al. 1987). Involucrin is a marker of terminal keratinization in keratinocytes and it is usually distributed in the granular cells or adjacent upper spinous layer cells (Warhol et al. 1982; Said et al. 1984; Walts et al. 1985; Sumitomo et al. 1986; Itoiz et al. 1986). Distribution patterns of involucrin and EMA in urinary bladder and their neoplastic lesions have not been also pre-

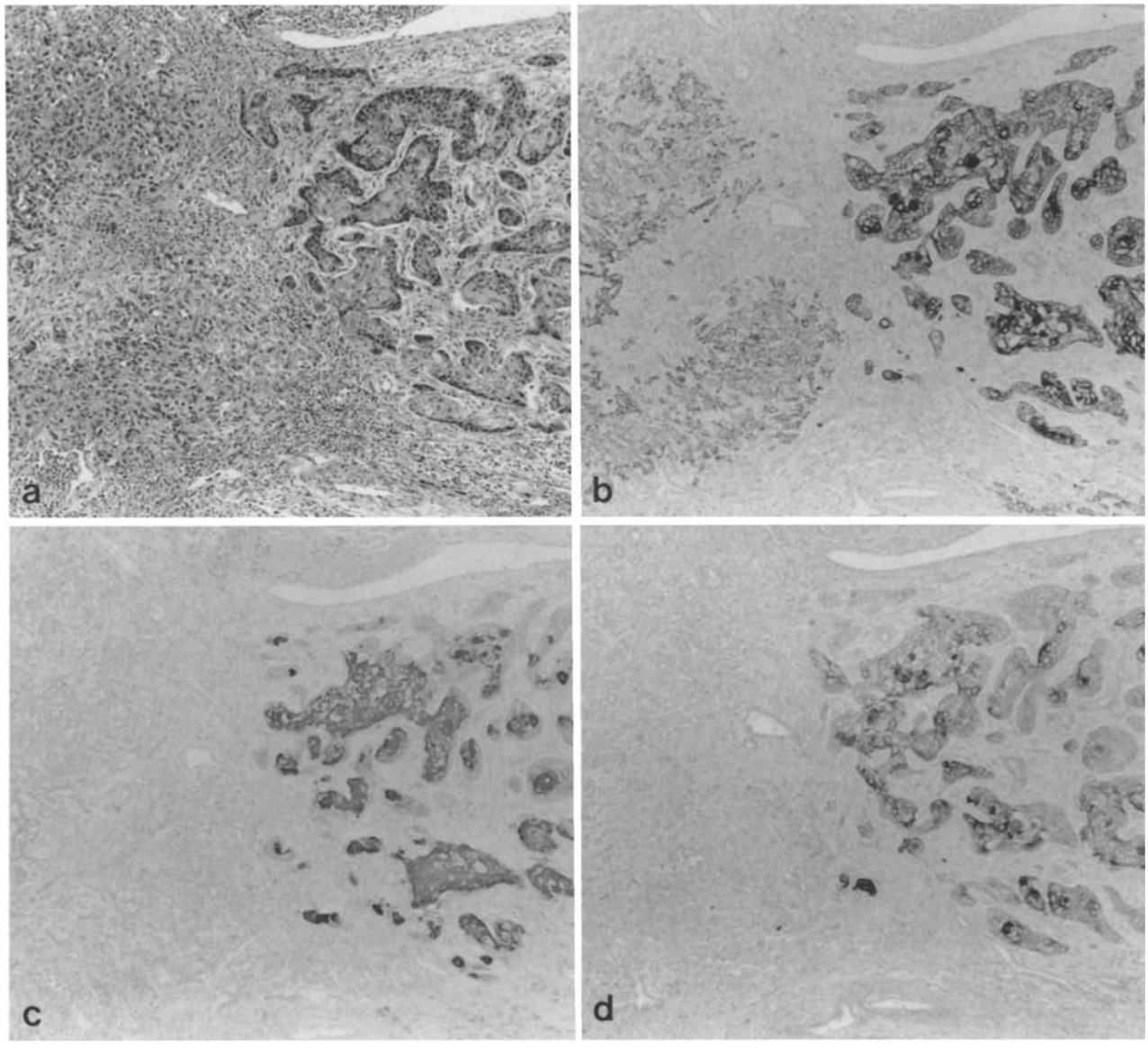

Fig. 9a–d. Squamous cell carcinoma (×40). **a** H&E staining. There are two types of squamous cell carcinoma; The right side is keratinized type (G 2) and the left side is non-keratinized type (G 3). **b** KL1 staining. Keratinized tumour cells displays marked staining to KL1, whereas non-keratinized tumour cells slight staining. **c** Involucrin staining. Keratinized tumour cells show marked staining, however, non-keratinized tumour cells are not reactive. **d** EMA staining. EMA reactions are positive in keratinized tumour cells. Immunohistochemical expression are different between keratinized and non-keratinized type

viously reported, although it has been reported that involucrin and EMA can be detected in skin epidermoid carcinomas and in EMA staining other epithelial tumours (Warhol et al. 1982; Said et al. 1984; Cordwell et al. 1985; Heyderman et al. 1985; Pinkus and Kurtin 1985; Tubura et al. 1985; Walts et al. 1985; Zotter et al. 1985; Itoiz et al. 1986; Sumitomo et al. 1986; Tatemoto et al. 1987a, b).

In the present material from Egypt, involucrin staining in the normal urothelial epithelium was of slight intensity, while changed epithelium was irregularly present in intermediate layer cells. This finding suggests that umbrella cells may not be fully keratinized. Squamous metaplastic changes were occasionally evident in bladder epithelium, and keratin and involucrin expressions within them were relatively similar to those of the terminal keratinized cells (upper spinous and granular cells) seen in the other squamous epithelia, except for PKK1 staining, which was positive in the basal cells of stratified squamous epithelium (Nakai and Mori 1986). The expression of keratins and involucrin in G 1 and G 2 type squamous cell carcinomas may be rather similar to that of squamous

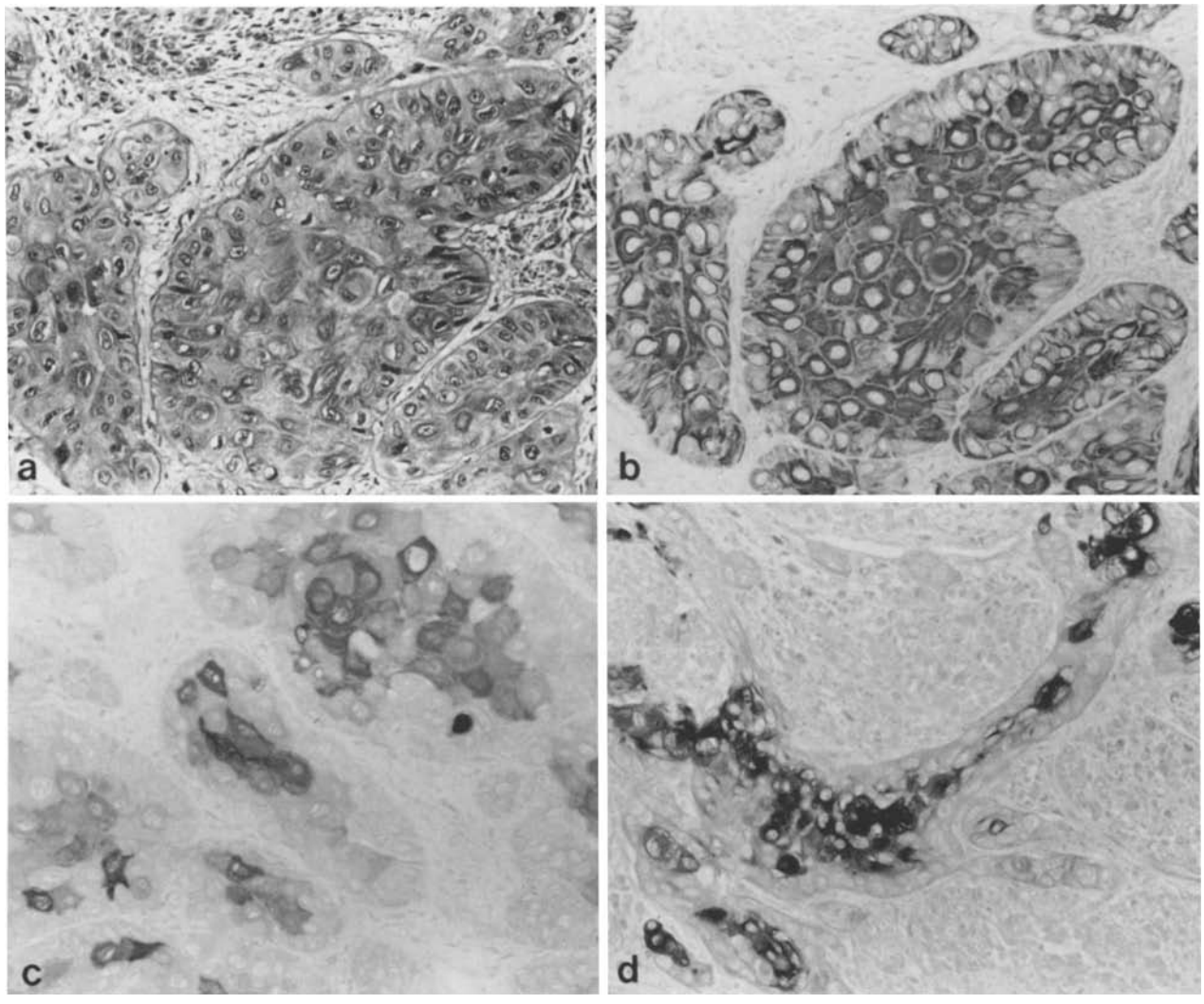

Fig. 10a–d. Squamous cell carcinoma (×100). **a** H&E staining. Moderate keratinized squamous cell carcinoma (G 2). There are no intense hornification into tumour focus. **b** KL1 staining. Intensely KL1 reaction is distributed in the central located neoplastic cells, while no or slight reaction in peripheral tumour cells, corresponding to basal of intact squamous cell epithelium. **c** Involucrin staining. Positive involucrin staining is confined to keratinizing neoplastic cells on the central focus. **d** EMA staining. Strong positive EMA reaction is also limited to keratinizing tumour cells. EMA distribution are usually resembled to that to involucrin

metaplastic epithelium. The intestinal type of urothelium was another variant of transitional epithelial change. The high columnar cells were occasionally vacuolated. These columnar cells gave particularly abundant KL1 keratin staining, while their reaction to TK and PKK1 reagents was slight or negative. Basally located cells in such epithelium gave only a moderate TK reaction and gave none with KL1 and PKK1. Involucrin staining was strong in some limited columnar cells of glandular structures and in the cuboidal or round cells of duct-like structures. Bilharzial bladder carcinoma may also arise from transitional phase or metaplastic form of intestinal columnar epithelium because the signs of keratinization were seen in premetaplastic columnar epithelium. In the process of carcinogenesis in the intestinal type, the first stage appears to be the formation of involucrin in columnar cells or superficial cuboidal cells; and in the second stage squamous metaplasia may occurs in almost all columnar cells.

In conclusion, the gradual change or irregular distribution of keratin proteins in both metaplastic and carcinomatous process could be detected. The low molecular weight keratins disappeared as a first change, which was found in hyperplastic or premalignant lesions in other squamous epithelium. Occasionally, the differential diagnosis of either G 2 or G 3 carcinoma, including mixed lesions, may be possible by the irregular finding of KL1 keratin. Involucrin expression may be a most useful marker of terminal keratinization or may

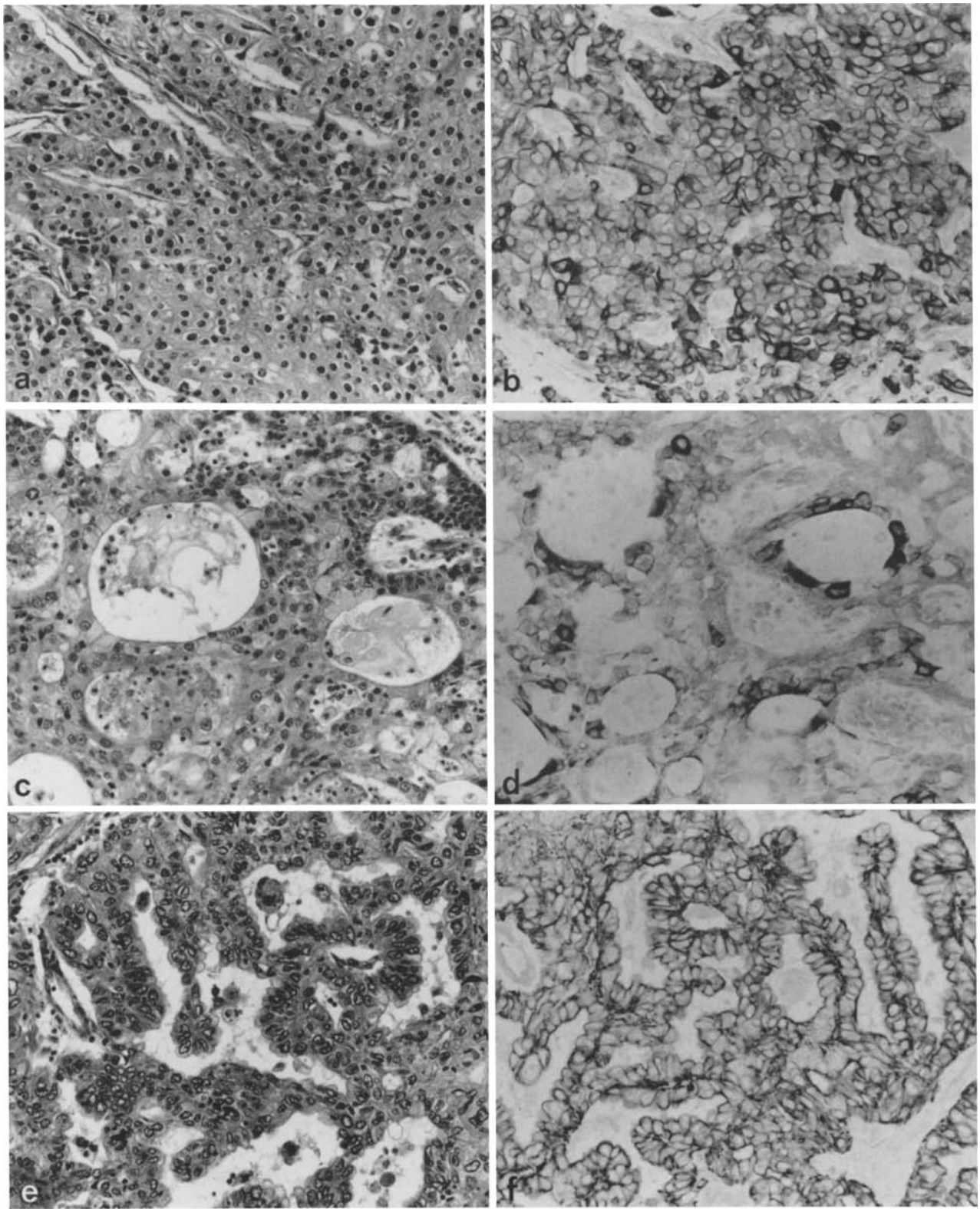

Fig. 11a–f. Variant of bladder carcinoma (×100). **a** H&E staining. Non-keratinized squamous cell carcinoma (G 3). There are diffusely proliferated non-keratinized tumour cells. **b** KL1 staining. Most of neoplastic cells show moderate reaction to KL1, however strongly positive cells are scattered into tumour focus. **c** H&E staining. Neoplastic transformation areas from glandular, intestinal mataplastic epithelia and G 3 carcinomatous lesions are mixture. **d** Involucrin staining. There are striking positive cells scattered into glandular epithelia. Mucous cells of cystic surface and other tumour cells are negative to involucrin staining. **e** H&E staining. Papillary proliferation of tumour cells. **f** KL1 staining. All the neoplastic cells display uniform staining to KL1 with moderate levels

be used to detect potential keratinization. Involucrin staining could then be employed to assess keratinizing potential in urothelial carcinomas.

Urothelial squamous cell carcinomas were occasionally positive for EMA and this feature coincided with abnormal keratinization due to malignant transformation in neoplastic cells, and with involucrin expression. EMA may also, therefore be a useful marker of differential diagnosis in metaplastic lesions, G 1 and G 2 carcinoma.

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