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Production of β-human chorionic gonadotropin by prostatic adenocarcinoma and transitional cell carcinoma of the upper urinary tract

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Summary. Ectopic production and secretion of hormones by a wide variety of tumours has been known for many years. Recently human chorionic gonadotropin (HCG) production and/or secretion have been noted in 15 cases with prostatic adenocarcinoma (Fukutani *et al.* 1983; Papapetrou *et al.* 1980; Broder *et al.* 1977; Menon & Stefani 1980; McManus *et al.* 1976) and in two with upper urinary tract transitional cell carcinoma (Fukutani *et al.* 1983; McManus *et al.* 1976). In this study we utilised the indirect immunoperoxidate technique to demonstrate β-HCG production in prostatic adenocarcinoma and upper urinary tract urothelial tumours. Of 100 cases of prostatic adenocarcinoma β-HCG production was demonstrated in nine cases, eight of which were poorly differentiated, and of 14 urothelial tumours of the upper urinary tract β-HCG production was present in two high grade transitional cell carcinomas.

Keywords: β-human chorionic gonadotropin, prostatic adenocarcinoma, transitional cell carcinoma of ureter and renal pelvis, immunoperoxidase technique

The frequency and types of tumours associated with human chorionic gonadotropin (HCG) production and secretion are greater than has been previously appreciated (McManus *et al.* 1976) and more recently β-HCG production and secretion have been demonstrated in wide variety of tumours in addition to the commonly associated gonadal, placental and extragonadal chorioncarcinoma and we have recently demonstrated β-HCG production in 12 of 104 bladder transitional cell carcinomas (Shah *et al.* 1986). To our knowledge, only 15 cases of prostatic adenocarcinoma producing HCG have been reported in the literature (Fukutani *et al.* 1983; Papapetrou *et al.* 1980; Broder *et al.* 1977; Menon & Stefani 1980; McManus *et al.* 1976) and in far fewer cases of upper urinary tract transitional cell carcinomas (Fukutani *et al.* 1983; McManus *et al.* 1976). We, therefore, undertook this retrospective study utilizing the indirect immunoperoxidase technique on paraffin processed tissue to demonstrate β-HCG production in

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prostatic adenocarcinoma and in transitional cell carcinoma of the upper urinary tract.

Materials and methods

We obtained from the files in the Pathology Department at the East Birmingham Hospital 100 biopsies of prostatic adenocarcinoma that had been received between 1976 and 1979 as well as 14 cases of transitional cell carcinoma of the upper urinary tract received between 1981 and 1985. Of the latter there were equal numbers of renal pelvic and ureteric tumours. All tissues had been fixed in 4% solution of formaldehyde in saline and were processed for paraffin section and cut at 3 μm. Sections of placental tissue served as positive controls while four sections of benign prostatic hyperplasia and five sections of normal urothelium served as negative controls.

Human chorionic gonadotropin is a glycoprotein hormone composed of two non-covalently bound alpha and beta sub-units. Because the alpha chains of luteinizing hormone and HCG are virtually identical in their amino acid sequence, the beta chain of HCG has been chosen as the antigen as this subunit confers the immunologic and biologic specificity of HCG.

The indirect immunoperoxidase technique entailed the following steps: Sections were dewaxed through xylene to ethanol, placed in 0·3% hydrogen peroxide in methanol for 15 min at room temperature to block endogenous peroxidase, washed in Tris buffer at pH 7.6, then incubated with normal swine serum (diluted 1/5) for 10 min, the excess then tipped off and the sections were then overlaid with rabbit anti-β-HCG diluted 1 in 25 in Tris buffer for 20 min.

After a thorough wash in buffer the sections were covered with peroxidase conjugated swine anti-rabbit immunoglobulin, diluted 1 in 10 and incubated for 20 min. Following a further wash in Tris buffer the peroxidase complex was visualized by reaction with a freshly prepared solution of DAB (3-3′ di-aminobenzidine) and hydrogen peroxide. Sections were counterstained with Mayer's haemalum, dehydrated in 74% alcohol, cleared in xylene and mounted in synthetic medium.

As it had been shown that prior trypsinization reduced intensity of the staining reactions, this step was omitted (Curran & Gregory 1979). All reactions took place at room temperature. All antisera were diluted with Tris buffer at pH 7.6. The anti-β-HCG was shown to be blocked by β-HCG preadsorption. The other usual controls were applied (Crocker & Smith 1984).

All positive cases were subsequently examined in clinical detail and all available previous and/or subsequent biopsies were also tested for β-HCG production.

Table 1. Cases of prostatic adenocarcinoma

Degree of differentiation	No. of cases	No. positive for β-HCG	(%)	Documented follow up (mean 4 years)	No. dying of adenocarcinoma
Well	15	0	0	3	0
Moderate	20	1	5	13	3 (0)*
Poorly	65	8	12.3	29	16 (5)
Total	100	9	9	45	19 (5)

* ( ) Patients with β-HCG tumour positivity.

Results

All four sections of benign prostatic hyperplasia and all five sections of normal urothelium, as expected, were negative for β-HCG staining. Of the 100 prostatic biopsies tested nine were positive for β-HCG, shown in Table 1. The tumour in case 1 was moderately differentiated while the remaining eight tumours were poorly differentiated adenocarcinoma, shown in Tables 1 and 2. The staining for β-HCG was always focally distributed, being present in small groups of cells and even as isolated single cells, amounting to less than 10 cells per high power field. In all cases the staining had a coarse granular appearance and was localized to the cytoplasm of mononucleate cells: the nuclei and stroma being uniformly negative (Figs 1–4). In addition there was also a more generalized diffuse fine granularity involving 30–60% of the cells. None of the biopsies contained multinucleate cells and the surrounding non-tumorous glandular tissue was consistently negative. In three cases (cases 7, 8 and 9) clinical records were not available for study. Of the remaining six, only in three cases were previous and/or subsequent biopsies taken and all were positive (cases 1, 2 and 6), shown in Table 2. In none of the six cases had serum and/or urine been tested for the presence of β-HCG. Five of these six patients have died; four of carcinomatosis with associated bronchopneumonia and one of carcinomatosis associated with a cerebrovascular accident. All these five patients died within 3 years of presentation and postmortem examinations were not performed. Only one of these six patients is alive and well 7 years after the initial presentation of a moderately differentiated adenocarcinoma of prostate (case 1). With regard to the group as

Table 2. Clinical and Pathological details of β-HCG positive prostatic adenocarcinoma

Case	Age (years)	Degree of differentiation	Previous or subsequent tumour β-HCG status	Treatment	Prognosis
1	72	Moderate	positive	Surgery	Alive and well 7 years.
2	74	Poor	positive	Surgery + stilbestrol	Died 2 years. Carcinoma and bronchopneumonia.
3	79	Poor	No biopsies	Surgery	Died 3 years. Carcinoma and cerebrovascular accident.
4	64	Poor	No biopsies	Surgery	Died 2 years. Carcinoma and bronchopneumonia.
5	50	Poor	No biopsies	Surgery + stilbestrol	Died 2 years. Carcinoma and bronchopneumonia.
6	74	Poor	positive	Surgery + stilbestrol	Died 2 years. Carcinoma and bronchopneumonia.
7	—	Poor			
8	—	Poor		No details available	
9	—	Poor			

Fig. 1. CASE 4. Prostatic adenocarcinoma. Bladder neck tissue infiltrated by poorly differentiated adenocarcinoma. H & E, ×40.

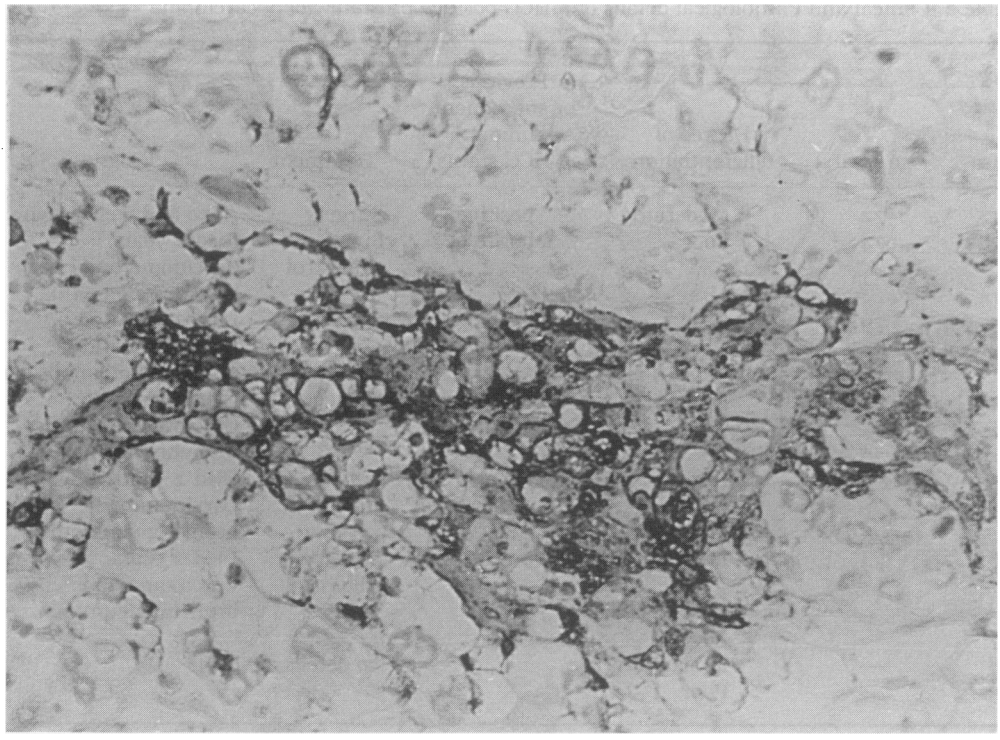

Fig. 2. CASE 4. Prostatic adenocarcinoma. Large group of intensely positive mononucleate cells. Immunoperoxidase, ×400.

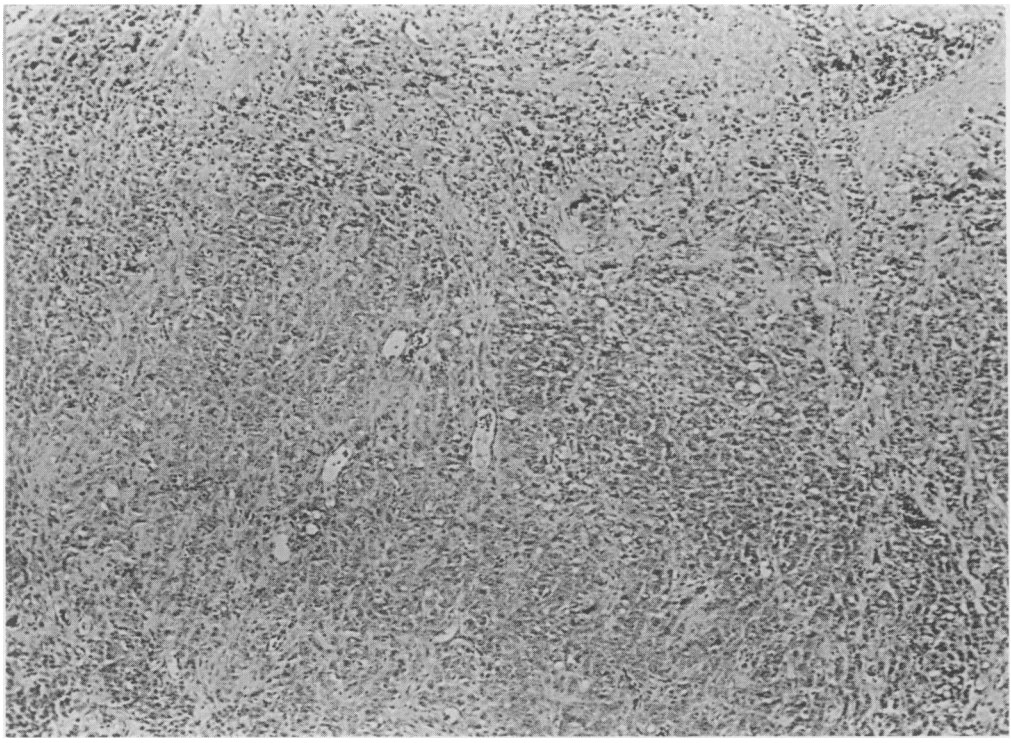

Fig. 3. CASE 7. Prostatic adenocarcinoma. Extensive infiltration by poorly differentiated adenocarcinoma. H & E, ×40.

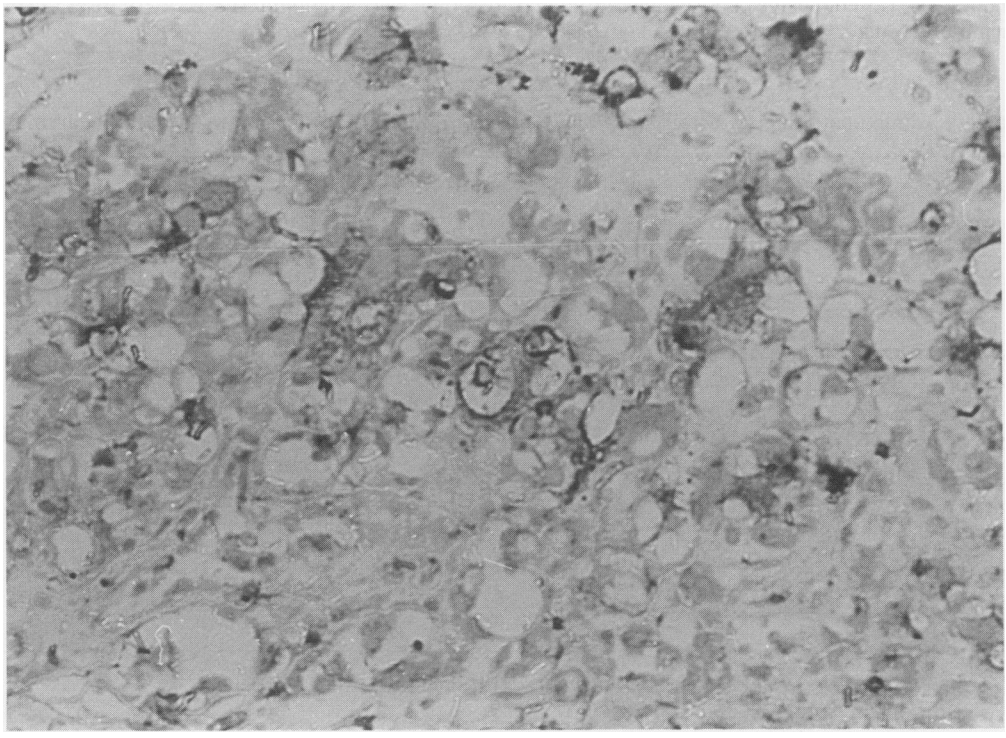

Fig. 4. CASE 7. Prostatic adenocarcinoma. Small group of intense focal positivity within mononucleate cells. Immunoperoxidase, ×400.

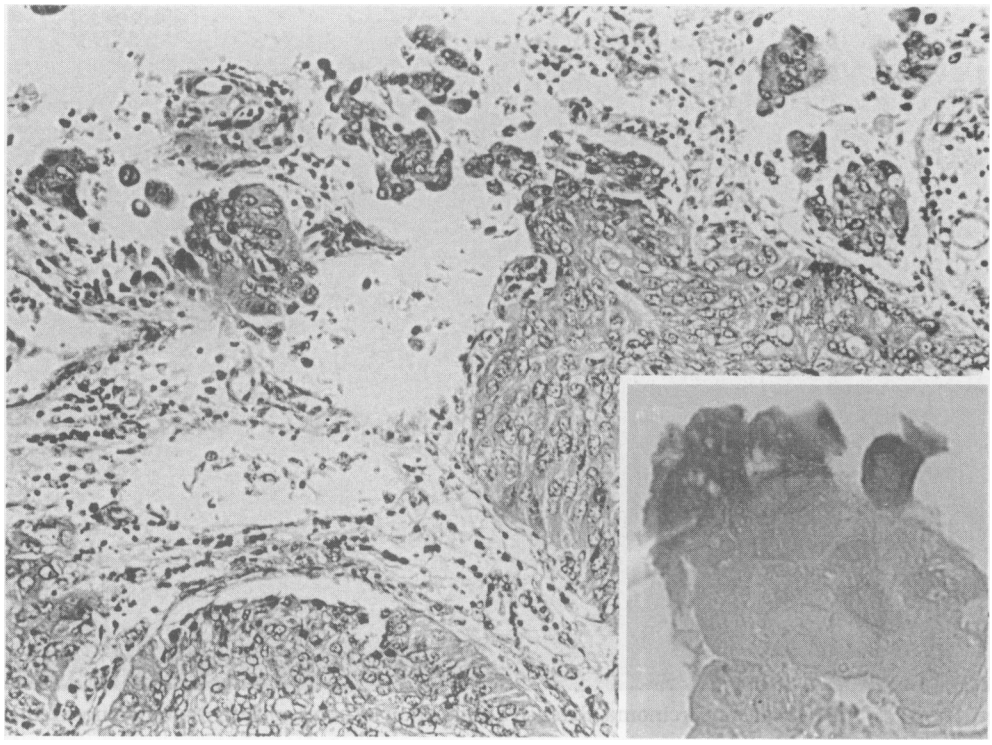

Fig. 5. CASE 1. Renal pelvis. Poorly differentiated transitional cell carcinoma. H & E, ×40. **Inset**, A small group of intensely positive mononucleate cells. Immunoperoxidase, ×400.

Table 3. Clinico-pathological details and β-HCG status in upper renal tract transitional cell carcinoma

Case/age in years/sex	Laterality	Site	Grade	β-HCG Status	Prognosis*
1/81/F	R	P	3–4	Positive	D 6 months
2†/63/F	R	U	3–4	Positive	D $2\frac{1}{2}$ years
3/63/M	L	U	2	Negative	AW 1 year
4/57/M	L	U	1	Negative	AW 2 years
5/81/F	L	U	1–2	Negative	AW 1 year
6/64/M	L	U	1	Negative	AW 2 years
7/66/M	R	U	1	Negative	D 2 years with R
8/83/M	L	P	2-3	Negative	No follow up
9/57/M	R	P	1–2	Negative	AW 2 years
10/60/M	L	P	1	Negative	AW 3 years
11/71/M	L	P	1–2	Negative	AW $1\frac{1}{2}$ years
12/81/M	R	PU	2	Negative	No follow up
13/71/M	R	PU	2–3	Negative	AW 1 year
13/67/M	L	U	2–3	Negative	Records lost

* A, alive; R, recurrence; D, dead; W, well; R, right; L, left; U, ureter; P, pelvis.
† History of Acetophenetidine (Phenacetin) abuse.

a whole, adequate documented follow up exists for 45 patients, with a mean of 4 years (see Table 1). Almost one third of the patients with poorly differentiated adenocarcinoma that died within 4 years were positive for β-HCG production.

One of the ureteric and one of the pelvic transitional cell carcinomas (Fig. 5) were positive for β-HCG production. Both these cases were high grade invasive transitional cell carcinomas. The staining had a coarse granularity, involving small groups of cells, less than 10 cells at any one high power field. It was localized to the cytoplasm of mononucleate cells, the nuclei and stroma being uniformly negative. All 14 cases were subsequently examined in clinical detail, shown in Table 3.

Discussion

Chorioncarcinoma is a relatively rare, highly malignant neoplasm which generally arises from the gestational trophoblastic tissue or within the ovary, testis or other extragonadal sites (Javadpour 1979). Less commonly, it has been documented within tumours arising in a wide variety of organs, usually as a component of an undifferentiated or poorly differentiated carcinoma, the ectopic β-HCG production being related to the degree of tumour differentiation (McManus *et al.* 1976; Shah *et al.* 1986; Javadpour 1979; Wirt *et al.* 1984).

To our knowledge in only 14 cases of prostatic adenocarcinoma has there been an associated detection of HCG in urine and/or serum by radio-immune-assay and in only one further case has the presence of β-HCG been detected in the tissues using immunoperoxidase technique on frozen material (Fukutani *et al.* 1983; Papapetrou *et al.* 1980; Broder *et al.* 1977; Menon & Stefani 1980; McManus *et al.* 1976).

In this retrospective study of 100 cases of prostatic adenocarcinoma, in nine cases of otherwise typical adenocarcinoma β-HCG production was detected within tumour cells. There were no histological features characteristic of chorioncarcinoma and we, therefore, regard the β-HCG positivity as an expression of hormone production. This phenomenon appears to be only partly related to the degree of tumour differentiation, all except one being poorly differentiated adenocarcinomas. Only one patient is alive and well 7 years later, the other five dying within 3 years. Within the poorly differentiated adenocarcinoma group, β-HCG positivity seems to indicate a worse prognosis.

Our results with regard to transitional cell carcinoma of the upper urinary tract (two of 14 cases showing β-HCG production) are similar to those that we have reported in association with transitional cell carcinoma of the urinary bladder (12 of 104 cases showing β-HCG production (Shah *et al.* 1986). To our knowledge there has only been a single previous report of β-HCG production by a transitional cell carcinoma of renal pelvis (McManus *et al.* 1976) and none by a ureteric tumour. However, β-HCG secretion has been demonstrated by urinary radioimmunoassay in one case of transitional cell carcinoma of ureter (Fukutani *et al.* 1983) and by serum radioimmunoassay in four cases of transitional cell carcinoma, site not specified (Lange *et al.* 1976).

There are several hypotheses for the histogenesis of ectopic β-HCG producing neoplastic cells in various tumours; these include an origin from totipotential cells, from germ cell rests, metaplasia and retro-differentiation. The latter hypothesis seems to be the most likely as the production of β-HCG is usually associated with the less differentiated tumour (McManus *et al.* 1976; Wirt *et al.* 1984; Civantos & Rywlin 1972).

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References

BRODER L.E., WEINTRAUB B.D., ROSEN S.W., COHEN M.H. & TEJADA F. (1977) Placental proteins and their subunits as tumor markers in prostatic carcinoma. *Cancer* **40**, 211–216.

CIVANTOS F. & RYWLIN A.M. (1972) Carcinomas with trophoblastic differentiation and secretion of chorionic gonadotrophins. *Cancer* **29**, 789–797.

CROCKER J. & SMITH P.J. (1984) Immunohistochemical localisation of factor VIII-related antigen in Hodgkin's disease. *J. Clin. Pathol.* **37**, 37–44.

CURRAN R.C. & GREGORY J. (1979) The unmasking of antigens in paraffin sections of tissue by trypsin. *Experientia* **33**, 1400–1401.

FUKUTANI K., LIBBY J.M., PANKO W.B. & SCARDINO P.T. (1983) Human chorionic gonadotropin detected in urinary concentrates from patients with malignant tumors of the testis, prostate, bladder, ureter and kidney. *J. Urology* **129**, 74–77.

JAVADPOUR N. (1979) The value of biologic markers in diagnosis and treatment of testicular cancer. *Semin. Oncol.* **6**, 37–47.

LANGE, P.H., MCINTIRE K.R., WALDMANN T.A., HAKALA T.R. & FRALEY E.E. (1976) Serum alpha fetoprotein and human chorionic gonadotropin in the diagnosis and management of nonseminomatous germcell testicular cancer. *New Engl. J. Med.* **295**, 1237–1240.

MENON M. & STEFANI S.S. (1980) Evaluation of human chorionic gonadotrophin B levels in prostatic carcinoma. *Urol. Int.* **35**, 291–293.

MCMANUS L.M., NAUGHTON M.A. & MARTINEZ-HERNANDEZ A. (1976) Human chorionic gonadotropin in human neoplastic cells. *Cancer Res.* **36**, 3476–3481.

PAPAPETROU P.D., SAKERELOU N.P., BRAOUZI H. & FESSAS P.H. (1980) Ectopic production of human chorionic gonadotrophin (HCG) by neoplasms: the value of measurements of immunoreactive HCG in urine as a screening procedure. *Cancer* **45**, 2583–2592.

SHAH V.M., NEWMAN J., CROCKER J., CHAPPLE C.R., COLLARD M.J., O'BRIEN J.M. & CONSIDINE J. (1986) Ectopic β-human chorionic gonadotropin production by bladder urothelial neoplasia. *Arch. Pathol. Lab. Med.* **110**, 107–111.

WIRT D.P., NAGLE R.B., GUSTAFSON H.M., PHILPOTT P.J., KUIVINEN N.A. & SCHUCHARDT M. (1984) The probable origin of an anemone cell tumor: Metastatic transitional cell carcinoma producing HCG. *Ultrastructural Pathol.* **7**, 277–288.