Journal of Clinical Pathology, 1979, **32**, 368-372

An immunohistochemical study of the significance of HCG secretion by large bowel adenocarcinomata

C. H. BUCKLEY AND H. FOX

From the Department of Pathology, The University, Manchester M13 9PT, UK

SUMMARY A series of five benign and 60 malignant colonorectal neoplasms has been examined immunohistochemically for the presence of HCG. This hormone was not demonstrated in any of the benign tumours but was present in 43% of the malignant neoplasms. The incidence of HCG secretion was unrelated to the sex of the patient but tended to be decreased in patients of advanced age. The HCG-containing tumours, which were predominantly from the left side of the large intestine, had all penetrated the full thickness of the bowel wall while a significant proportion of those tumours lacking HCG were still confined to the bowel wall. Despite the greater degree of local aggressiveness shown by the HCG-secreting tumours there was no correlation between HCG production and the presence of local metastases but, as the presence of HCG is associated with local invasion, it is suggested that preoperative immunohistochemical studies of HCG in biopsies of large bowel neoplasms may be of value in the planning of surgical procedures.

The introduction of a specific and highly sensitive radioimmunoassay technique for the measurement of plasma levels of human chorionic gonadotrophin (HCG) has allowed for the demonstration of secretion of this placental hormone by a wide variety of non-trophoblast containing neoplasms (Rosen *et al.*, 1968; Weintraub and Rosen, 1971; Braunstein *et al.*, 1973). That the hormone is actually synthesised by the neoplastic cells seems virtually certain, but the biological and prognostic significance of tumour secretion of HCG is not yet well defined. Kahn *et al.* (1977) and Walker (1978) have, however, shown that pancreatic islet cell and breast carcinomas which synthesise HCG appear to behave in a more aggressive manner, as indicated by the presence of metastases, than do similar neoplasms which lack this secretory capacity.

It is well established that a proportion of colonorectal carcinomas, variously estimated as being between 8·5 and 41%, secrete HCG (Braunstein *et al.*, 1973; Goldstein *et al.*, 1974; Rosen *et al.*, 1975; Gailani *et al.*, 1976) and we report here an immunohistochemical study of HCG in large bowel neoplasms in which the clinicopathological and prognostic significance of the ability to synthesise this hormone was assessed.

Received for publication 18 October 1978

Material and methods

A total of 65 colonic and rectal tumours (5 benign and 60 malignant) were examined from 61 patients. One patient, a 39-year-old man with ulcerative colitis, had two discrete adenocarcinomata and three patients had an adenocarcinoma with a separate benign adenomatous polyp.

The tissue was fixed in 4% formaldehyde solution either by opening the intestine and immersing the specimen in fixative, or by instilling formalin into the lumen of the closed bowel and then immersing the specimen. Representative blocks of the tumours were dehydrated, cleared, and embedded in paraffin wax. 5 μm sections were cut and those for morphological examination were stained with haematoxylin and eosin. The morphology of the malignant tumours is indicated in the Table.

The immunological studies were carried out using the PAP method described by Sternberger *et al.*

Table *Morphology of 60 malignant neoplasms*

Tumour differentiation	*Number*	*% of Total*
Well differentiated	37	62
Moderately differentiated	8	13
Poorly differentiated	10	17
Undifferentiated	3	5
Mucoid	1	1·5
Carcinoma in situ in an adenoma	1	1·5

(1970), except that the washing procedures between applications of antisera were reduced to two brief rinses and a 5-minute wash over a magnetic stirrer.

Commercially produced anti-HCG antiserum raised in rabbits to the whole HCG molecule (Miles) was used.

The sections were dewaxed in xylene and brought to alcohol. Endogenous peroxidase activity was blocked by immersing the sections in a solution of 0·3% 30 vol. hydrogen peroxide in methanol for 30 minutes.

The non-specific background staining was reduced by treating the sections with normal, non-immune, non-conjugated swine serum at a dilution of 1 in 5 for 5 minutes; it was further reduced by bringing all antisera to the required dilution in 1 in 20 swine serum in tris buffered saline. The non-immune swine serum was tipped off the slides, and the sections were wiped but not washed before the application of the primary antiserum at a dilution of 1 in 120 for 45 minutes. Swine anti-rabbit IgG serum (1 in 20) (Dakopatts) was then applied for 15 minutes and finally rabbit peroxidase anti-peroxidase complex (Dakopatts) 1 in 60 for 15 minutes. Peroxidase staining was achieved using the method of Graham and Karnovsky (1966) using 3,3 diaminobenzidine tetrahydrochloride (Sigma) at a concentration of 50 mg/100 ml in tris buffered saline to which was added 0·01 ml of 30 vol. hydrogen peroxide per 100 ml substrate solution. Harris's haematoxylin was used as a nuclear stain. Washing of all sections was carried out in tris buffered saline.

The reactivity of the HCG antiserum with related hormones was investigated. The antiserum was reacted at increasing dilutions with similar quantities of radioactively labelled preparations of highly purified HCG, luteinising hormone (LH), follicle stimulating hormone (FSH), and thyroid stimulating hormone (TSH). Gross reactivities were calculated by comparing the antiserum dilutions at which 50% of maximal radioactive binding to antibody occurred. Taking HCG as 100%, the gross reactivity of LH was 85%, FSH 60%, and TSH 20%. Control sections were treated with normal rabbit serum, and second and third trimester chorionic villi were stained for HCG using the same primary antiserum. In addition, sections were stained with antiserum to the specific β-subunit of FSH, TSH, and LH with negative results, to confirm that we were in fact demonstrating the specific β-subunit of HCG and not the α-subunit which is shared by HCG, FSH, TSH, and LH.

Absorption of the primary antiserum with whole HCG diminished the intensity of the staining at dilutions of 1 in 120. It was thought that the retention of some very slight staining was indicative of complex deposition, and dilutions to 1 in 200 entirely abolished this.

Results

PRESENCE OF HCG

Benign tumours

No HCG was demonstrated in any of the adenomatous polypi.

Malignant tumours

HCG was present in 26 (43%) of the 60 colonorectal carcinomata. The HCG-containing cells in those tumours giving a positive reaction were usually either solitary or focal but sometimes well-defined clusters within single or adjacent acini (Figure). The staining was seen in both the cytoplasm and the cell membrane, or in the cytoplasm alone, but in none of the tumours examined was the staining limited to the cell membrane. The HCG-containing cells tended to be seen in well-differentiated tumour tissue though three poorly differentiated and a mucoid tumour did contain the hormone; in the single mucoid carcinoma the HCG-containing cells were seen as clusters lying within pools of mucus.

The HCG-containing cells were present only in the more superficial part of the neoplasm (that is, in the tissue protruding into the lumen of the bowel) in 13 cases, were confined to the deep penetrating areas of the neoplasm in 10, and were present in both superficial and deep areas in 13.

CLINICOPATHOLOGICAL CORRELATES OF HCG SECRETION

Age

HCG production occurred in neoplasms from patients of all ages, ranging from the third to the ninth decades; HCG synthesis was, however, seen most frequently in tumours from patients aged 41-60, the incidence in such cases being 52% (11 of 21 tumours). In carcinomata from older patients, there was a progressive decline with age in the proportion of HCG-secreting neoplasms.

Sex

HCG secretion was apparent in 18 of the 39 tumours from male patients (46%) and in 8 of the 21 (38%) of those from female patients.

Site of tumour

Only 3 of the 17 large bowel carcinomata situated proximal to or at the splenic flexure contained HCG (18%) while of the 43 neoplasms distal to this site HCG was demonstrated in 23 (53%).

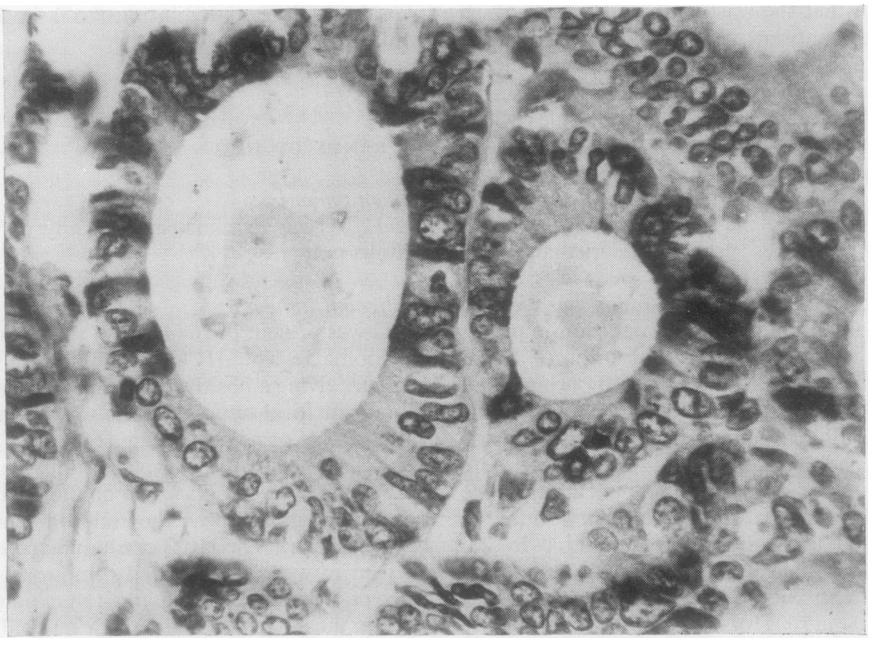

Figure *HCG secreting cells within a group of malignant acini. Immunoperoxidase stain (×300).*

Degree of local invasion

All the tumours that contained HCG had, without exception, penetrated the full thickness of the bowel wall and breached the covering serosa, many extending widely into the pericolic fat. By contrast, all those tumours that had not penetrated as deep as the serosa and were thus confined within the bowel wall gave a negative reaction to HCG. It was, of course, true that a failure to secrete HCG was not necessarily associated with a lesser degree of invasiveness for nearly two-thirds of such neoplasms had in fact penetrated the bowel wall; nevertheless the converse to this was not the case, for all the HCG-containing neoplasms were locally aggressive.

Metastases

Twenty-eight of the 60 (47%) malignant large bowel tumours were associated with metastases. Twelve of the 26 (46%) HCG-containing tumours had metastasised while 16 of the 34 (47%) HCG-negative neoplasms had metastasised.

Local immune response

No difference in the local lymphoplasmacytic response was observed between those tumours secreting HCG and those from which HCG was absent.

Discussion

HCG secretion appears to be a feature only of malignant neoplasms of the large bowel, a situation akin to that noted in the breast in which HCG production is a clear marker of malignancy (Horne *et al.*, 1976). The incidence of HCG-secretion, as determined by immunohistochemistry, in large bowel neoplasms is rather higher than that recorded in series in which evidence of HCG secretion was based solely upon radioimmunoassay of plasma levels; this is indicative both of the greater sensitivity of the histological method and of the fact that in many tumours only a few isolated cells appear to be producing the hormone. Our series also differs from those reported by workers using radioimmunoassay methods in showing no relation between the sex of the patient and tumour secretion of HCG, and in the decreased incidence of HCG secretion in neoplasms from patients of advanced age.

In this study those cells producing HCG were morphologically indistinguishable from immediately adjacent cells lacking this facility, and we saw no evidence that HCG secretion was associated with the presence of morphologically recognisable choriocarcinomatous foci within large bowel carcinomata. It is a matter of debate, of course, whether a cell

secreting HCG should be considered as behaving functionally as trophoblast or not, but the ability of epithelial cells to secrete this hormone has been variously attributed to retrodifferentiation, neoantigen formation as a manifestation of neoplastic de-differentiation, and desuppression of a normally suppressed genome (Pick, 1926; Goldenberg *et al.*, 1976).

In previously reported studies of the significance of tumour secretion of HCG elsewhere in the body, there has been a measure of agreement that this particular hormonal capacity is associated with an aggressive form of tumour growth and a poor, or relatively poor, prognosis. Thus, Tormey *et al.* (1977) found that patients with an HCG secreting breast carcinoma had a poorer response to chemotherapy, or shorter remissions, than had those whose tumours did not secrete HCG, while Walker (1978) noted a clear correlation between HCG secretion by breast carcinomata and the presence of lymph node metastases. Similarly, Kahn *et al.* (1977) found that a high proportion of functional malignant islet cell tumours secreted HCG while the benign tumours did not. In our present series, the same tendency was apparent, for although we were not able to demonstrate any correlation between HCG secretion by large bowel neoplasms and the presence of metastases there was a considerably increased degree of local invasion by those tumours secreting this substance. The reasons for this are obscure though it is of interest that HCG is known to modify both cell-mediated and humoral immune responses to antigenic stimulation (Contractor and Davies, 1973; Fabris *et al.*, 1977), and it has been argued that HCG masks the malignant cells from the patients' T-cell response, thus allowing for a greater proliferative and invasive capacity (McManus *et al.*, 1976); this is an attractive hypothesis but it must be doubted if the extremely small quantities of HCG synthesised by many of these neoplasms could be capable of significantly influencing an immune response. Certainly our own observations failed to detect a demonstrable reduction in lymphoplasmacytic response to the tumours producing HCG.

The practical significance of the correlation between HCG secretion and tumour invasiveness is that preoperative knowledge of HCG secretion by colonorectal neoplasms could alert the surgeon to the strong possibility that the tumour has already breached the bowel wall; Wood *et al.* (1977) have shown a significant reduction in survival time of patients with colonorectal tumours which have invaded locally, regardless of the presence or absence of local lymph node metastases, and hence prior suspicion of wall penetration should allow for a more considered plan of surgical attack. Information of this type can be gained from preoperative radioimmunoassay of serum levels, but this gives an artificially low figure of HCG secretion, the true incidence of which is most accurately assessed by immunohistochemical techniques. Preoperative staining of a biopsy specimen of a tumour for HCG may, therefore, be of considerable value, and in this respect it is of note that HCG secretion is found particularly in neoplasms of the left side of the colon and that in 62% of such neoplasms the foci of positive cells are present in the more superficial parts of the neoplasms; in other words, HCG secretion occurs most commonly in those tumours accessible to preoperative sampling and in those parts of the neoplasms most easily obtained for biopsy.

A series of surgical biopsies from 24 colonorectal neoplasms, which we have already stained using the method described in this paper, gives every indication that the technique lends itself to routine diagnostic application, and no difficulties have so far arisen from fragmentation of the tissue, size of the biopsy tissue, or orientation of the specimen.

We are grateful for the technical help given to us in the preparation of this paper by I. Laing, PhD, MRCPath, principal biochemist, St Mary's Hospital, Manchester, and J. S. Davies, FIMLS, senior medical laboratory scientific officer, The Department of Pathology, University of Manchester.

References

Braunstein, G. D., Vaitukaitis, J. L., Carbone, P. P., and Ross, G. T. (1973). Ectopic production of human chorionic gonadotrophin by neoplasms. *Annals of Internal Medicine*, **78**, 39-45.

Contractor, S. F., and Davies, H. (1973). Effect of human chorionic somatomammotrophin and human chorionic gonadotrophin on phytohaemagglutinin-induced lymphocyte transformation. *Nature New Biology*, **243**, 284-285.

Fabris, N., Piantanelli, L., and Muzzioli, M. (1977). Differential effect of pregnancy or gestagens on humoral and cell-mediated immunity. *Clinical and Experimental Immunology*, **28**, 306-314.

Gailani, S., Chu, T. M., Nussbaum, A., Ostrander, M., and Christoff, N. (1976). Human chorionic gonadotrophins (HCG) in non-trophoblastic neoplasms. *Cancer*, **38**, 1684-1686.

Goldenberg, D. M., Pant, K. D., and Dahlman, H. L. (1976). Antigens associated with normal and malignant gastro-intestinal tissues. *Cancer Research*, **36**, 3455-3463.

Goldstein, D. P., Kosasa, T. S., and Skarim, A. T. (1974). The clinical application of a specific radioimmunoassay for human chorionic gonadotrophin in trophoblastic

and nontrophoblastic tumors. *Surgery, Gynecology and Obstetrics*, **138**, 747-751.

Graham, R. C., Jr., and Karnovsky, M. I. (1966). The early stages of absorption of injected horseradish peroxidase in the proximal tubules of mouse kidney: ultrastructural cytochemistry by a new technique. *Journal of Histochemistry and Cytochemistry*, **14**, 291-302.

Horne, C. H. W., Reid, I. N., and Milne, G. D. (1976). Prognostic significance of inappropriate production of pregnancy proteins by breast cancers. *Lancet*, **2**, 279-282.

Kahn, C. R., Rosen, S. W. Weintraub, B. D., Fajans, S. S., and Gorden, P. (1977). Ectopic production of chorionic gonadotropin and its subunits by islet-cell tumors. *New England Journal of Medicine*, **297**, 565-569.

McManus, L. M., Naughton, M. A., and Martinez-Hernandez, A. (1976). Human chorionic gonadotropin in human neoplastic cells. *Cancer Research*, **36**, 3476-3481.

Pick, L. (1926). Über die chorionepithelähnlich metastasierende Form des Magencarcinoms. *Klinische Wochenschrift*, **5**, 1728-1729. Cited by Regan, J. F., and Cremin, J. H. (1960). Chorionepithelioma of the stomach. *American Journal of Surgery*, **100**, 224-233.

Rosen, S. W., Becker, C. E., Schlaff, S., Easton, J., and Gluck, M. C. (1968). Ectopic gonadotropin production before clinical recognition of bronchogenic carcinoma. *New England Journal of Medicine*, **279**, 640-641.

Rosen, S. W., Weintraub, B. D., Vaitukaitis, J. L., Sussman, H. H., Hershman, J. M., and Muggia, F. M. (1975). Placental proteins and their subunits as tumor markers. *Annals of Internal Medicine*, **82**, 71-83.

Sternberger, L. A., Hardy, P. H., Jr., Cuculis, J. J., and Meyer, H. G. (1970). The unlabeled antibody enzyme method of immunohistochemistry. Preparation and properties of soluble antigen-antibody complex (horseradish peroxidase-antihorseradish peroxidase) and its use in identification of spirochetes. *Journal of Histochemistry and Cytochemistry*, **18**, 315-333.

Tormey, D. C., Waalkes, T. P., and Simon, R. M. (1977). Biological markers in breast carcinoma: 2. Clinical correlations with human chorionic gonadotrophin. *Cancer*, **39**, 2391-2396.

Walker, R. A. (1978). Significance of α-subunit HCG demonstrated in breast carcinomas by the immunoperoxidase technique. *Journal of Clinical Pathology*, **31**, 245-249.

Weintraub, B. D., and Rosen, S. W. (1971). Ectopic production of human chorionic somatomammotropin by nontrophoblastic cancers. *Journal of Clinical Endocrinology and Metabolism*, **32**, 94-101.

Wood, C. B., Gillis, C. R., and Blumgart, L. H. (1977). Tumour staging of colo-rectal cancer (Abstract). *Digestion*, **16**, 286.

Requests for reprints to: Professor H. Fox, Department of Pathology, University of Manchester, Stopford Building, Oxford Road, Manchester M13 9PT.