Eur J Cancer Clin Oncol. Vol. 18, No. 3, pp. 227–235, 1982.
Printed in Great Britain.

0277-5379/82/030227-08$03.00/0

Tumor Markers in Carcinoma and Premalignant States of the Stomach in Humans*

J. M. SKINNER† and R. WHITEHEAD
Department of Pathology, Flinders University of South Australia, Bedford Park, South Australia, 5042

Abstract—*Tissue sections taken from areas of carcinoma, areas of intestinal metaplasia in stomachs bearing carcinoma and areas of intestinal metaplasia in stomachs showing atrophic gastritis only were examined for eight markers: a tumour-derived colon-specific antigen (tCSA), carcinoembryonic antigen (CEA), α-fetoprotein (AFP), pregnancy-specific β-glycoprotein 1 (SP1), human placental lactogen (HPL), human β chorionic gonadotrophin (β-HCG), transferrin (TF) and ferritin (FE). In terms of the number of markers demonstrated in each of the three categories, there is a close similarity between the cells of adenocarcinoma and cells of intestinal metaplasia in cases of cancer, but not to similar metaplastic cells in atrophic gastritis cases. In addition, it appears that the presence of tCSA and SP1 is closely linked to carcinoma, though only approximately half of such cases contain these markers. It would also appear that there are two types of morphologically identical intestinal metaplasia, one related to cancer, the other not. No difference was found between so-called intestinal type and diffuse type carcinomas.*

INTRODUCTION

CARCINOMA of the stomach has shown a steady decline in incidence in developed countries since the turn of the century, but still remains an important cause of morbidity. Diagnosis still tends to be made when the disease is well advanced and the prognosis remains poor.

It has long been held that there is a relationship between the presence of intestinal metaplasia in the stomach and the risk of development of cancer [1]. Intestinal metaplasia is a constant feature of the atrophic gastritis seen in pernicious anaemia and such patients have a four-fold increase in incidence of carcinoma of stomach as compared to the rest of the population [2]. Intestinal metaplasia is common in stomachs also affected by carcinoma [3], particularly when it is of the 'intestinal' type [4]. In the years following a variety of operative procedures to the stomach, widespread metaplasia regularly occurs and there is a higher than expected rate of development of cancer of the stomach in these patients [5].

More recently, investigators have described certain dysplastic features in the gastric mucosa and argued their premalignancy [6]. Others have noted that in the intestinal type of cancer as originally described [4], characteristic large, as opposed to small, intestinal mucosubstances are present in both the tumour and in the areas of metaplasia [7].

There are, however, no reliable markers of premalignancy in the stomach. The findings relating intestinal metaplasia and particularly dysplasia to cancer have been made by extrapolation following the histological or histochemical examination of stomachs in which carcinoma has already developed. Longitudinal studies showing the development of intestinal metaplasia or dysplasia into cancer, as with uterine cervical cancer, have not been made. Furthermore, definitions of dysplasia by their subjective nature are made difficult to standardise.

The advent of immunohistochemical methods [8] has allowed the study of a variety

Accepted 23 October, 1981.
*The work was supported by grants from the National Health and Medical Research Council (Aust.) and the Anti Cancer Foundation of the South Australian Universities.
†Reprints: Dr. J. M. Skinner, Department of Pathology, Flinders University of South Australia, Bedford Park, South Australia, 5042.

of so-called tumour markers in different situations, including the gastrointestinal tract.

Whilst early hopes that markers such as carcinoembryonic antigen and others would prove specific for carcinoma, particularly in adults, have not materialised [9], they do appear to be markers of abnormal or incomplete differentiation. They reflect a phenotype which is different from normal tissue. There is some support for the hypothesis that incomplete differentiation is an important factor in the growth of tumours, possibly by loss of feedback inhibition of cell division by products of normal mature cells [10]. It was decided to study a range of tumour markers as an indicator of abnormal differentiation, in both gastric carcinoma and in gastric mucosa showing atrophic gastritis and intestinal metaplasia. The markers chosen fall into three broad categories.

The first is a tumour-associated antigen (tCSA) produced from colon carcinoma by a modification of the method of Goldenberg *et al.* [11] for colon specific antigen.

The second includes a group of oncofetal antigens, viz. carcinoembryonic antigen (CEA) and α-fetoprotein (AFP), which have been demonstrated in a variety of human tumours [12–14], and placental antigens, which are also detectable in early fetal tissue and in some tumours of adults, e.g. pregnancy-specific β-glycoprotein 1 (SP1), human placental lactogen (HPL) and β human chorionic gonadotrophin (β HCG) [15–17].

The third group is made up of two markers of small intestinal differentiation, i.e., ferritin (FE) and transferrin (TF).

MATERIAL AND METHODS

Cancer cases

Eighteen cases of adenocarcinoma diagnosed initially in multiple biopsy tissues were studied. Nine of the cases subsequently came to gastrectomy. The tissues examined included the tumour (2 or more blocks) and areas of intestinal metaplasia, in one block of the antral, cardiac and body regions.

In the nine other cases diagnosed on gastric biopsy, material was also available from the cancer and the same mucosal regions. In only thirteen of the cancer cases was intestinal metaplasia detected.

Non-cancer cases with intestinal metaplasia

Seventeen cases were studied in which atrophic gastritis and intestinal metaplasia were demonstrated in biopsies collected from the same areas of the stomach as above. There was no evidence clinically, radiologically or histologically of gastric cancer. The majority of biopsies were from cases of gastric ulcer, but in one case the patient had pernicious anaemia.

Handling of tissues

The gastrectomy specimens were received fresh from the operating theatre wrapped in polythene on a bed of ice. They were opened along the greater curve and the mucosa gently washed free of debris using isotonic, isosmolar phosphate buffer, pH 7.14, with added sucrose. The specimen was then fixed in phosphate-buffered 4 per cent formaldehyde solution at pH 7 for 12–24 hr and then sampled. Biopsies were placed immediately into phosphate-buffered formaldehyde solution for 4–12 hr before further processing. It was recognised that this fixation process affects the sensitivity of the immunohistochemical method and therefore only significant amounts are detected [11]. The material was then processed through graded alcohols and xylol to Paraplast$^{T.M.}$ in the routine manner and sections cut 3 μm thick. After dewaxing, one section was stained by the haemotoxylin eosin method and one by P.A.S./Alcian blue, pH 1.0, for routine diagnosis. Subsequent serial sections of the 'ribbon' were used in immunohistochemical techniques after dewaxing and 30 min of washing in 0.2 M phosphate-buffered saline, pH 7.4.

The antibodies

The tCSA was prepared in the laboratory from carefully dissected colon carcinoma by the method of Goldenberg [18]. The high molecular weight fraction obtained from separation on G100 Sephadex was used to immunise rabbits in the standard manner [19]. After a single booster injection, the rabbits were bled and the serum prepared and fractionated on DEAE cellulose (DE 52 Whatman) to obtain the IgG fraction. This was absorbed with phenol extract of spleen [20] to remove non-specific cross-reacting antibodies (NCA), and with cells obtained from the buffy coat of pooled human peripheral blood and a fresh suspension of liver cells to remove nonspecific activity. The material was then further absorbed with material prepared by the Goldenberg technique from normal colon mucosa which had been dissected away from underlying tissues.

The antibody obtained (anti-tCSA) gives strongly positive results with colon cancers and tubulovillous adenomas, but usually negative or occasionally very weak results with normal colon epithelium [21].

Anti-CEA and -AFP were obtained as IgG

fractions, from commercial sources (Dako Reagents), then absorbed with phenol extract of spleen, buffy coat and liver cells as described above. After this treatment, the anti-AFP gives negative results on normal gut epithelium but remains positive on foetal liver. The undiluted anti-CEA gave positive results, though faint, on normal colon mucosa in 2 out of 4 controls.

Anti-SP1, (Hoechst), -HPL (Hoechst) and -β HCG (Hoechst) were obtained from commercial sources and further purified before use by ion exchange chromatography (DE 52 Whatman). The IgG fraction of the eluate was concentrated by dialysis against 10% polyethylene glycol, mol. wt. 6000 (PGE 6000), overnight at 4°C. The antibodies give positive staining of placental syncytiotrophoblast and are negative on normal gut epithelium.

Anti-ferritin (FE) and anti-transferrin (TF) were purchased from Dako Reagents, IgG fraction-purified as above and absorbed with washed human peripheral blood buffy coat cells prior to use on the tissue sections. Prepared in this way and at high dilution (1/400), these antibodies give positive staining of small intestinal absorptive cells, but not epithelium in the stomach or colon. There was no cross-reactivity with granulocytes.

Antibody specificity

The specificity of the laboratory-prepared anti-tCSA is shown above.

The anti-CEA (Dako) was absorbed with CEA prepared from colon by perchloric acid extraction [18] and gave negative results on sections of foetus and colon carcinoma.

Anti-AFP (Dako) (1/20) was adsorbed with an equal volume of the corresponding human α fetoprotein control (Dako Reagents) used neat, i.e., in excess. Results on control foetal liver were negative or very weak.

Anti-SP1 (1/10) (Hoechst) was adsorbed with purified SP1 (from Dr. H. Bohn) and gave negative results on syncytiotrophoblast (Fig. 1). As the pure SP1 was available in limited amount, the SP1 glycoprotein standard (Hoechst) was used as a check on the later vials of antibody used (all from the same production batch).

Anti-β HCG specificity was checked by absorption with pure β HCG, but in the case of anti-HPL the purified antigen was not available to us and the manufacturers' (Hoechst) controls were accepted. However, pregnancy sera and saline extracts of placenta abolished staining of control material.

As the three antibodies above are all of placental origin, cross-absorptions of anti-SP1 with β HCG, anti-β HCG and SP1, and anti-HPL with both β HCG and SP1 were performed. The cross-absorbed antisera gave positive results on the placental control material. Anti-ferritin specificity was checked by prior absorption with pure ferritin and anti-transferrin with pure transferrin, both in excess. The normal staining of ileal absorptive cells was abolished in each case. Cross-absorptions caused no diminution in positive staining.

Immunohistochemical technique

The PAP method as outlined by Burns [22] was used throughout. The concentration of antibody to be used in each case was determined by the results achieved using positive control material from foetus, placenta or adult small bowel, as appropriate.

The concentrations finally employed were the lowest which gave positive results and absence of background staining in control material. The final dilutions of processed reagents were: for CEA, 1/100; tCSA, 1/20; AFP, 1/20; SP1, 1/10; HPL, 1.20; β HCG, 1/20; F, 1/400; and TF, 1/400.

Controls

Negative controls were sections of normal gastric body mucosa and normal colon from cases of diverticular disease which appeared macroscopically and microscopically normal. Positive controls for CEA and AFP were sections of human foetus between 12 and 30 mm long, for tCSA, sections of carcinoma of colon, for SP1, HPL and β HCG, sections of fresh human placenta, and for FE and TF, sections of ileum from operation specimens.

The effectiveness of the H_2O_2/alcohol blocking reagent for endogenous peroxidase was checked using the diaminobenzidine reagent alone. The results were consistently negative.

The presence of heterophile antibodies or nonspecific binding proteins in the swine anti-rabbit serum was checked by using the PAP reagent followed by diaminobenzidine. Results were always negative.

Interpretation of histochemical staining

In each case, a simple positive or negative result was recorded. No attempt was made to quantify the number of positive cells, which varied between 5 and 90% of the tumour.

No attempt was made to assess the staining intensity as the avidity and antibody concentrations of the reagents used was not known. However, in general, the stronger staining was given by anti-CEA and anti-tCSA, and the weakest by anti-HPL in the dilutions used.

Histological interpretation

The diagnosis of atrophic gastritis and intestinal metaplasia was made using criteria outlined by Whitehead [23], and the classification of carcinoma used was that of Laurén [4]. It is recognised that this is difficult in biopsy tissues, but endoscopic information concerning the macroscopic form of the tumours was taken into account.

RESULTS

The staining of epithelial cells in both cancer and intestinal metaplasia was usually more intense along the cell luminal membrane, but was also present in a granular distribution in the cytoplasm. In general, the strongest staining was seen with anti-CEA and anti-tCSA, and the weakest with anti-HPL (Figs 1(a and b) and 2 illustrate the results). There was strong positive CEA and tCSA staining in association with mucin secretion in the carcinomas. Where multiple markers were positive it was usually, but not exclusively, in the same cell groups, though in the absence of a double staining technique it is more difficult to be certain that any individual cell has more than one. The staining in the test sections was usually as strong as in the control sections, except in the case of SP1 and HPL, where the control placentas stained more strongly than the test sections, falling to approximately the same intensity on a further four-fold dilution of antibody. In the case of anti-TF, the extracellular and vascular compartments were always positive as transferrin is present in serum, and a positive result was recorded only when epithelial cells were stained. Some cases stained with anti-FE showed background staining of this nature, but it was always weak. Again, only positive epithelial cells were recorded.

In Table 1, CEA is the most commonly found marker, and by and large, antigens were most frequently expressed in cancer tissue as opposed to intestinal metaplasia. There is no significant difference between the groups for each marker considered, except for the case of SP1 and tCSA. Using a two-way contingency test with each of the three groups recorded as SP1-positive or -negative, the value for $\chi^2 = 13.41$ (FE = 2), $P < 0.01 > 0.001$, i.e. there is a difference at the 5% level. The results for tCSA are $\chi^2 = 15.12$ (FE = 2), $P < 0.001$. There were five cases in which both SP1 and tCSA were positive, and in four of these the associated intestinal metaplasia was positive for both markers. In one case the intestinal metaplasia was positive for both, but the associated cancer had only tCSA.

In the analysis of Table 2, the cells were grouped into two because of the small numbers in each cell, those with low numbers, i.e. 0 to 3 markers, and those with high numbers, i.e. 4 to 8. Using the 3×2 two-way contingency analysis, $\chi^2 = 10.55$, giving a value for P of 0.005. There is a significant difference between the cancer and the intestinal metaplasia with cancer group, and the intestinal metaplasia with atrophic gastritis group.

As far as the number of markers demonstrated is concerned, the intestinal metaplasia seen in association with carcinoma is similar to carcinoma and different from the intestinal metaplasia in association with atrophic gastritis only.

The antibodies to placental products SP1, HPL and β HCG pose a particular problem as, even with the controls used, it is possible that the PAP immunohistochemical technique is identifying a placental product different from all three which has not been adsorbed out in preparation. That is, there could be the same or similar antigenic contaminants of the apparently pure antigens used in immunisation. Table 3 shows, however, that although a number of cases did possess all three markers, other combinations also existed.

Examination of the routinely stained sections shows no obvious difference in the type of intestinal metaplasia present. In both groups there were significant numbers of absorptive

Table 2. Number of markers positive in each disease category

No. of markers	Low (0–3)	High (4–8)
Cancer (18)	5	13
IM/Ca (13)	4	9
IM/No Ca (17)	13	4

Table 1. Number of cases showing each tumour marker

	SP1	HPL	CEA	AFP	tCSA	FE	TF	HCG	Total
Cancer	9	9	18	8	10	11	11	10	18
IM/Ca	4	4	11	6	7	8	8	4	13
IM/No Ca	0	4	14	2	0	6	8	5	17

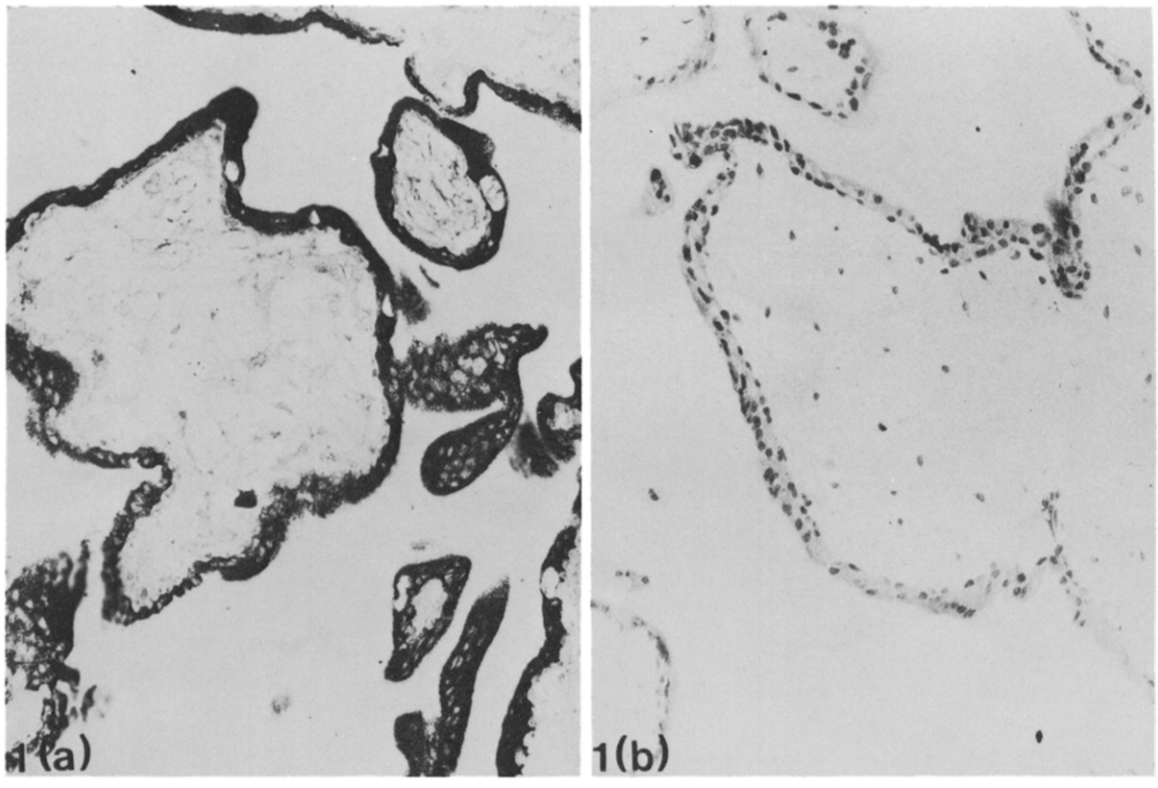

Fig. 1. (*a*) *Ten-week human placenta stained for β-HCG using the PAP immunoperoxidase technique* (×120). (*b*) *The same placenta stained with antibody to β-HCG previously adsorbed with β-HCG, showing abolition of the positive brown staining. Because the methyl green counterstain used appears indistinct in black and white photographs, haematoxylin has been used in this negative preparation to demonstrate the nuclei* (× 120).

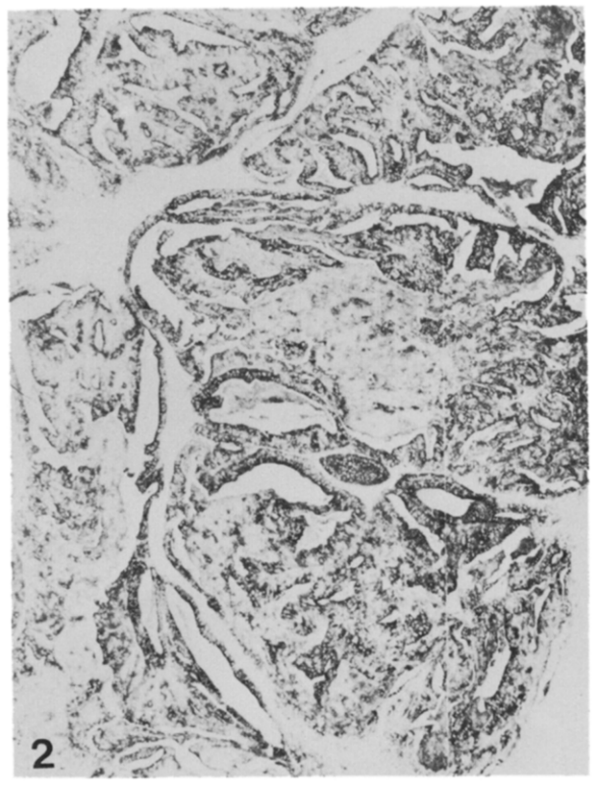

Fig. 2. *A human gastric carcinoma stained with anti-CEA in the immunoperoxidase technique* (× 100).

Table 3. Number of cases positive for combinations of placental antigens

	SP1	HPL	HCG	HP1 SP1	β HCG SP1	β HCG HPL	SP1 β HCG HPL	Negative	Total
Cancer	4	2	4	2	1	3	2	0	18
IM/Ca	1	1	2	1	1	1	1	5	13
IM/No Ca	0	1	2	0	0	3	0	11	17

cells with brush border, as well as goblet cells and Paneth cells, and mitotic activity towards the base of the crypts. In both groups there were areas of mucosa with a majority of goblet cells and few absorptive cells which, in the absence of Paneth cells, have an appearance more closely mimicking the large rather than the small intestine. Mitototic activity in these areas tended to occur higher in the tubular structures. These areas did not show any particular antigen profile and ferritin and transferrin, small intestinal markers, were found as commonly in this type as in the so-called small intestinal type of metaplasia.

Cancer type

Only 4 cases were of the diffuse type, 12 were intestinal and 2 were unclassifiable. There was no difference in antigen profile between the groups.

DISCUSSION

The cells of gastric carcinoma have a notably different tumour marker profile than normal cells and the metaplastic cells seen in cases of atrophic gastritis alone. Despite the range in the number of positive cells present in different cases, there was a similarity in the result, regardless of whether the tissue used was obtained from a surgical specimen or a biopsy procedure. This would indicate that because of the distribution of positive cells, tissue sampling did not affect our results. Surprisingly, the intestinal metaplasia in the cancer subjects shows a profile similar to that of the cancer cells and quite different from that in atrophic gastritis. Claims have been made previously that premalignant intestinal metaplasia is 'colonic' rather than 'small intestinal' in type. This has been based either upon the paucity of absorptive type cells and absence of Paneth cells, such as is typical of colonic mucosa [24], or on the presence of colonic, rather than small intestinal mucosubstances [24, 25]. Using the former criteria, areas with 'colonic type' metaplasia were found in this study, but they were not exclusively associated with cancer. Furthermore, the markers of small intestinal cells, i.e. transferrin and ferritin, were commonly found in areas of intestinal metaplasia, whether associated with cancer or not.

It has previously been reported that CEA is found in a variety of cancers and pre-malignant conditions [18], but does not distinguish between them. As a diagnostic aid it is of limited value, therefore, to the histopathologist, even though its presence in association with changes in mucosubstances has been claimed to occur in cells which have severe dysplastic appearances [26].

Although the markers SP1, HPL and β-HCG have been detected at higher frequencies in breast cancer [26], they are surprisingly common. The possibility remains that some weak, common, cross-reacting substance is present, though the finding of a single marker only from this group in 10 of the 18 positive carcinomas strengthens the significance of the overall findings.

It is of interest that tCSA, a good marker of colonic cancer [21], is detected in the stomach in both malignant and non-malignant, though not normal, cells. However, despite the view that intestinal type of gastric cancer derives from cells of colonic type intestinal metaplasia, tCSA was found in only 6/12 cases of intestinal type cancer and in four of the non-intestinal type in this study. It is perhaps worth noting that ultrastructural microscopic evidence for intestinal metaplasia is present in almost all gastric carcinomas [27] and that cells of small intestinal differentiation are seen equally in carcinomas classified as intestinal or diffuse [28]. The strict distinction between intestinal and non-intestinal types of gastric carcinoma is thus somewhat artificial.

tCSA was not seen in cases of intestinal metaplasia unassociated with cancer and in this respect is similar to SP1. This study does indicate that there are two forms of intestinal metaplasia and that one may be premalignant.

Our results do not lend support to the notion that the premalignant form is colonic in type.

On the other hand, they do show that it may be possible to predict whether or not a form of intestinal metaplasia is premalignant.

None of the markers used is specific for carcinoma. SP1 and tCSA show a significant correlation with cancer in statistical contingency tests, but the association is not strong, only 50% of cancers showing a positive result.

REFERENCES

1. MORSON BC. Carcinoma arising from areas of intestinal metaplasia in the gastric mucosa. *Br J Cancer* 1955, **9**, 377–385.
2. SHEARMAN DJC, FINLAYSON NDC, WILSON R, SAMSON RR. Carcinoma of the stomach and early pernicious anaemia. *Lancet* 1966, **ii**, 403–405.
3. CORREA P, CUELLO C, DUQUE E. Carcinoma and intestinal metaplasia of the stomach in Colombian migrants. *J Natl Cancer Inst* 1970, **44**, 297–306.
4. LAURÉN P. The two histological main types of gastric carcinoma: diffuse and intestinal type carcinomata. *Acta Pathol Scand* 1965, **A64**, 31–49.
5. STALSBERG H, TAKSDAL S. Stomach cancer following gastric surgery for benign conditions. *Lancet* 1971, **ii**, 1175–1177.
6. MORSON BC, SOBIN LH, GRUNDMANN E, JOHANSSEN A, NAGAYO T, SERCK-HANSEN A. Precancerous conditions and epithelial dysplasia in the stomach. *J Clin Path* 1980, **33**, 711–721.
7. TEGLBJAERG PS, NIELSEN HO. "Small intestinal type" and "colonic type" intestinal metaplasia of the human stomach. *Acta Pathol Microbiol Scand* 1978, **A86**, 351–355.
8. NAKANE P. Simultaneous localisation of multiple tissue antigens using the peroxidase-labelled antibody method: A study on pituitary glands in the rat. *J Histochem Cytochem* 1968, **16**, 557–560.
9. GOLD P, WILSON T, ROMERO R, FREEDMAN SO. Immunology and colonic cancer: Further evaluation of the radioimmunoassay for carcinoembryonic antigen of the human digestive system as an adjunct in cancer diagnosis. *Dis Colon Rectum* 1973, **16**, 358–365.
10. NICHOLSON GL, POSTE G. The cancer cell: Dynamic aspects and modification in cell surface organisation. *N Engl J Med* 1976, **295**, 253–259.
11. GOLDENBERG DM, PANT KD, DAHLMAN HL. Antigens associated with normal and malignant gastrointestinal tissues. *Cancer Res* 1976, **36**, 3455–3463.
12. MCINTIRE KR, WALDEMENN TA, MOERTEL CG, GO VLW. Serum α fetoprotein in patients with neoplasms of the gastrointestinal tract. *Cancer Res* 1975, **35**, 991–996.
13. ISAACSON P, LE VANN H. P. The demonstration of carcinoembryonic antigen in colorectal carcinoma and colonic polyps using an immunoperoxidase technique. *Cancer* 1976, **38**, 1348–1356.
14. SHARKEY RM, HAGIHARA PF, GOLDENBERG DM. Localisation by immunoperoxidase and estimation by radioimmunoassay of carcinoembryonic antigen in colonic polyps. *Br J Cancer* 1977, **25**, 179–189.
15. MCMANUS LM, NAUGHTON MA, MARTINEZ-HERNANDES A. Human chorionic gonadotrophin in human neoplastic cells. *Cancer Res* 1978, **36**, 3476–3481.
16. BUCKLEY CH, FOX H. Immunohistochemical study of the significance of H.C.G. secretion by large bowel adenocarcinomata. *J Clin Pathol* 1979, **32**, 368–372.
17. GRUNDZINSKAS JG, COOMBES RC, RATCLIFFE JG *et al.* Circulating levels of pregnancy specific beta 1 glycoprotein in patients with testicular, bronchogenic and breast carcinomas. *Cancer* 1980, **45**, 102–103.
18. GOLDENBERG DM. Oncofetal and other tumour-associated antigens of the human digestive system. *Curr Top Pathol* 1976, **63**, 289–342.
19. HERBERT WJ. Mineral oil adjuvants and the immunisation of laboratory animals. Appendix 3. In: WEIR DM, ed. *Handbook of Experimental Immunology.* Oxford, Blackwell Scientific Publications, 1978, Vol. III.
20. PANT KD, DAHLMAN HL, GOLDENBERG DM. Further characterisation of CSA, an antigen associated with gastrointestinal and ovarian tumours. *Cancer* 1978, **42**, 1626–1634.
21. SKINNER JM, WHITEHEAD R. Tumour-associated antigens in polyps and carcinoma of the human large bowel. *Cancer* 1981, **47**, 1241–1245.
22. BURNS J. Background staining and sensitivity of the unlabelled antibody enzyme (PAP) method. Comparison with the peroxidase labelled antibody sandwich method using formalin fixed, paraffin embedded material. *Histochemistry* 1975, **43**, 291–294.

23. WHITEHEAD R. *Mucosal Biopsy of the Gastrointestinal Tract.* Philadelphia, W. B. Saunders, 1979.
24. JASS JR, FILIPE MI. Sulphomucins and precancerous lesions of the human stomach. *Histopathology* 1980, **4**, 271–279.
25. MONTERO C, SEGURA DI. Retrospective histochemical study of microsubstances in adenocarcinomas of the gastrointestinal tract. *Histopathology* 1980, **4**, 287–291.
26. WURSTER K, RAPP W. Histological and immunohistochemical studies on gastric mucosa. I. The presence of CEA in dysplastic surface epithelium. *Pathol Res Pract* 1979, **164**, 270–281.
27. SASANO N, KATSUHIRO N, NOTONOBU A, KANEGOSHI A. Ultrastructural cell patterns in human gastric carcinoma compared with non-neoplastic gastric mucosa. Histogenetic analysis of carcinoma by mucin histochemistry. *J Natl Cancer Inst* 1969, **43**, 783–802.
28. NEVALAINEN TJ, JÄRVI OH. Ultrastructure of intestinal and diffuse type gastric carcinoma. *J Pathol* 1977, **122**, 129–136.