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Immunocytochemical studies of blood group A, H, I, and i antigens in gastric mucosae of infants with normal gastric histology and of patients with gastric carcinoma and chronic benign peptic ulceration

ANUPSHEELA KAPADIA,* TEN FEIZI,* DEREK JEWELL,† JEAN KEELING,† AND GERARD SLAVIN*

*From *The Clinical Research Centre, Watford Road, Harrow, Middlesex, and †John Radcliffe Hospital, Headington, Oxford, UK*

SUMMARY Indirect immunofluorescence studies of blood group A, H, I, and i antigens were performed on the gastric mucosae and tumour tissues of patients with gastric carcinoma, on the mucosae of patients with chronic benign gastric ulceration, and on the mucosae of infants who had died of causes other than gastrointestinal disease. The following findings were of particular interest:

(1) Normal 'secretor' type mucosae were distinguishable from 'non-secretor' type mucosae by the uniform staining of the A or H antigens at the surface and in the pits. Normal 'non-secretor' type mucosae showed little staining of the H or A antigens but, instead, there was staining with anti-I(Ma) antibody. Staining with anti-I(Step) and anti-i(Den) did not show a clear correlation with the 'secretor'/'non-secretor' status of the normal mucosae.

(2) Apparently normal areas of gastric mucosae of patients with gastric carcinoma or the normal part of gastric mucosae of patients with benign gastric ulcer frequently showed focal areas of loss or gain of the blood group antigens as is often seen in gastric carcinoma tissues.

(3) In the mucosae of patients with intestinal metaplasia there was marked loss of A/H antigens in 'secretors' and I(Ma) antigen in 'non-secretors'.

(4) Staining characteristics of tissues from gastric carcinoma were: (*a*) Focal loss of the expected A/H or I antigens was observed with much variation in staining from area to area, but only a minority showed complete loss of the expected staining. (*b*) A majority of the carcinomas from 'secretors' showed foci of substantial staining with anti-I(Ma) in contrast to normal 'secretor' mucosae. This is probably due to incomplete biosynthesis of A/H determinants. (*c*) Incompatible A-like staining by a rabbit anti-A serum was observed in one out of nine adenocarcinomas from blood group B or O persons. (*d*) A few cases showed substantial i antigen staining.

The aberrant expression of blood group A, H, I, and i antigens in neoplastic as well as in some areas of morphologically normal mucosa of patients with benign and malignant diseases of the stomach is discussed in the context of current biochemical knowledge.

Changes in the expression of blood group ABH antigens are well known to occur in carcinoma tissues. The most common is a diminution or loss of the genetically predicted antigens.[1 2] Inappropriate antigen expression has also been described, for example, the presence of A-like antigen in gastric cancer tissues of blood types O or B persons.[3] In gastric carcinoma, changes also occur in the amount of one of the blood group I antigenic determinants, termed I(Ma), which is detected by the monoclonal anti-I antibody of a patient (Ma) with chronic cold agglutinin disease.[4 5] This antigenic determinant consists of an oligosaccharide chain which serves as precursor or carrier of the blood group ABH antigens.[6] Although this oligosaccharide structure occurs commonly in gastric mucins, its antigenicity is usually strongly expressed in 'non-secretors' of blood group ABH antigens, but not in 'secretors' due to masking by the blood group ABH monosaccharides.[4] Thus quantitative precipitin assays of

the I(Ma) antigenic determinant can distinguish the gastric mucins and mucosal extracts of 'non-secretors' from those of 'secretors'. In 'secretors' with gastric carcinoma, however, precipitating I(Ma) activity is found in the majority of extracts.[4 5] This is presumed to be due to incomplete biosynthesis of the ABH active oligosaccharide chains.

Abnormalities in the blood group carbohydrate antigens are readily detectable biochemical parameters of abnormal gene expression. In the present work the blood group H, A, and I(Ma) antigenic determinants have been studied by immunofluorescence in normal and pathological gastric tissues.

The monoclonal anti-I and anti-i antibodies from other patients with cold agglutinin disease (for example, patients Step and Den) have been shown to recognise antigenic determinants on oligosaccharide precursors of blood group ABH antigens which are distinct from the precursor chain recognised by anti-I(Ma).[7–9] There has been no information thus far on the localisation of these antigenic determinants in the mucus-secreting cells of the stomach. The present studies were extended to include the cytochemistry of these antigenic determinants in the gastric tissues.

Material and methods

GASTRIC TISSUES

Gastric carcinoma

Tissues from 24 patients with gastric carcinoma were studied. They were obtained at gastrectomy (18 patients) or during diagnostic endoscopy (6 patients). Immunofluorescence staining was performed on fresh frozen tissues in all but one of the patients. In addition, formalin-fixed, paraffin-embedded sections were studied in 12 of the patients. Samples of non-neoplastic gastric mucosa distant from the tumours were also studied in the majority of patients. These included samples of body and antrum in five patients, body only in 10 patients, and antrum only in eight patients. The source of the tissues and the mode of examination are summarised in Tables 1 to 3.

Gastric ulcer

Tissues were obtained from six patients undergoing surgery for intractable benign peptic ulcers. These included the ulcer site in all six patients and uninvolved mucosa from body and antrum in four of the patients. Frozen sections and formalin-fixed, paraffin-embedded sections were studied in two patients, paraffin only in two, and frozen sections only in two patients (Table 4).

Infants with normal gastric histology

In the absence of volunteers, it is difficult to obtain normal gastric mucosae from individuals free from any gastric pathology as controls. We chose, therefore, to examine the gastric mucosae from infants and young children obtained at necropsy on the premise that chronic gastric disease was unlikely in this group. Formalin-fixed, paraffin-embedded samples of normal gastric mucosa from seven infants (age range 9 days-1 year) were from either the body or antrum of the stomach and were obtained one to three days after death from infants who had died of cardiac and respiratory arrest after accidental death, pulmonary hypoplasia, or bronchopulmonary dysplasia.

IMMUNOFLUORESCENCE

Cryostat sections, 5 μ thick, were air-dried and stored at $-70°C$ in the presence of silica gel desiccant until required. Formalin-fixed, paraffin-embedded sections were dewaxed and cleared through xylene and alcohol.

For immunofluorescence staining of the I and i antigens, two human anti-I sera from patients Ma and Step (anti-I(Ma) and anti-I(Step)) and an anti-i serum from patient Den (anti-i(Den)) and a control serum were used followed by fluorescein-conjugated rabbit anti-human IgM. For immunofluorescence of blood group A and H antigens the rabbit antisera batches 773 and 130, respectively,[10] were used (kindly provided by Dr Winifred Watkins) followed by fluorescein-conjugated goat anti-rabbit IgG. Details of these reagents, the staining procedure, and specificity controls have been described previously.[11] A rabbit antiserum[11a] against Forssman antigen was kindly provided by Dr Senitiroh Hakomori and was used at a 1:20 dilution. When control human serum or normal rabbit serum were used instead of the anti-blood group sera, no fluorescence or weak fluorescence was observed. In certain mucosae with intestinal metaplasia, weak or moderate staining of the goblet-cell vacuoles was observed with normal rabbit serum. The intensity of specific fluorescence with the anti-blood group sera was graded from weak to strong above that observed with control sera. The inappropriate immunofluorescence staining with anti-A and anti-H sera (see Results section) was abolished by absorption[11] with blood group A erythrocytes and H-substance, respectively.

Haematoxylin and eosin staining was performed on every tenth section for histological assessment.

DETERMINATION OF 'SECRETOR' STATUS

The 'secretor' status of the patient was determined by examination of saliva for the presence of H, A, or

Table 1 *Focal loss and gain of blood group antigen staining in the mucus-secreting cells of the surface and pits of gastric mucosae at a distance from the tumours ('secretors')*

Patient (blood type)	*Tissues examined*	*'Abnormal' patterns of immunofluorescence*		
		*Loss of A or H antigen**	*Gain of I(Ma) antigen*	*A antigen in O or B persons*
'Secretors' with normal histology				
O1 (O)	Body (f,p)			
Ri (O)	Body (f)	F		
Nu (O)	Body (f)		F	
	Antrum (f)			
Pa (A)	Body (f,p)		F	
We (A)	Body (f)			
Ha (A)	Body (b)			
Mo (B)	Antrum (b)			
'Secretors' with gastritis				
Wi (O)	Body (f,p)		F	U
Bu (O)	Body (f)		F	
	Antrum (f)	F	F	F
Go (AB)	Body (p)		F	
	Antrum (f)			
'Secretors' with intestinal metaplasia (non-metaplastic areas)				
Wo (O)	Body (f)		F	U
Ba (O)	Body (f,p)	F		
Ti (O)	Antrum (b)	F	F	
Ni (A)	Body (f)			
	Antrum (f,p)	F	F	
Tr (A)	Body (f,p)	F		
Pos (A)	Antrum (p)	C		
Le (A)	Antrum (b)	F	F	
Gr (A)	Antrum (b)	F		
De (B)	Antrum (b)		F	F
Areas with overt intestinal metaplasia in 'secretors'				
Ba (O)	Antrum	F		
Ti (O)	Antrum	F		
Ni (A)	Antrum	C		
Tr (A)	Antrum	C		
Pos (A)	Antrum	C		
Le (A)	Antrum	F		
Gr (A)	Antrum	F		
De (B)	Antrum			F

*Loss of A antigen in blood group A persons and H antigen in blood group O persons.
f = fresh frozen sections; p = formalin-fixed, paraffin-embedded sections; b = fresh frozen sections of biopsies obtained at endoscopy.
F = focal loss or gain of antigen staining; C = complete loss with anti-A staining; U = uniform staining.

Table 2 *Focal loss and gain of blood group antigen staining in the mucus-secreting cells of the surface and pits of gastric mucosae at a distance from the tumours ('non-secretors')*

Patient (blood type)	*Tissues examined*	*'Abnormal' patterns of immunofluorescence*			
		Loss of I(Ma) antigen	*Strong staining of*		*A antigen in O or B persons*
			A antigen	*H antigen*	
'Non-secretors' with normal histology					
Do (O)	Body (b)	F			F
'Non-secretors' with intestinal metaplasia (non-metaplastic areas)					
Hu (A)	Body (p)				
	Antrum (f)		F	F	
He (A)	Antrum (f,p)	F	F		
Vr (A)	Antrum (f,p)	F			
Areas with overt intestinal metaplasia in 'non-secretors'					
Hu (A)	Body	C		F	
	Antrum				
He (A)	Antrum	C			
Vr (A)	Antrum	C	F		

Symbols and abbreviations as in Table 1.

Table 3 *Immunofluorescence staining patterns in gastric carcinoma*

Patient (blood type)	*Tumour cell type (differentiation)*	*Immunofluorescence*		
		Loss of A or H antigen	*I(Ma) antigen staining*	*A antigen in O or B persons*
'Secretors'				
Ol (O)	(P) Intestinal (f,p)	F	F	
Wi (O)	Intestinal (f,p)	F	F	
Bu (O)	(W) Intestinal (f)		U	
Ri (O)	Intestinal (f,p)	F	F	
Ti (O)	(P) Intestinal (b)	F		U
Ni (A)	(P) Intestinal (f,p)	F		
Nu (A)	(W) Intestinal (f)	F	F	
Pos (A)	(P) Intestinal (p)	C	F	
We (A)	(P) Intestinal (f)	F		
Tr (A)	(W) Intestinal (f,p)	F	F	
Le (A)	(W) Intestinal (b)	F	F	
Gr (A)	(W) Intestinal (b)	F	F	
Ev (A)	Anaplastic (f)	F		
De (B)	(W) Intestinal (b)	F	F	
Wo (O)	(P) Diffuse (f)		F	
Ba (O)	(P) Diffuse (p)	F	F	
Go (AB)	(P) Diffuse (f,p)	F	F	
Mo (B)	(P) Diffuse (b)		U	
Pa (A)	(P) Indeterminate (f,p)	C		

		Loss of I(Ma) antigen	*Staining of*	
			A antigen	*H antigen*
'Non-secretors'				
Hu (A)	(W) Intestinal (f,p)	F	F	F
He (A)	(W) Intestinal (f,p)	F		F
Vr (A)	(P) Diffuse (f,p)	C	F	F

(P) = poorly differentiated; (W) = well differentiated.
Other symbols and abbreviations as in Table 1.

Table 4 *Focal loss and gain of blood group antigen staining in the mucus-secreting cells of the surface, pits, and ulcer site in patients with chronic benign gastric ulceration*

Patient (blood type)	*Tissues examined*	*'Abnormal' patterns of immunofluorescence*		
		Loss of A or H antigen	*Gain of I(Ma) antigen*	*A antigen in O or B persons*
'Secretor' without intestinal metaplasia				
Sq (A)	Ulcer (in body) (p)	F	F	
'Secretors' with intestinal metaplasia (non-metaplastic areas)				
Tru (O)	Body (f,p)		F	U
	Antrum (f)	F		
	Ulcer (in antrum) (p)		F	
So (A)	Ulcer (in antrum) (p)			
Cl (B)	Body (f)		F	F
	Antrum (f)		F	F
	Ulcer (in body) (f)		F	F
Areas with overt intestinal metaplasia in 'secretors'				
Tru (O)	Antrum			
Cl (B)	Antrum			F

		Loss of I(Ma) antigen	*Strong staining of*	
			A antigen	*H antigen*
'Non-secretors' with intestinal metaplasia (non-metaplastic areas)				
Gow (O)	Body (f)	C		U
	Antrum (f)	C		F
	Ulcer (in antrum) (f)	C		U
Ta (B)	Body (f)			U
	Antrum (f,p)	F		U
	Ulcer (in antrum) (f,p)	F		U

Symbols and abbreviations as in Table 1.

B antigens.[4] The secretor status of the infants studied was deduced from the immunofluorescence staining of the A and H antigens in the mucus cells of the superficial gastric mucosa.

Results

A comparison of immunofluorescence staining of cryostat sections and paraffin sections showed comparable results for the mucus-secreting cells at the surface and in the pits of the gastric mucosae. The paraffin sections showed less staining compared to the frozen sections in the specialised gastric glands. These will be described in detail elsewhere. The detailed immunofluorescence data on each individual patient are summarised in Appendix Figures 1 to 3 (pages 336-7).

'NORMAL' GASTRIC MUCOSAE

Immunofluorescence staining of A, H, and I(Ma) antigens in 'secretors'

The predominant staining in histologically normal appearing mucosae of 'secretors' was as previously described.[12-15] There was bright staining of the H or A antigens in persons of blood groups O or A, respectively (Fig. 1a). Most areas were not stained with anti-I(Ma) (Fig. 1b). However, in a high proportion of non-neoplastic mucosae at a distance from gastric carcinoma we observed divergences from these normal patterns with focal areas of unexpected antigen loss or gain (Fig. 2).

Of the seven samples of gastric mucosa from infants, five behaved as 'secretors' and showed uniform H or A antigen staining in the mucus-

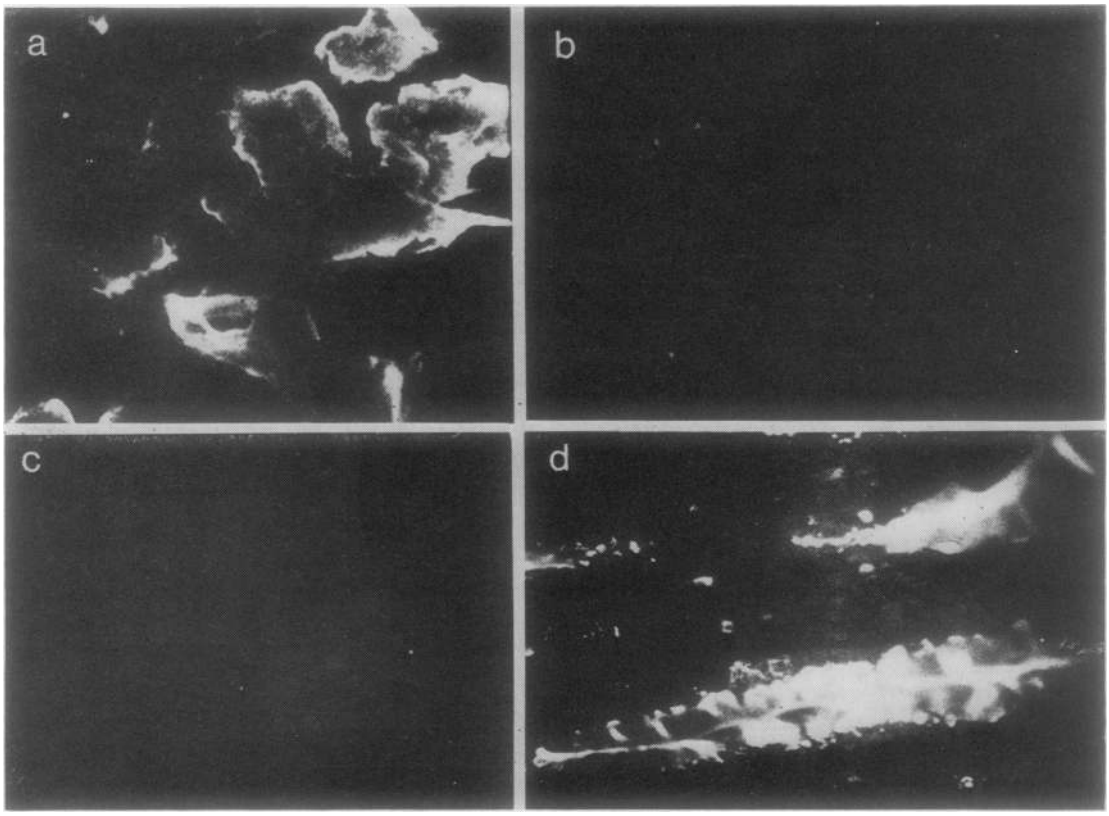


Fig. 1 *Immunofluorescence staining of the surface mucosa of the stomach of a blood group O 'secretor', case O1*, showing staining with anti-H serum* (a) *and lack of staining with anti-I(Ma)* (b). *In contrast, the mucosa of a blood group A 'non-secretor', case Hu, shows lack of staining with anti-A serum* (c) *and strong staining with anti-I(Ma)* (d) *× 180.*

*Full details of each patient designated are given in Appendix Figs 1 to 3 and Tables 1 to 4.

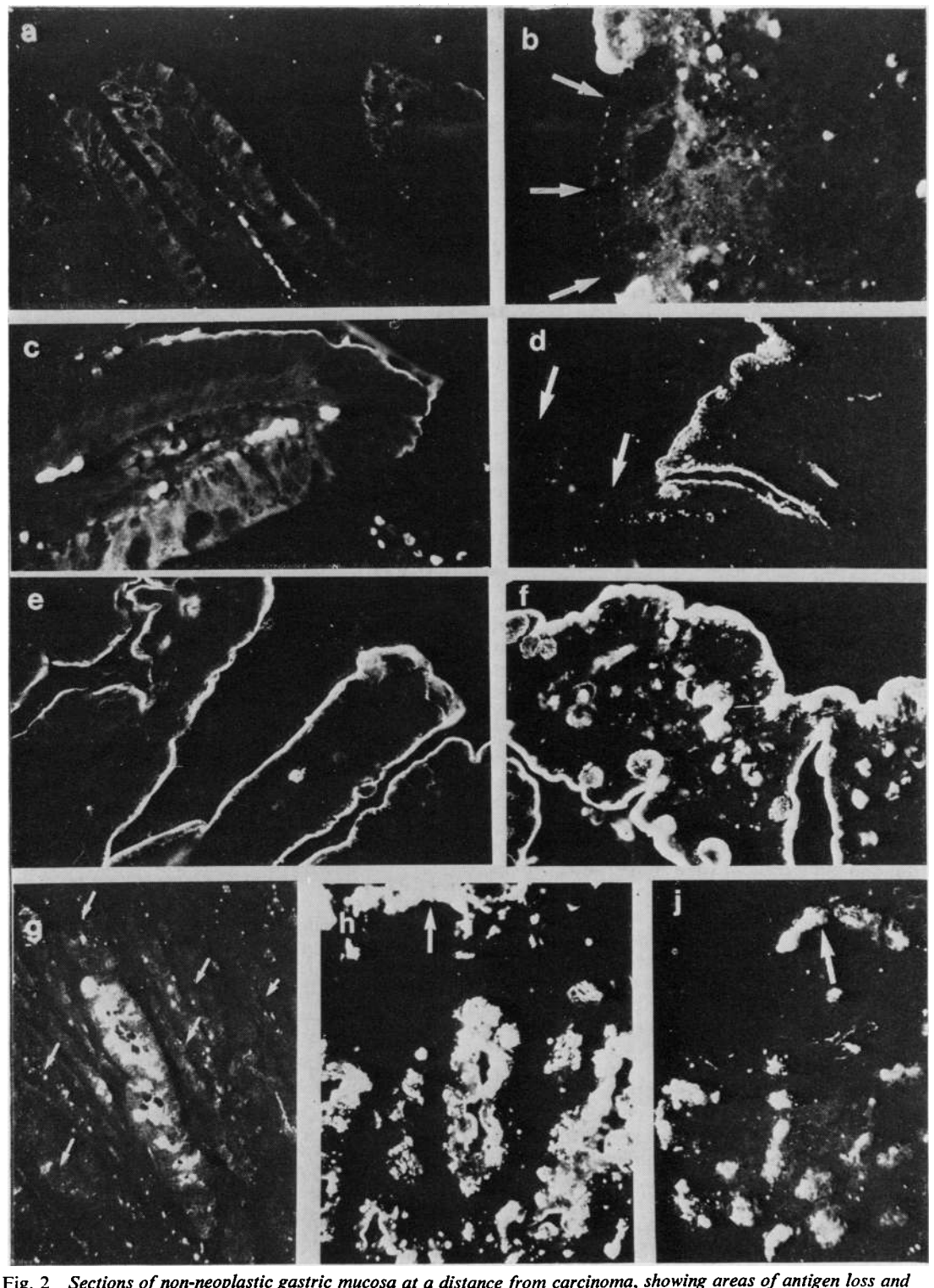


Fig. 2 *Sections of non-neoplastic gastric mucosa at a distance from carcinoma, showing areas of antigen loss and gain. Blood group A secretor, case Tr, showing lack of A antigen staining in overtly metaplastic area* (a) *and abrupt loss of A antigen staining in a normal antral mucosa* (arrowed) (b). *Intestinal type mucosa in a 'non-secretor', case He, showing diminished I(Ma) staining in goblet cells and linear staining in a brush-border distribution* (c). *An area of histologically normal mucosa in a 'non-secretor', case Vr, with intestinal metaplasia elsewhere, showing normal I(Ma) staining in a part of the surface, linear apical staining in a pit, and lack of staining in the adjacent surface,* arrowed (d). *Blood group A 'secretor' case Pos, showing intense apical staining* (e) *and both apical and goblet cell staining* (f) *with an anti-H serum; there was total lack of staining of this patient's mucosa with anti-A serum. One gastric pit showing I(Ma) staining among unstained pits* (arrowed) *in a 'secretor', case Pa* (g). *'Non-secretor' of blood group A, case Vr, showing an area of strong immunofluorescence of surface* (arrowed) *and pits with anti-A* (h). *'Secretor' of blood group O, case Wi, showing focal areas of A antigen staining on surface* (arrowed) *and pits* (j). × *180* (a, b, e-h); × *63* (d, j); × *298* (c).

secreting cells of the surface and pits (Fig. 3a), but there was no staining with anti-I(Ma), (Fig. 3b).

Immunofluorescence staining of A, H, and I(Ma) antigens in 'non-secretors'

In 'non-secretors', A or H staining was usually lacking in the greater part of the surface (Figs 1c and 3c), though moderate or bright staining in the deeper regions of the pits was often seen. The predominant staining on the surface and in the pits of 'non-secretor' mucosae was of the I(Ma) antigen (Figs 1d and 3d).

Immunofluorescence staining in 'secretors' and 'non-secretors' with anti-I(Step) and anti-i (Den)

Unlike the observations with anti-I(Ma) there was no clear relationship with 'secretor'/'non-secretor' status and the overall staining with anti-I(Step) and with anti-i(Den). The immunofluorescence patterns observed indicate that the antigenic determinants recognised by the latter sera are expressed independently from that recognised by anti-I(Ma).

The mucosae of all seven infants showed some staining with anti-i(Den); in the five 'secretors' the staining was predominantly in the mucus cells of the pits (Fig. 3g) while in the 'non-secretors' there was also some staining in the surface mucus cells (Fig. 3h). In the non-neoplastic mucosae of patients with gastric carcinoma and benign gastric ulcers, there was some immunofluorescence with the anti-i serum in more than half of the patients irrespective of 'secretor' status. The staining was usually focally distributed. In seven patients, samples of both antral and body mucosa were examined: there was staining in both samples in two patients, and there was staining in one or other sample in the remaining five. As with the infants the staining was most commonly in the mucus cells of the pits in 'secretors' (Fig 4a, b); staining of the surface mucus cells was more commonly observed in 'non-secretors' but occasionally it was also observed focally in 'secretors' (Fig. 4c).

The distribution of staining with anti-I(Step) often resembled that with anti-i(Den), being predominantly in the gastric pits in 'secretors'. However, a higher proportion of the samples showed focal or more uniform staining at the surface. It was not uncommon to find isolated mucus cells staining strongly among cells lacking in staining with this serum (Fig. 4d-f).

ANTIGENIC CHANGES IN NON-NEOPLASTIC MUCOSAE IN PATIENTS WITH GASTRIC CARCINOMA

Antigen loss

Focal areas lacking in staining of the expected A/H or I(Ma) antigens were observed in three out of 11 patients whose non-neoplastic mucosae appeared histologically normal or showed gastritis (Tables 1 and 2).

Loss of antigen was most pronounced in the mucosae of patients with intestinal metaplasia. Staining of the expected antigens was totally lacking or markedly diminished in the overtly metaplastic sites in 10 out of 11 patients (Tables 1 and 2; Fig. 2a, c). Moreover, focal antigen loss was frequently observed even in adjacent areas of their mucosa which were not overtly metaplastic (Tables 1 and 2; Fig. 2b, d). Sometimes residual staining of the mucosa was linear and confined to apical brush border-like areas of the mucosal cells (Fig. 2c-e). In 'secretors' of blood group A, the loss of A antigen staining was accompanied by a loss of H staining in two cases; in a third patient substantial staining of H remained (Fig. 2e, f). In areas with overt intestinal metaplasia, loss of A or H antigen in 'secretors' was not necessarily associated with the appearance of I(Ma). In their lack of A, $H^{14\ 15}$ and I(Ma) activities (Picard J and Feizi T, unpublished observations) these areas resembled the mucosa of the distal colon. Mucosae showing intestinal metaplasia were usually not stained with anti-I(Step) and anti-i(Den).

Antigen gain

Focal areas of antigen gain* were also observed in non-neoplastic mucosae, for example in 'secretors' focal areas of I(Ma) staining were commonly observed (Fig. 2g); and in two out of three 'non-secretors' there were focal areas of strong immunofluorescence of A or H antigens in the mucus-secreting cells of the surface and pits, with an appearance indistinguishable from that of the mucosae of 'secretors' (Fig. 2h). Aberrant A-like antigen staining was observed in five out of 13 patients of blood groups O and B (Fig. 2j). These samples with aberrant A-like antigen were not stained when tested with the anti-Forssman serum.

ANTIGENIC CHANGES IN GASTRIC CARCINOMA

Variable antigen loss

The most common finding (in 16 out of 22 cases) was focal antigen loss with much variation of staining from area to area (Table 3; Fig. 5a-f). Total or almost total lack of the expected antigen staining

*The term antigen gain is used here to denote antigens normally not detectable, irrespective of whether these antigens appear as a result of *de-novo* synthesis or whether they become revealed from a cryptic state as a result of incomplete biosynthesis of oligosaccharide chains.

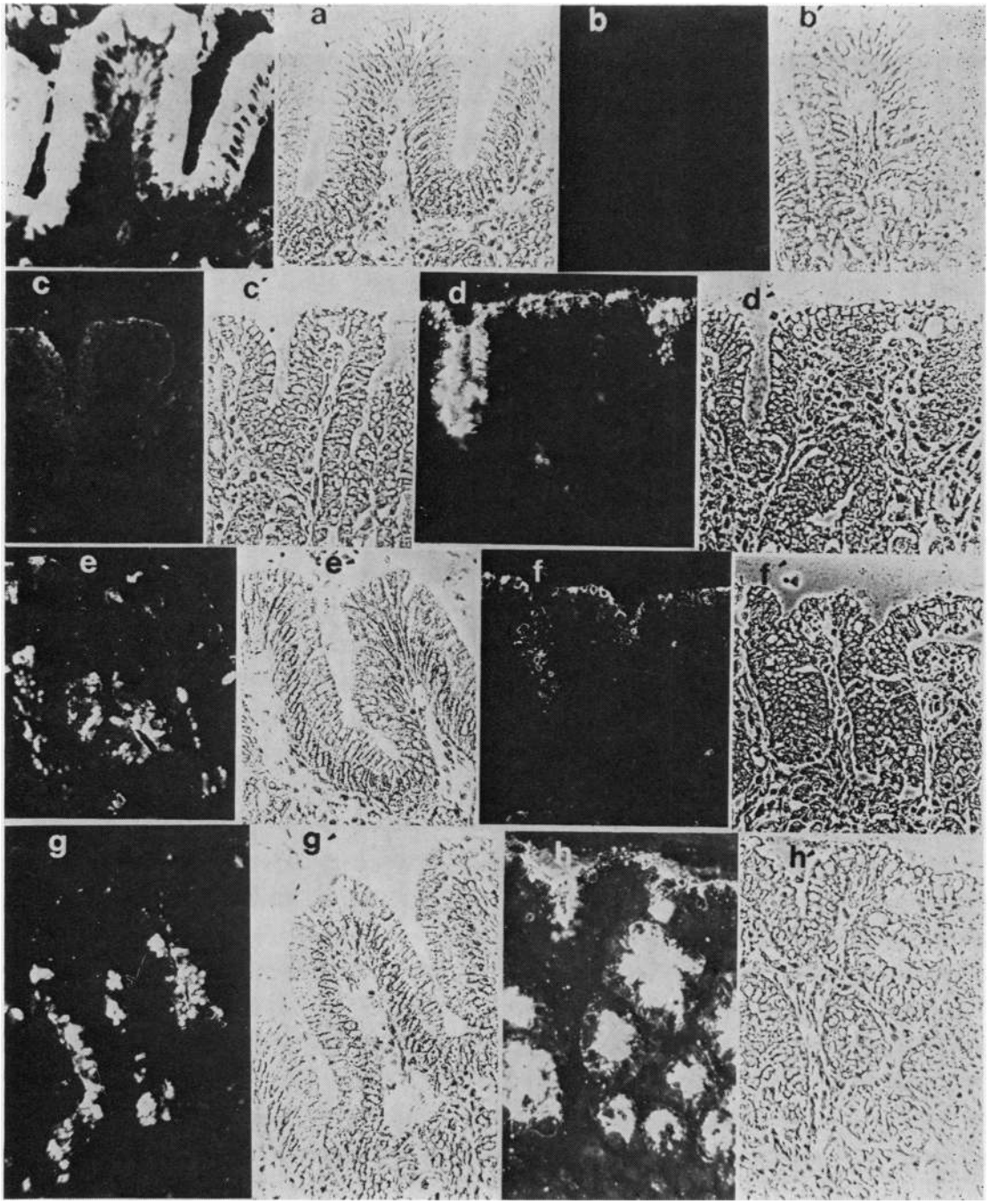


Fig. 3 *Immunofluorescence staining of surface and pits of gastric mucosa* (a-h) *and corresponding phase contrast microscopy* (a′-h′) *of sections from infants with normal gastric histology. Strong staining with anti-H serum in a 'secretor'* (a) *contrasts with very weak staining in a 'non-secretor'* (c). *Staining with anti-I(Ma) serum in a 'non-secretor'* (d) *contrasts with lack of staining in a 'secretor'* (b). *Staining with anti-I(Step) confined to certain cells in the surface and pits in a 'secretor'* (e) *and a 'non-secretor'* (f). *Staining with anti-i(Den) serum in certain cells in pits in a 'secretor'* (g) *and in both the surface and pits in a 'non-secretor'* (h). *There is some immunofluorescence of erythrocytes in the lamina propria in* (e) *and* (g). × *180.*

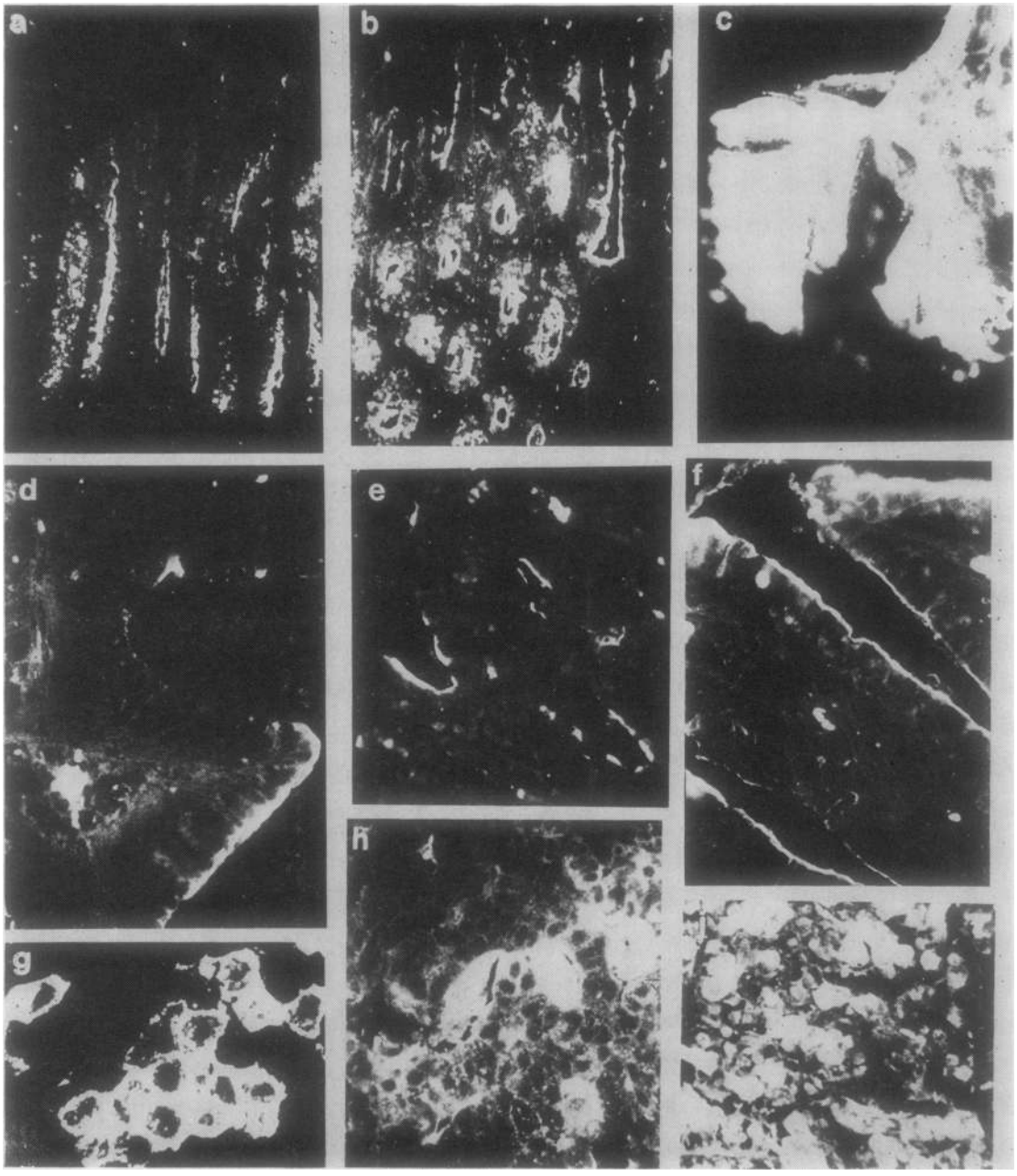


Fig. 4 *Immunofluorescence staining with anti-i serum(Den) and anti-I serum(Step). Non-neoplastic mucosae of three 'secretors' with gastric carcinoma showing staining with anti-i(Den) in the gastric pits, case Pa antrum* (a), *case Wi antrum* (b), *and in the surface mucus of one small area of body mucosa, case O1* (c). *Non-neoplastic mucosa of three 'secretors' showing staining with anti-I(Step) focally in the cells of the surface and pits, case Wi* (d) *and Pos* (e), *and more uniform staining of the surface and pits, case Tru* (f). *Tumours of three patients showing: cytoplasmic staining with anti-i(Den), case O1 'secretor'* (g); *cytoplasmic and luminal contents staining with anti-i(Den), case He 'non-secretor'*, (h); *variable cytoplasmic staining with anti-I(Step) in a 'secretor' case Go* (j). × *180* (c-j); × *63* (a, b).

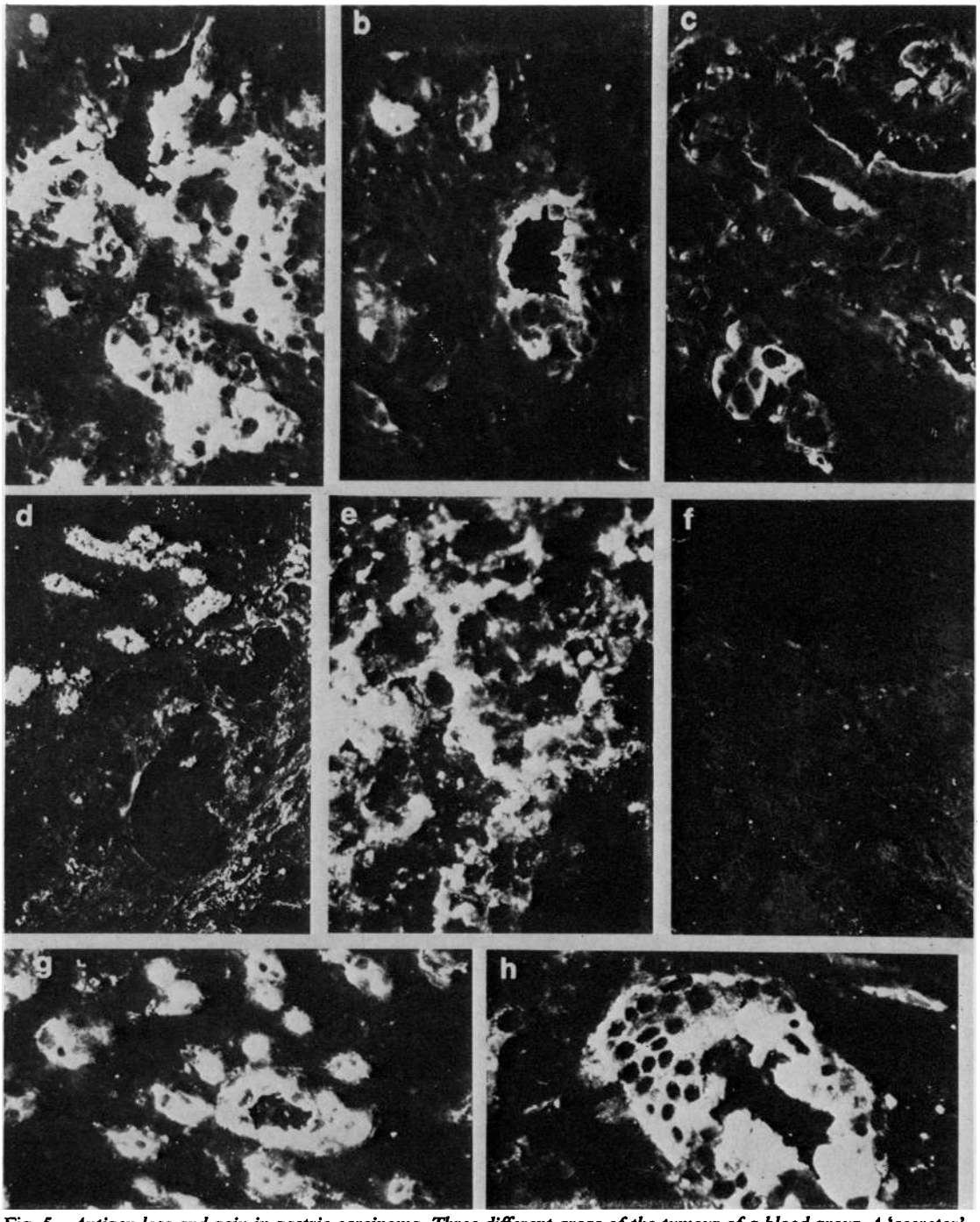


Fig. 5 *Antigen loss and gain in gastric carcinoma. Three different areas of the tumour of a blood group A 'secretor', case Pos, showing variable staining with anti-H: focal areas of cytoplasmic staining* (a), *luminal surface staining* (b *and* c). *This patient's tumour was totally devoid of A antigen staining. Tumour of a blood group O 'secretor', case Wi, showing occasional foci of H staining* (d). *Two areas of the tumour of a blood group A 'secretor', case Tr, showing cytoplasmic and pericellular staining with anti-A in one area* (e) *and total lack of staining in another* (f). *Tumours of two 'non-secretors' of blood group A, cases Vr* (g) *and Hu* (h), *showing gland-like structures staining with anti-A serum.* × *180* (a-c, e, g, h); × *63* (d, f).

was seen in only three out of the 22 carcinomas examined (Table 3).

Antigen gain

The majority (14 out of 19) of carcinomas from

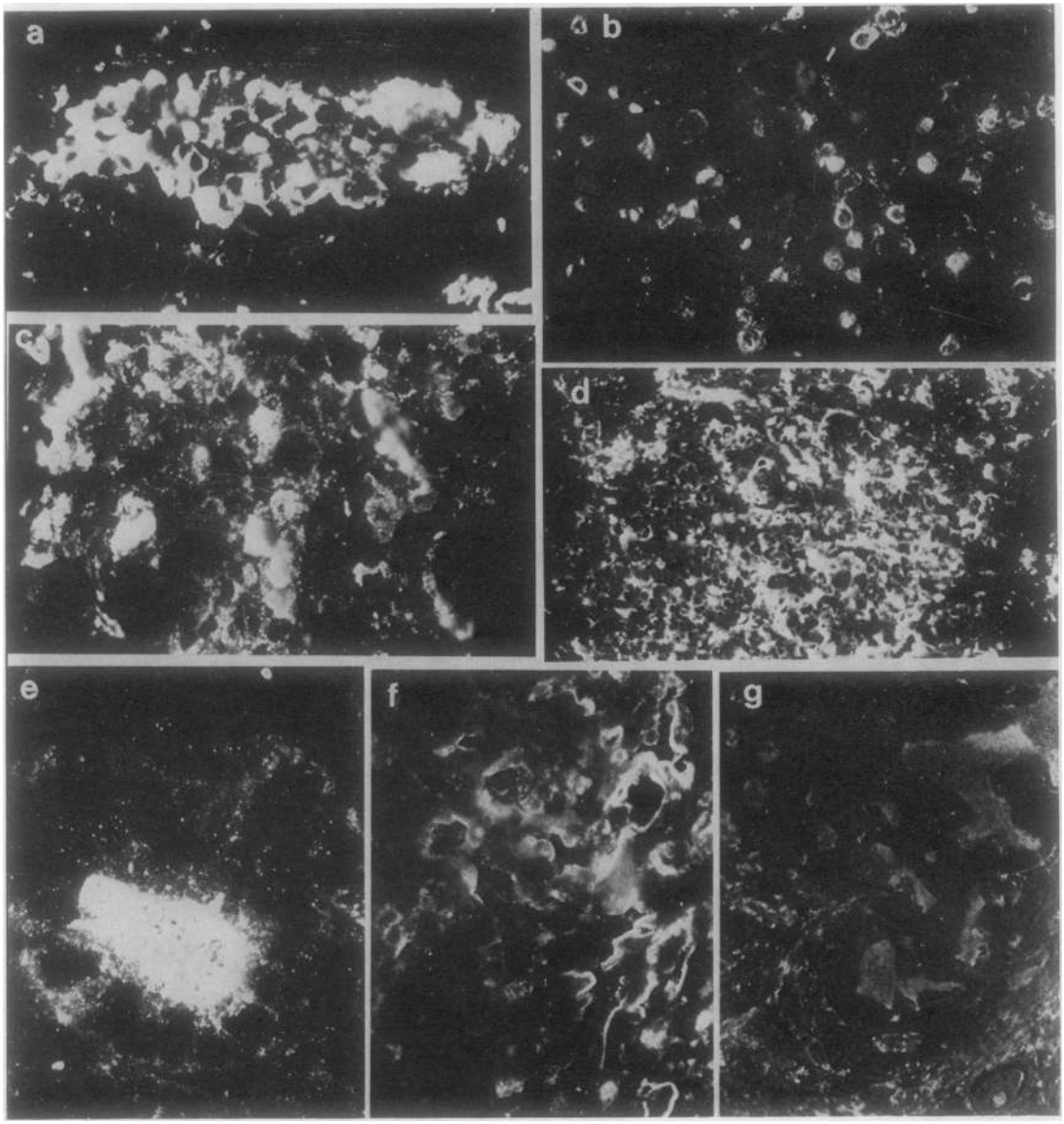


Fig. 6 *Different patterns of I(Ma) antigen staining in gastric carcinomas in 'secretors'. Two areas of the tumour of a blood group O 'secretor', case O1, showing intense cytoplasmic staining of a cluster of tumour cells in the lamina propria* (a) *and variable cytoplasmic staining of the tumour cells in another area of solid tumour* (b). *Tumours of three blood group O 'secretors' showing cytoplasmic and some extracellular staining, case Ba* (c)*; case Wi* (d), *and staining of the luminal contents of a gland-like structure, case Bu* (e). *Tumour of a 'secretor' of blood group A, case Tr, showing accentuation of staining at the luminal aspects of tumour cells* (f) *and staining of secreted material in another area* (g). × *180* (a-c, e, f); × *63* (d, g).

'secretors' showed substantial I(Ma) staining (Table 3). This was seldom uniform but varied in intensity from area to area and from cell to cell within a given tumour. The staining was predominantly cytoplasmic in some tumours (Fig. 6a-d), extracellular in others (Fig. 6e, g), or sometimes accentuated at the luminal aspects of gland-like structures (Fig. 6f).

The tumour tissues of three 'non-secretors' of blood group A showed focal areas of A and H type staining (2 cases) and of H staining only (1 case) (Fig. 5g, h). Aberrant A-like antigen staining was observed in one out of nine adenocarcinomas from persons of blood groups O or B (Table 3).

Eight of the 19 gastric carcinomas from 'secretors' and two out of three from 'non-secretors' showed focal areas of staining with anti-i(Den) and anti-I (Step) in addition to staining with anti-I(Ma) (Fig. 4g-j). Often the areas staining with the three antisera were not the same in a given tumour. In the remaining tumours there was I(Ma) staining only (2 tumours); I(Ma) and I(Step) staining (4 tumours); and I(Ma) and i(Den) staining (1 tumour).

FOCAL ANTIGENIC CHANGES IN PATIENTS WITH CHRONIC BENIGN GASTRIC ULCERATION

Focal loss or gain of blood group antigens was not confined to patients with gastric carcinoma but was observed in five out of six patients with chronic benign gastric ulceration. All but one of these patients had intestinal metaplasia (Table 4). The most striking changes were areas of strong H staining in the uninvolved mucosa and ulcer site in 'non-secretors' (Fig. 7a, b). Unexpected blood group A staining was observed in two patients of blood groups O and B (Fig. 7d). Focal areas of I(Ma) staining were observed in three of the 'secretors'.

Discussion

The gastric mucosa is an ideal tissue for the study of

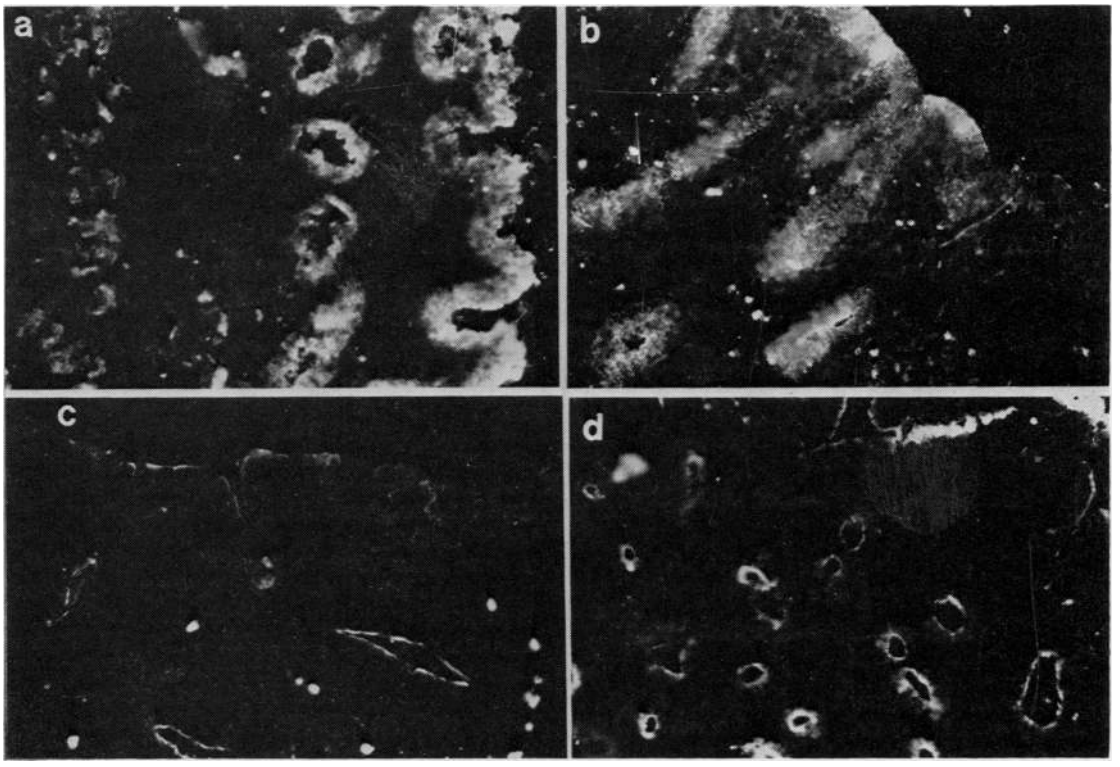


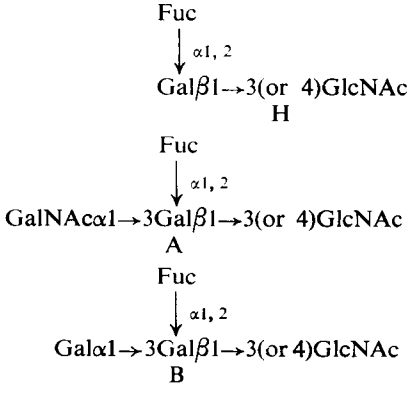
Fig. 7 *Antigenic 'atypia' in gastric mucosa of patients with chronic benign gastric ulceration. Body mucosa* (a) *and ulcer site* (b) *of a 'non-secretor' of blood group B, case Ta, showing uniform staining with anti-H serum. Body mucosa of a blood group O 'secretor', case Tru, showing linear staining with anti-I(Ma) in the luminal aspects of the mucosa* (c). *Antral mucosa of blood group B 'secretor'* (d) *showing some cytoplasmic and some luminal staining with anti-A serum in the surface and pits.* × *180.*

altered blood group antigen expression in benign and malignant disease. Gastric juice and biopsy tissues obtained at endoscopy are readily available, and when gastrectomy is performed for carcinoma, there is opportunity to examine both the tumour tissue and non-neoplastic tissue. The immunofluorescence technique is of special value in revealing focally occurring changes in antigen expression.

EXPRESSION OF A, H, I, AND i ANTIGENS IN NORMAL GASTRIC MUCOSAE IN RELATION TO 'SECRETOR'/'NON-SECRETOR' STATUS

The antisera used in the present studies recognise the blood group A and H antigens and I and i antigens. The Ii antigens are expressed on certain oligosaccharides which carry the A, B, and H antigens. In agreement with previous immunofluorescence data[12–15] and antigenic analysis of gastric mucosal glycoproteins,[4] the present studies demonstrate that normal 'secretor' type mucosae express the H or A antigens while normal 'non-secretor' mucosae express the antigenic determinant recognised by anti-I(Ma). Such a clear correlation was not observed in reactivities with anti-I(Step) and anti-i(Den).

The blood group antigenic determinants A, B, and H are formed when the monosaccharides *N*-acetylgalactosamine, galactose, and/or fucose are transferred by specific glycosyl transferases on to oligosaccharide precursor chains having the terminal sequence Galβ1→3GlcNAc (type 1 chain) or Galβ1→4GlcNAc (type 2 chain)[16 17] as shown below.



The amount of fucosyl transferase present in secretory cells is controlled by the 'secretor' gene. Thus, in those having the 'secretor' gene, the blood group H active structures are normally synthesised in large amounts and occur as terminal structures of oligosaccharide chains of the gastric glycoproteins. In those having blood group A or B genes, the appropriate *N*-acetylgalactosaminyl or galactosyl transferases are present but they can function only in the presence of blood group H-active structures as acceptors. Thus in persons lacking the 'secretor' gene ('non-secretors') the A and B transferases remain redundant on account of the lack of the fucosyl transferase.

Types 1 and 2 precursor chains themselves occur as the end structures of oligosaccharides which vary in size and branching and are attached to the peptide moiety of the gastric glycoproteins.[17 18] In human gastric mucins there is little information on the precise sequences of the precursor chains beyond the terminal disaccharides. However, the sequence Galβ1→4GlcNAcβ1→6 is known to occur.[19] This oligosaccharide sequence is the antigenic determinant recognised by the monoclonal anti-I antibody of patient Ma,[6] and it has so far been detected only in branched oligosaccharide chains.[8 18] In 'secretors', the H, A, or B monosaccharides mask the antigenicity of the I(Ma) determinant,[6] but in 'non-secretors' it is available for reaction with anti-I(Ma). The type 1 precursor sequence Galβ1→3GlcNAcβ1→3, which has also been detected in human gastric mucins,[19] is apparently not involved in I and i antigen specificities.[20]

The precursor chains recognised by anti-i(Den) and anti-I(Step) are distinct from those recognised by anti-I(Ma). Anti-i(Den) recognises a linear sequence made up of two type 2 chains as follows: Galβ1→4GlcNAcβ1→3Galβ1→4GlcNAcβ1→3...[7] Anti-I(Step) can cross-react with this linear sequence although it reacts best with the branched sequence

...[7–9]

The immunofluorescence data provide evidence for the presence of these 1→4, 1→3 linked sequences in the gastric pits where rapidly proliferating cells are known to occur.[21] It is possible that the linear oligosaccharide sequence in these cells is a transiently occurring structure which becomes branched or further glycosylated in more mature cells.

CHANGES IN NON-NEOPLASTIC MUCOSAE OF PATIENTS WITH GASTRIC CARCINOMA AND CHRONIC BENIGN PEPTIC ULCERATION

The present studies have shown that gastric mucosae at a distance from malignant and chronic benign gastric ulcers often show focal areas of loss or gain of blood group antigens. The most marked example of antigen gain was the occurrence of 'secretor-like' areas with strong H antigen staining in 'non-secretors'. The focal changes revealed by immunofluorescence are not necessarily detected by antigenic analysis of glycoprotein-rich extracts of these tissues (Picard J, Feizi T, unpublished observations).

It is possible that the blood group antigens on glycosphingolipids[2 22] contribute to some of the immunofluorescence staining in the mucus cells; these would be excluded in the glycoprotein-rich extracts. The early studies of Hartmann[23] showed that low-grade blood group H activity occurs in a proportion of gastric mucosal extracts such that it is not clearly possible to describe them as being 'secretor' or 'non-secretor' type. These were saline extracts of postmortem tissues, and the salivary 'secretor status' of these subjects and information on gastric disease were not available.

The present studies have also shown striking examples of A-like antigen activity in non-neoplastic mucosae of persons of blood groups O or B. Previous studies have stressed the association of A-like antigen with neoplasia,[3] but one patient was described who showed this change in association with benign peptic ulcer.[24] Our studies indicate that the aberrant A-like antigen activities are not due to the presence of Forssman antigen which may cross-react with anti-A sera.[17] Further investigations are required to determine the chemical nature of the A-like antigen.

Histologically normal looking areas of the mucosa of certain patients showed these changes; this raises the possibility that change in blood group antigen expression may be an early biochemical abnormality in gastric mucosal disease, and the regulatory effect of the 'secretor' gene may be disturbed in gastric disease. Thus, caution is required in regarding the apparently uninvolved mucosae of patients with gastric ulcer or gastric carcinoma as 'normal' tissues. These studies indicate the need to study in detail truly healthy gastric mucosae. The mucosae of infants in the present studies only partially fulfil this requirement for some of the features in these samples, for example, the presence of i antigen may be a normal feature in the mucosae of infants (as on their erythrocytes[28]) but not in those of healthy adults. Loss of blood group activities have been observed previously in non-neoplastic mucosae adjacent to carcinoma of the right colon[25] and of the larynx.[26] In studies of experimental colon cancer in rats after administration of the carcinogen 1,2 dimethylhydrazine, alterations of glycosyl transferase activities were noted not only in the tumours but also in apparently uninvolved colonic mucosae.[27]

In patients with intestinal metaplasia, which is considered a precancerous state[29–32] antigen loss was most striking. Furthermore, it was not confined to areas of mucosa showing overt morphological changes with goblet cells and/or intestinal brush borders. It is of interest that intestinal sucrases, which appear focally in the gastric mucosae of patients with intestinal metaplasia, show a lack of blood group activities.[33] This is in contrast to the sucrases isolated from normal small intestine which express the blood group activities in accordance with blood group and 'secretor' status. Thus the loss of blood group antigens may be added to the list of biochemical changes that occur in mucus cells in intestinal metaplasia; these include the appearance of sulphated glycoproteins[34 35] and other intestinal type glycoprotein antigens.[36] Decreased reactivities for blood group antigens have also been demonstrated in premalignant lesions of the oral epithelium.[37]

CHANGES IN GASTRIC CARCINOMA

Antigen loss in gastric carcinoma tissues is by no means uniform; persistence of blood group antigen staining in gastric carcinoma tissues has been described previously.[12 13 22 38] On the other hand, marked depletion of the expected antigens has been clearly demonstrated in glycoprotein-rich extracts of some gastric tumours.[39–41] However, in certain tumour extracts very high blood group activities have been detected.[4] The present morphological studies provide an explanation for these varied results and clearly demonstrate the variation in antigen expression that can occur from one area to another within a tumour. In agreement with earlier studies of gastric[16] and bladder carcinomas[42] we have observed no correlation in the expression of blood group antigens with the histological type or degree of differentiation of the tumours.

These immunofluorescence studies have confirmed that the tumour cells are the source of the increased I(Ma) antigen activities that were previously detected in glycoprotein-rich extracts of gastric tumours of the majority of 'secretors'.[4 5] The expression of this antigen was also variable from area to area and even from cell to cell within a given area. There was no clear relationship between the presence of I(Ma) activity and the lack of A or H activities in the tumours.

Diminished blood group activities could be caused either by incomplete biosynthesis of oligosaccharide chains or by inappropriate substitutions of the oligosaccharides. Alternatively, there may occur increased degradation of oligosaccharide chains in the diseased tissues. Enzymological studies[43 44] thus far have provided evidence for incomplete biosynthesis in adenocarcinoma tissues. The increased level of I(Ma) antigen in the tumours of 'secretors' is also compatible with incomplete biosynthesis.

The influence of sulphation and sialylation of oligosaccharide chains on the expression of these various blood group activities[8] needs to be investi-

gated. They may be responsible in part for the paucity of blood group antigens A, H, I, and i in intestinalised gastric mucosae as well as in the mucosa of normal distal colon of man. From about the time of birth it is well known that there occurs a marked diminution of the blood group ABH antigens in the mucosa of the distal two-thirds of the colon.[45]

POSSIBLE BIOLOGICAL AND CLINICAL IMPLICATIONS OF ABNORMAL EXPRESSION OF BLOOD GROUP ANTIGENS

Perhaps the most important implication of this study is that the abnormalities of blood group antigens in tissues may reflect abnormal expression of glycosyl transferase genes and may be an early manifestation of mutagenic injury(ies) to the gastric mucosa. Further investigations are required in order to ascertain whether antigenic atypia correlates with nuclear ploidy.[46] On the other hand, the changes observed may merely reflect an imbalance of glycosyl transferases, of their substrates, and of their acceptors in metabolically disturbed cells. Such imbalance could give rise to antigen deletion; it may even result in altered specificity of glycosyl transferases, giving rise to inappropriate antigen expression.[47]

An important consideration with respect to possible immunotherapeutic targeting of antibodies to incompatible antigens (for example, to inappropriately expressed A antigen in neoplastic or preneoplastic cells) is the focal expression of these antigens. This would preclude effective ablation of all the diseased cells.

Clearly, the cytochemical changes described are not of immediate value in the routine diagnosis of overt gastric neoplasia. Further investigations are required to determine to what extent they may be of value as early biochemical markers of mutational or metabolic injury. Antigenic analyses of glycoproteins of gastric juice for levels of 'neo-antigens', for example, I(Ma) antigen in 'secretors' or A antigen in blood groups O or B persons, are currently under way to determine the levels of these antigens in various diagnostic groups, Moreover, detailed structural analysis of the oligosaccharide moieties of gastric glycoproteins in health and disease may also provide important information. Improved techniques of oligosaccharide isolation and microscale structural analysis applicable to intestinal glycoproteins are now available.[18 48]

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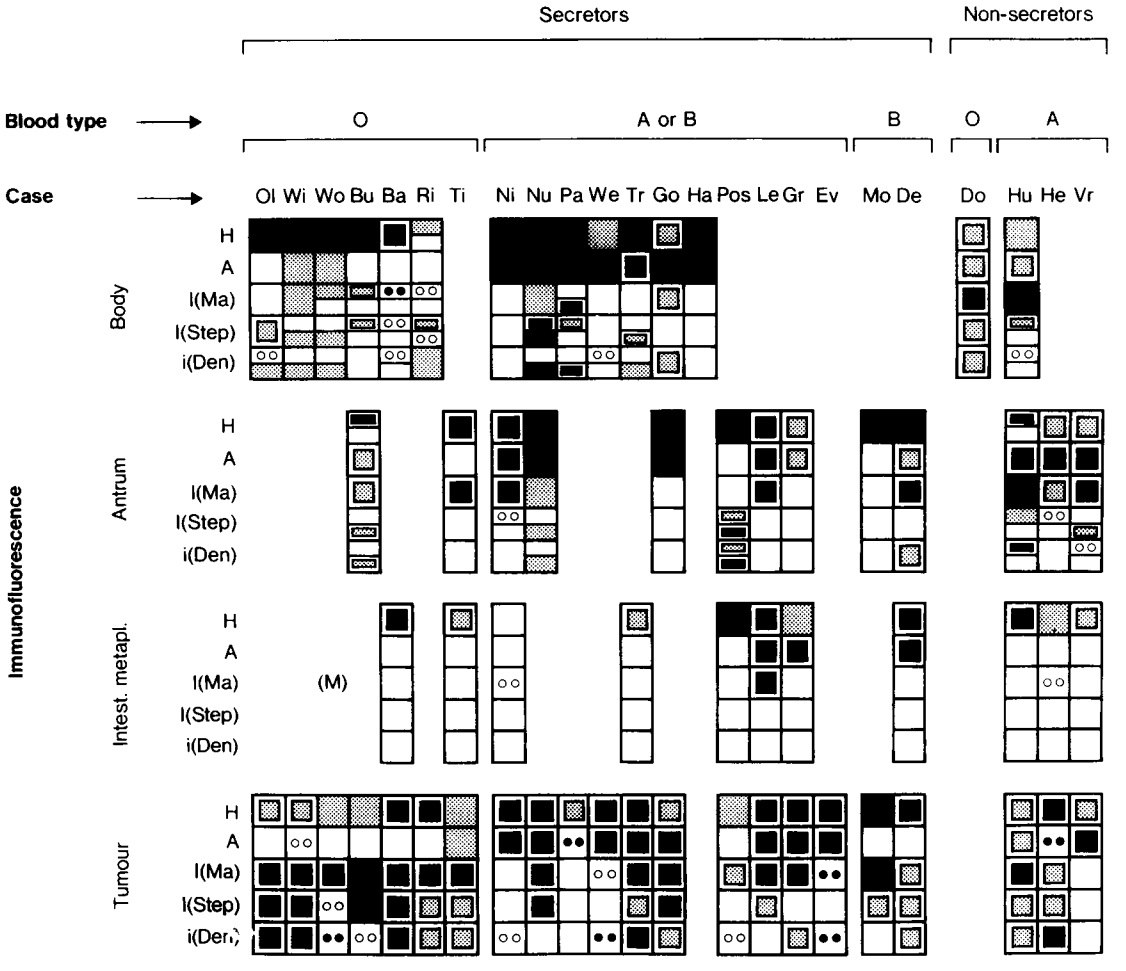
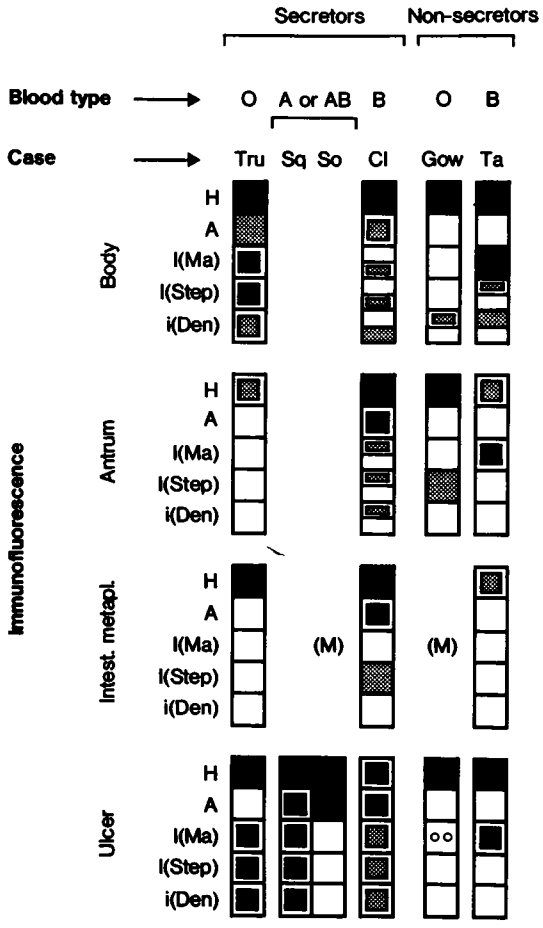
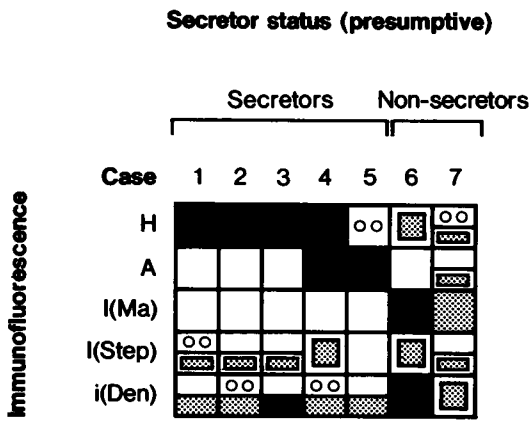
Requests for reprints to: Dr Ten Feizi, Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ.

Appendix



Appendix Fig. 1 *Schematic presentation of immunofluorescence staining of the mucus-secreting cells of gastric mucosa and tumour tissues of 24 patients with gastric cancer. The data are a composite of observations on formalin-fixed, paraffin-embedded sections and/or cryostat sections of fresh frozen tissues described in Tables 1-3. When available, the immunofluorescence data on different samples from the same patients have been aligned. In addition, the staining in areas of overt intestinal metaplasia are separately described and appropriately aligned. See key on page 337 for explanation of symbols.*





Appendix Fig. 2 (left) *Schematic presentation of immunofluorescence staining of mucus-secreting cells in the gastric mucosae and ulcer site in patients with chronic benign gastric ulceration.*

Appendix Fig. 3 (above) *Schematic presentation of immunofluorescence staining of the mucus-secreting cells of the surface and pits of the gastric mucosae of seven infants with normal gastric histology.*

Symbols for strong immunofluorescence:

- Uniform staining in surface and pits; Uniform staining in pits only;
- Focal staining in mucus cells of surface and pits; Focal staining in pits only;
- Focal staining in the surface only; Occasional strongly stained cells in surface and pits;
- Occasional strongly stained cell in the surface only;

Symbols for moderate or weak immunofluorescence:

- Uniform staining in surface and pits; Uniform staining in pits only; Uniform staining of surface only;
- Focal staining in surface and pits; Focal staining in pits only; Focal staining in surface only;
- Staining of occasional cells in surface and pits; Staining of occasional cells in pits only;
- Staining of occasional cells in surface only

(M) Denotes intestinal metaplasia noted elsewhere but not in the samples examined