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The Variability of Blood Group Antigens in Gastric Carcinoma as Demonstrated by the Immunoperoxidase Technique

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Summary. In this study the immunoperoxidase technique was used to demonstrate the blood group antigens A, B and H in normal gastric mucosa and primary and metastatic gastric carcinomas. No clear relationship between tumour differentiation and preservation of blood group antigens was found. In some well differentiated tumours there was a marked loss of blood group substance, whereas in other poorly differentiated tumours, both primary and metastatic, it was easily detectable. In some tumours an inverse relationship between A or B and H activity was noted. This observation is consistent with the theory that in some malignant cells there is a blocking of the normal step-by-step elongation of the carbohydrate portion of blood group substances.

Key words: Immunoperoxidase – Blood group antigens – Carcinoma.

Introduction

The changes which occur when a cell becomes malignant have been the subject of intensive study over many years. Much attention has been focused on cell surface antigens in general and blood group substances in particular. Glynn et al. (1957) and Glynn and Holborow (1959) reported the persistence of blood group substance in gastric carcinoma and this finding was supported in a larger series by Eklund et al. (1963). However, Masamune et al. (1958), Cowan (1962), Davidsohn et al. (1966) and Sheahan et al. (1971) all reported total or partial loss of blood group reactivity in gastric neoplasms.

Kovarik et al. (1968) developed the mixed cell agglutination technique of Coombs et al. (1956) for studying the localisation of blood group substances in formalin fixed paraffin embedded material. This enabled Davidsohn et al. to undertake retrospective studies of large numbers of cases using archive material. Studies on carcinoma of the lung (1969), cervix (1969, 1973, 1975), pancreas

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(1971), stomach (1971), bladder (1973 and 1979) and oral mucosa (1978) all showed loss of blood group antigens in both primary and secondary tumour. Davidsohn (1972) also reported a direct relationship between loss of blood group substance and the degree of anaplasia of a tumour as well as its tendency to metastasize. He suggested that loss of blood group substance was a useful diagnostic and prognostic indicator. This view has been supported in longterm follow-up studies of carcinoma of the bladder (Bergman and Javadpour 1978; Lange et al. 1978). However, Denk et al. (1974) using the same technique failed to confirm any correlation between blood group substance and differentiation in carcinomas of the stomach and colon.

The aim of this investigation was to study the behaviour of blood group substances in gastric carcinoma using the immunoperoxidase technique. Particular attention was paid to the relationship between the A and B blood group substances and their precursor H substance.

Materials and Methods

Purified human blood group substances A, B and H were kindly supplied by Professor Winifred Watkins (M.R.C. Research Centre, Harrow).

Specific antisera to human blood group substances were obtained by the immunization of New Zealand White rabbits. The blood group of each rabbit was initially determined using the method of Glynn, Holborow and Johnson (1956). Half the rabbits tested were found to be A-type and half alpha-type.

The rabbits were immunized by intramuscular injection at four separate sites with 1 mg of purified blood group substance in Freund's complete adjuvant, made up to a total volume of 1 ml.

The rabbits were bled at regular intervals, the serum collected and incubated at 56 ° C for 30 min. to inactivate complement. The serum was then absorbed using washed human red cells to remove non-specific factors. Anti-A serum was absorbed using group B cells, anti-B serum was absorbed with group A cells, and anti-H serum was absorbed with group AB cells. The completeness of absorption was checked by a tube haemagglutination technique using the appropriate group cells. Absorption was repeated as necessary.

Booster injections, with 1 mg of blood group substance in Freund's incomplete adjuvant, were given 8 months later. Both A-type and alpha-type rabbits were immunized with A, B and H human blood group substances, but the atypical anti-A obtained from A-type rabbits was not used in the subsequent procedures.

Sections for examination were cut from paraffin embedded blocks of formalin-fixed tissue. Previous authors (Davidsohn 1971; Denk et al. 1974) have emphasised the suitability of this type of material for the study of blood group substances by a mixed-cell agglutination reaction, and Kovacs et al. (1976) used the immunoperoxidase technique successfully on similar material. Both surgical and post-mortem material was studied, and where possible separate blocks of normal gastric mucosa, tumour, and lymph nodes were examined. However, the degree of tissue autolysis in post-mortem specimens of gastric mucosa made evaluation difficult and therefore only surgical specimens were included in the final study. All the tumours were adenocarcinomas.

The immunoperoxidase staining technique used was an adaptation of the one used by Kovacs et al. (1976) for the localization of prolactin in chromophobe adenomas of the pituitary. The technique used for the localization of blood group substances differed from the original in the following respects:

The blood group antisera were found to be more effective if 30% normal goat serum in phosphate buffered saline (pH 7.2) was used as a dilutent instead of isotonic saline. By a series of titrations the optimum concentrations for the antisera were found to be 1 in 1000 for anti-A and anti-B and 1 in 50 for anti-H. At these concentrations, strong specific staining of cells bearing the appropriate antigens was achieved.

Unlike Kovacs original method, where the sections were treated with prolactin anti-serum at room temperature for 10 min, in our system optimum results with blood group anti-sera were obtained by treating the sections for 1 h at room temperature.

Endogenous peroxidase activity in the tissues was removed using the technique of Reid (1976) which is derived from the work of Strauss (1971) and Streefkerk (1972). This consists of treating the sections with a solution of 0.5% hydrogen peroxide in methanol containing 0.074% hydrochloric acid for 30 min prior to commencing the immunoperoxidase technique.

A consecutive section from each block studied was stained with haematoxylin and eosin, and used to assess the localization of the blood group antigens as revealed by the immunoperoxidase technique.

As Davidsohn et al. (1971) described, the tissues examined furnish a number of "built-in controls". The endothelial cells lining the lumina of small blood vessels, blood plasma, the epithelial cells of normal tissue adjacent to the carcinoma and other tissues normally containing blood group iso-antigens, all constitute a positive control. Negative controls are connective tissue and other tissues which normally lack the antigens.

Reagent controls consisted of neutralization of the blood group antisera with isologous purified blood group substance prior to incubation with the sections. By titration, isologous blood group substance was found to abolish specific staining of blood vessel endothelial cells at dilutions of up to 1 in 30.

Results

Of the 33 gastrectomy specimens studied, all had the appropriate blood group substance in the normal mucosa (Table 1). Sixteen specimens were from Group 0 patients, 14 Group A and 3 Group B. In 27 cases (87%) the blood group substance was easily detectable in the tumour. No correlation was found between degree of tumour differentiation and retention of blood group substance. Deeply invasive tumour, whether well differentiated and forming glands (Figs. 1 and 2) or poorly differentiated single cells (Figs. 3 and 4), retained stainable blood group substance.

In cases where blood group substance had been lost there was sometimes a dramatic contrast between the positively staining normal mucosa and adjacent unstained tumour (Figs. 5 and 6). This occurred even when the tumour was well or moderately differentiated.

Twelve of the 33 gastrectomy specimens had metastatic tumour detectable in the regional lymph nodes and 7 of these had retained the appropriate blood group substance. Strong staining could be obtained not only of well differentiated metastases, but also of isolated clumps of tumour cells in the peripheral sinuses of otherwise unaffected nodes (Figs. 7–10). Isolated tumour cells were easily

Table 1. Retention of blood group substances in normal mucosae and tumours

	Blood groups			Total
	0	A	B	
Gastrectomies	16	14	3	24
Blood group substances in normal mucosa	16	14	3	24
Blood group substance in tumour	13	12	2	27 (82%)

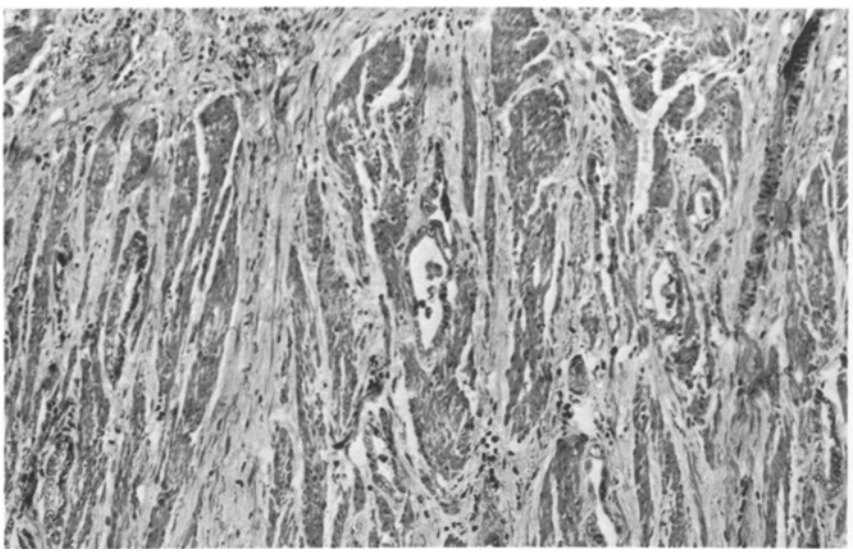

Fig. 1. Group 0 patient. Well differentiated carcinoma invading muscle. H & E; ×40

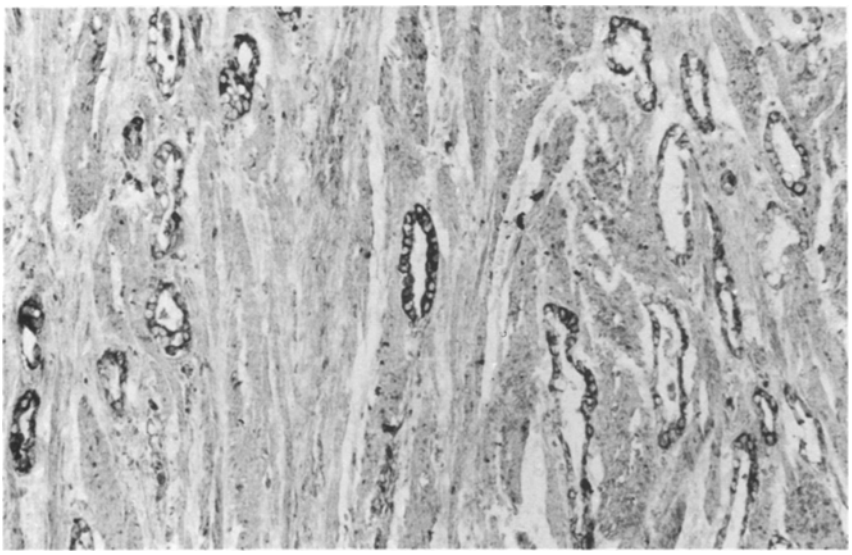

Fig. 2. Same patient. Immunoperoxidase stain with anti-H. Specific staining of tumour cells. Muscle and connective tissue negative. ×40

detectable because of the universally negative staining of lymphoid tissue, which does not produce blood group substance.

The results of staining for the H blood group substance were rather more complicated. In Group 0 patients, H substance was present in the normal gastric mucosa of all 16 specimens and in 13 of the tumours (Table 2). Similarly in Group A patients, H substance was present in the majority of normal and

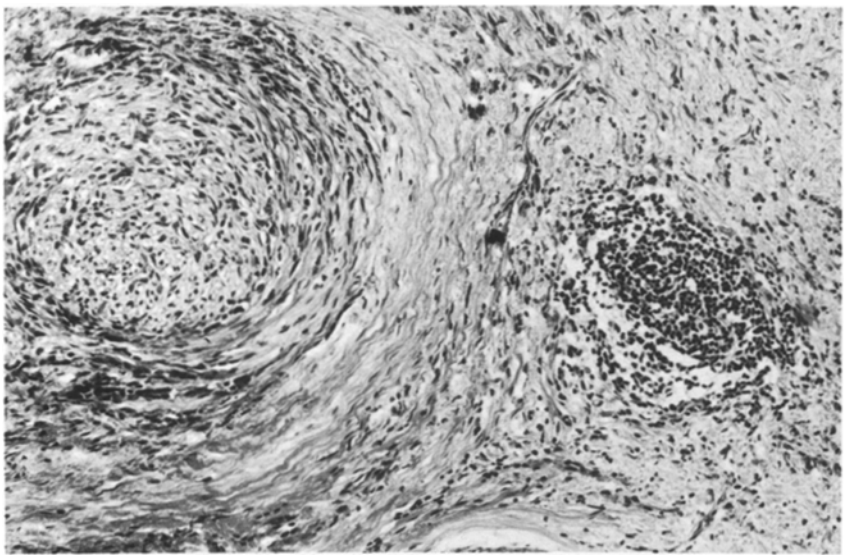

Fig. 3. Group B patient. Poorly differentiated tumour with single cells invading and surrounding a nerve to the left of the picture. Note lymphoid follicle to the right. H & E; ×40

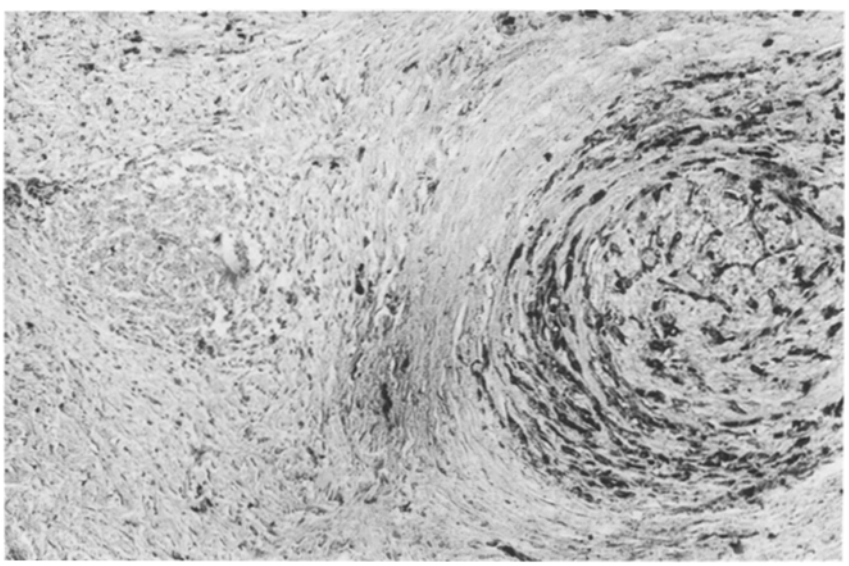

Fig. 4. Same section. Immunoperoxidase stain with anti-B. Specific staining of tumour cells. Connective tissue and lymphoid tissue negative. ×40

tumour specimens. However, in the 3 Group B patients, no H substance was detectable in the normal gastric mucosa, but in 2 cases it was detectable in the tumours.

Interestingly, in one Group A tumour and one Group B tumour, H substance appeared when the main blood group substance had been lost. However, no inappropriate A or B-like substances were detected in either the normal mucosa

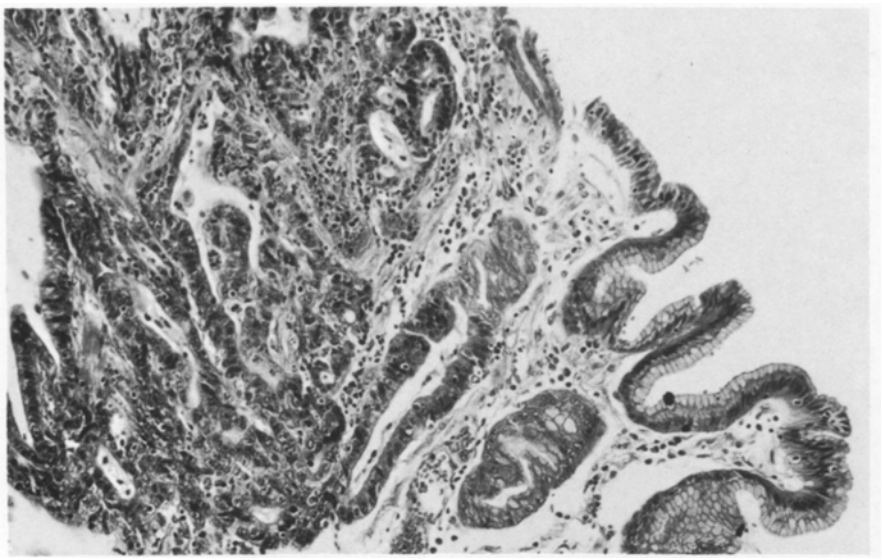

Fig. 5. Group B patient. Normal gastric mucosa to the right. Moderately differentiated carcinoma to the left. H & E; ×40

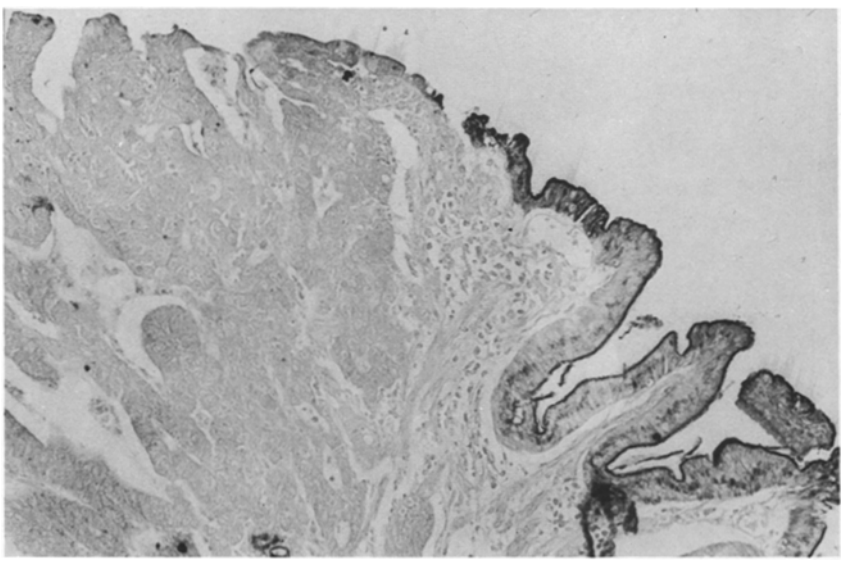

Fig. 6. Same section. Immunoperoxidase stain with anti-B. Note complete loss of blood group staining in tumour. Positive staining of normal mucosa. ×40

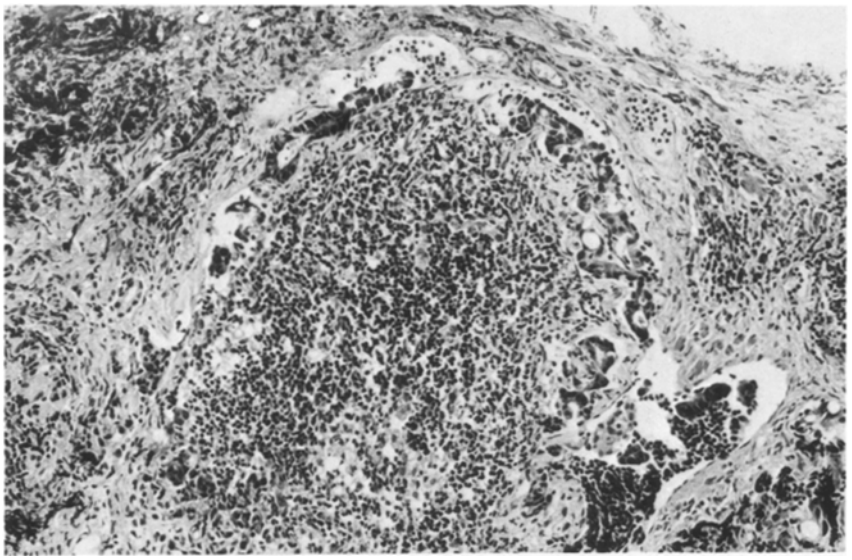

Fig. 7. Group A patient. Lymph node with clumps of metastatic tumour cells in peripheral sinus. H & E; ×40

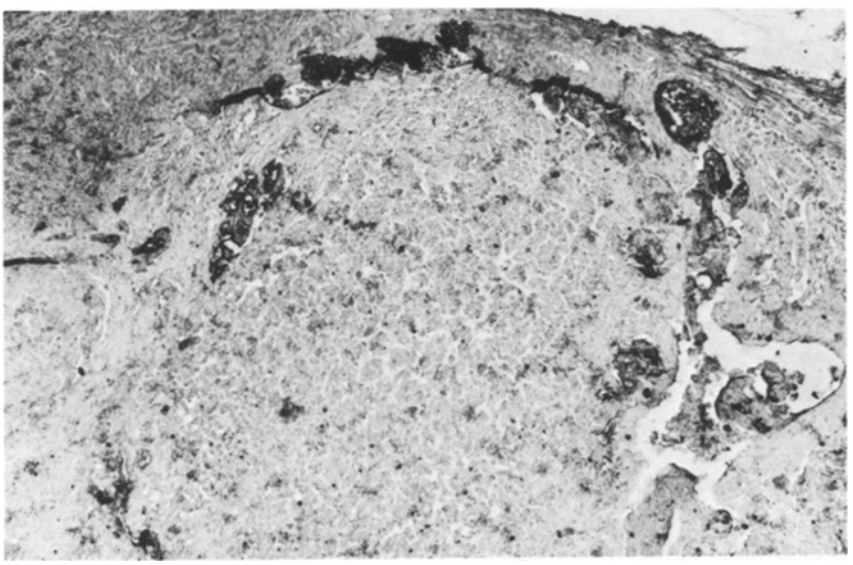

Fig. 8. Same section. Immunoperoxidase stain with anti-A. Strong positive staining of tumour cells. Lymphoid tissue negative. ×40

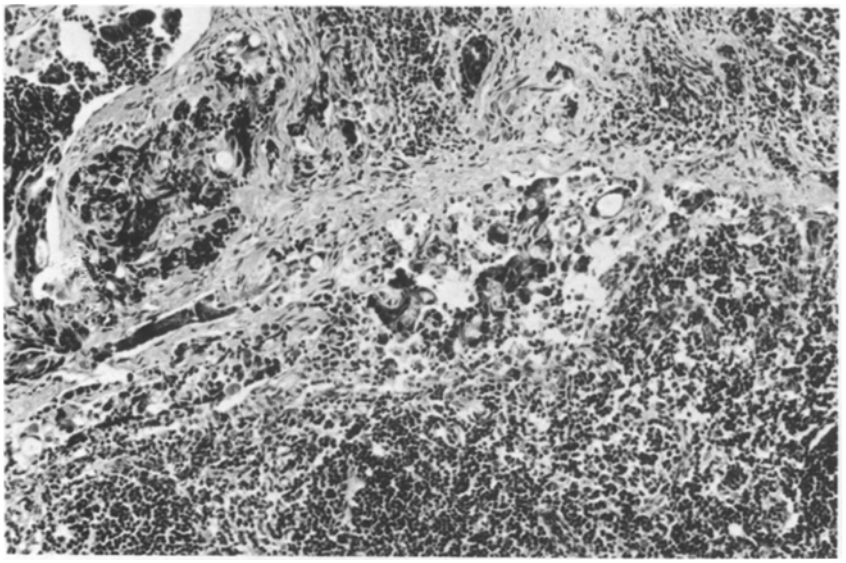

Fig. 9. Group B patient. Lymph node containing poorly differentiated metastatic carcinoma. H & E; ×100

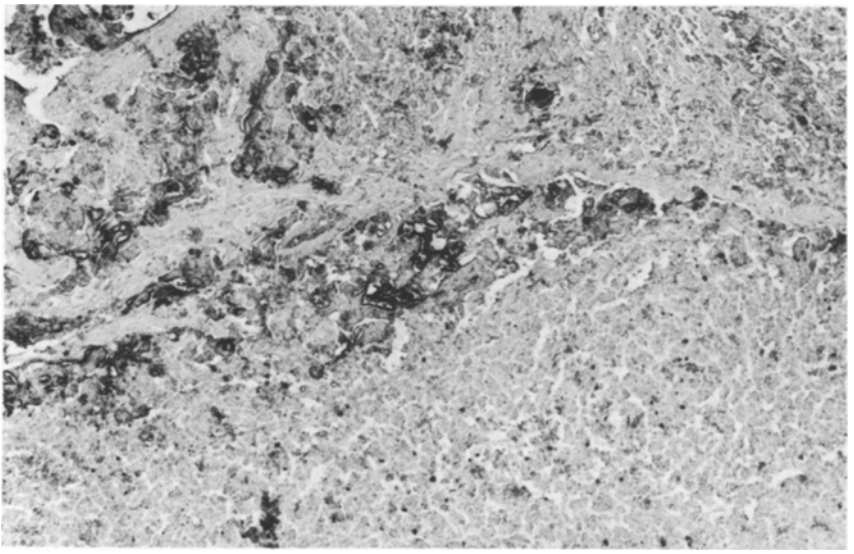

Fig. 10. Same section. Immunoperoxidase stain with anti-B. Strong positive staining of tumour cells. Lymphoid tissue negative. ×100

Table 2. The distribution of H substance in normal mucosae and tumours

	Blood groups			Total
	0	A	B	
Gastrectomies	16	14	3	33
H substance in normal mucosa	16	13	0	29
H substance in tumour	13	12[a]	1[a]	18

[a] H substance detected in 1 Group B and 1 Group A tumour where the blood group antigen was absent

or tumours of patients who did not have that blood group. This phenomenon had been reported by Häkkinen (1970).

In 4 cases of blood group 0 and 3 of group A, areas of intestinal metaplasia were found. These showed preservation of the appropriate blood group substance in all instances studied. Our sections included no superficial carcinoma, nor were areas of dysplasia encountered.

Discussion

The presence of blood group iso-antigens in a wide variety of epithelial and endothelial cells has long been established (Holborow et al. 1960; Szulman 1960, 1962 and 1964). Two forms are described: a water-soluble glycoprotein present in secretions; and an alcohol-soluble glycosphingolipid present in epithelial and endothelial cells. It was originally thought that the alcohol-soluble form was lost from formalin-fixed paraffin embedded tissue during processing. However, Kovarik et al. (1968) were able to show that by using a sensitive technique such as mixed cell agglutination, blood group substances were easily detectable in epithelial and endothelial cells. The development of immunoperoxidase techniques in recent years provides a new tool with which to look afresh at the question of changes in blood group iso-antigens in neoplasia. Immunoperoxidase methods provide a permanent and reproducible demonstration of specific cell products, (Heyderman 1979). We found, like Davidsohn and his group, that blood group substance was easily detectable in normal gastric mucosa. Where our results differed dramatically was in the retention of the blood group iso-antigen in primary and secondary gastric carcinoma. Davidsohn et al. (1966) found blood group substance in only 38% of 45 gastric carcinomas studied by immunofluorescence and in 1971 found only 5% total and 20% partial retention in 95 carcinomas studied by mixed cell agglutination. In our study, blood group substance was present in 87% of the 33 gastric carcinomas. This may be explained by the greater sensitivity of the immunoperoxidase technique, and a comparitive study would perhaps be useful. However, in the minority of cases where we found loss of blood group substance, the contrast between

normal mucosa and tumour was clear cut and dramatic, indicating that even with a sensitive technique genuine loss of antigen is demonstrable.

Davidsohn also showed a correlation between loss of blood group substance and loss of tumour differentiation and particularly the production of metastases. We were unable to confirm this, since we found well differentiated tumours without blood group substance and anaplastic ones with strong blood group staining. This agrees with the findings of Denk et al. (1974).

The behaviour of H substance has been extensively studied since it was first designated by Morgan and Watkins (1948). It is now recognized that H substance serves as a substrate upon which the A, B and 0 genes work. The 0 gene is an amorph and therefore in Group 0 individuals H substance remains unaltered. The A gene codes for the production of a glycosyltransferase responsible for the addition of N-acetylglucosamine to the H substance to form the A antigen. The A_1 gene codes for a more effective enzyme than the A_2 gene, thus in A_2 individuals a greater proportion of the H antigen remains unconverted. The B gene similarly codes for a glycosyltransferase which adds. D-galactose to the H substance. The subject has recently been reviewed by Watkins (1978). Szulman (1962) investigated the histological distribution of H substance in A and B individuals and found that its distribution exactly paralleled that of A or B substance. He also found that in some A_1, A, B and B individuals, H substance was undetectable. He interpreted this as evidence of near complete conversion of H substance by the glycosyltransferase enzymes in these individuals. Our study showed similar results although it was not possible to obtain details of the Group A sub-groups of our cases. The majority of Group A cases had detectable H substance in the normal mucosa, but one did not. It is tempting to postulate that this represents one of the A, individuals described by Szulman. Similarly we were unable to detect H substance in the normal mucosa of any of the three Group B cases studied.

In carcinomas, however, H substance appeared in two of the Group B tumours. In one Group A and one Group B tumour the H substance appeared when the main blood group substance had been lost. This correlates with the findings of Stellner et al. (1973) who demonstrated deficiencies of A and B transferases accompanying loss of A and B activity in adenocarcinomas. Watanabe and Hakomori (1976) demonstrated that the carbohydrate chains of blood group substances are synthesised step-by-step during the process of ontogenesis and that this programme of synthesis can be blocked during oncogenesis with loss of the final product and accumulation of precursor substances. Denk et al. (1974) showed the independant behaviour of A and B blood group substances in gastric carcinomata from AB individuals. The whole subject of the biochemical and genetic basis for changes in blood group antigens in malignancy has recently been reviewed by Salmon (1978).

In conclusion, the behaviour of blood group substances in gastric carcinoma demonstrated by the immunoperoxidase technique in this study shows considerable variation, as would be expected from the heterogeneous nature of all neoplastic processes. Loss of blood group substance in malignancy may not be as prevalent as had been previously thought. When it does occur there is evidence for the blocking of blood group antigen synthesis described by

other authors. This variation casts doubt on the value of blood group substances as a diagnostic and prognostic index.

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