Preliminary Communications

Gastric Antigens in Health and Disease. Behaviour in Early Development, Senescence, Metaplasia, and Cancer

[WITH SPECIAL PLATE FACING PAGE 96]

British Medical Journal, 1969, 3, 93–94

Summary: Immunofluorescence studies of the behaviour of gastric antigens in health and disease have shown that during foetal development both gastric and intestinal antigens are present in the gastric superficial mucous epithelium. The intestinal component disappears soon after birth; it re-emerges in senescence and in metaplasia and neoplasia, while the gastric antigen, which normally persists in adult life, is depleted in these circumstances. The loss of adult and the re-emergence of foetal antigen in both metaplasia and neoplasia suggest a possible fundamental relationship between these conditions; the phenotypic variation may reflect cytogenetic liability, which has malignant transformation as a final irreversible step.

INTRODUCTION

Immunofluorescent tracing with antisera against specific gastrointestinal components has permitted the study of the behaviour of human gastric mucosal cells in early development, senescence, intestinal metaplasia, and cancer. The components examined were an intestinal antigen (Nairn *et al.*, 1962), which is a normal constituent of foetal gastric mucous epithelium, and another mucous epithelium antigen found throughout life but only in the stomach (Lynraven and de Boer, 1969). The intestinal antigen reappeared in adult stomach in pathological states, and there was often an associated depletion of the specific gastric antigen.

METHODS

The anti-intestinal serum, prepared in rabbits by immunization with microsomal material from fresh surgical specimens of human colonic mucosa, has already been reported; it reacts with an acid mucopolysaccharide component of human and other mammalian intestinal mucosal cells (Nairn *et al.*, 1962; Nairn and de Boer, 1966). The antiserum specific for gastric mucous epithelium was prepared against rat stomach and reacted strongly with human and other mammalian gastric mucous epithelium (Lynraven and de Boer, 1969).

The histological material examined comprised fresh stomach specimens of five human foetuses whose ages ranged from 15 to 22 weeks, and fresh operation specimens from six patients with gastric or duodenal ulcer and from 12 patients with gastric cancer, including lymph node metastases from six of these. Formalin-fixed paraffin-embedded material was obtained less than 10 hours after death from necropsies of eight children of different ages and five adults aged 42 years or less with no history of gastric disease, and from gastric biopsies of six healthy individuals over the age of 60 years.

Immunofluorescent staining with general procedures and controls described elsewhere (Nairn, 1969) was effected by the "sandwich" method with fluorescein-labelled goat anti-rabbit-globulin. From the fresh specimens snap-frozen blocks were taken of the different parts of the stomach, and, in the case of the cancers, also from the centre of the tumour and from its edge to include both normal mucosa and tumour tissue. From the paraffin-embedded material 6μ sections after removal of the paraffin were stained for one hour—that is, for twice as long as the frozen sections. We had earlier confirmed that both antisera reacted specifically with paraffin sections, albeit less strongly than with the frozen sections.

Tissue was also examined by conventional histological and histochemical methods with haematoxylin and eosin, periodic-acid–Schiff reagents, and Alcian dyes.

RESULTS

In the stomach of the 15-week human foetus there was early development of gastric pits with differentiation of the first parietal cells at the base. The identity of these cells could be established by immunofluorescence with sera from pernicious anaemia patients, which contained gastric parietal cell antibody. The superficial mucous epithelium contained both the gastric and the intestinal antigens (Special Plate, Fig. 1); it did not react with the gastric parietal cell antibody. A similar distribution of staining was observed in the other foetal stomachs.

As shown in Table I the intestinal antigen persisted in the superficial gastric mucous epithelium after birth, but only for some months, whereas the gastric antigen was found in all the specimens throughout life. Too few specimens were available for examination to permit any useful assertions about the precise time period within which the intestinal antigen disappeared or about any factors concerned with the disappearance. Histochemical examination showed that acid mucopolysaccharides, which were present in the superficial gastric mucosa before and shortly after birth, had disappeared at this stage of early life. In none of the infant stomachs were there pathological lesions. The normal adult stomachs showed a constant antigenic picture—that is, the presence of gastric antigen in the superficial mucous epithelium and total absence of intestinal antigen.

TABLE I.—*Immunofluorescent Staining of Normal Stomach in Neonates, Children, and Adults*

Case No.	Age	Sex	Diagnosis	Gastric Antigen	Intestinal Antigen
1	6 hours	M	Prematurity, hyaline membranes	+	+
2	2 days	M	Prematurity, hyaline membranes	+	+
3	1 week	M	Bilateral renal dysplasia	+	+
4	4 weeks	F	Congenital heart disease	+	+
5	9 weeks	F	Pinealoma	+	+
6	5 months	F	Dural sinus thrombosis	+	–
7	20 months	F	Astrocytoma	+	–
8	4 years	F	Congenital heart disease	+	–
9	29 years	M	Subarachnoid haemorrhage	+	–
10	33 years	M	Diabetes mellitus	+	–
11	35 years	M	Lymphosarcoma	+	–
12	42 years	M	Myocardial infarct	+	–

One man, 39 years old, had intestinal antigen in the gastric mucosa but histological examination showed the presence of chronic atrophic gastritis. The biopsy specimens of a group of apparently healthy subjects over 60 years old disclosed different manifestations of chronic gastritis; their antigenic patterns are summarized in Table II. We have graded the gastritis according to the classification of te Velde *et al.* (1966), but, because of the limited amount of tissue available, we have not distinguished between the diffuse atrophic form and gastric atrophy.

TABLE II.—*Immunofluorescent Staining of Stomach Biopsy Specimens in Senescence*

Case No.	Age	Gastritis	Intestinal Metaplasia	Gastric Antigen	Intestinal Antigen
13	64	Superficial	–	+	–
14	67	Diffuse atrophic	+	+	+
15	73	Chronic multifocal	+	+	+
16	75	Superficial	–	+	+
17	77	Nil	–	+	–
18	87	Superficial	+	+	+

Intestinal metaplasia was present in three of the five biopsy specimens with gastritis, and intestinal antigens were demonstrated in these areas (Special Plate, Fig. 2). In one of the cases with superficial gastritis but no intestinal metaplasia intestinal antigen was found to be present in histologically normal-looking superficial epithelial cells. Intestinal antigens were not found in the remaining two specimens, one of which looked normal and the other showed superficial gastritis.

The association of gastritis and intestinal metaplasia or presence of intestinal antigens was striking in the group of patients with peptic ulcer and gastric cancer (Table III). The mucosa in these operation specimens showed different grades of gastritis, and areas of intestinal metaplasia were present in all stomachs but two, both with mucoid adenocarcinoma. Intestinal antigens could be demonstrated not only in mucosa with intestinal metaplasia (Special Plate, Fig. 3) but also in areas with histologically normal-looking gastric superficial mucous epithelium ; this was observed in the two specimens without overt metaplastic changes.

TABLE III.—*Immunofluorescent Staining of Gastric Mucosa in Disease*

Case No.	Age	Sex	Blood Group		Diagnosis	Gastritis	Intestinal Metaplasia	Intestinal Antigen	
			ABO	Rhesus				Stomach	Lymph Node Metastases
19	36	M	A	+	Duodenal ulcer	Chronic multifocal	+	+	
20	49	M	O	−	Gastric ulcer	Diffuse atrophic	+	+	
21	49	F	A	+	Gastric ulcer	Diffuse atrophic	+	+	
22	50	M	A	−	Gastric ulcer	Diffuse atrophic	+	+	
23	51	F	O	+	Gastric ulcer	Chronic multifocal	+	+	
24	52	M	A	+	Anastomotic ulcer	Chronic multifocal	+	+	
25	31	M	B	+	Adeno-carcinoma	Diffuse atrophic	+	+	
26	54	M	A	+	Mucoid carcinoma	Diffuse atrophic	+	+	+
27	56	F	B	+	Adeno-carcinoma	Diffuse atrophic	+	+	
28	58	M	O	+	Adeno-carcinoma	Diffuse atrophic	+	+	+
29	60	M	O	+	Scirrhous carcinoma	Diffuse atrophic	+	+	
30	63	F	O	+	Mucoid carcinoma	Chronic multifocal	+	+	+
31	64	M	A	+	Mucoid carcinoma	Diffuse atrophic	+	+	
32	65	M	O	+	Mucoid carcinoma	Superficial	−	+	
33	70	F	A	+	Mucoid carcinoma	Superficial	−	+	+
34	70	F	A	+	Mucoid carcinoma	Diffuse atrophic	+	+	+
35	73	F	AB	+	Adeno-carcinoma	Diffuse atrophic	+	+	
36	76	M	O	+	Scirrhous carcinoma	Diffuse atrophic	+	+	+

The gastric antigen was either absent altogether or considerably reduced in the metaplastic areas and in the other groups of cells containing intestinal antigens (Special Plate, Fig. 4). Double immunofluorescent staining of the gastric and intestinal antigens simultaneously with separate fluorescein- and rhodamine-labelled antisera and the use of sensitive microphotometry (Nairn *et al.*, 1969) confirmed that in the metaplastic zones some epithelial cells containing the intestinal antigen also had detectable quantities of the gastric antigen. Thus in the diseased stomachs there were islands of superficial mucous cells with irregularly varying proportions of both antigens and often the intestinal antigen alone.

In all the gastric cancers, irrespective of their histological appearance, intestinal antigen could be detected. As a rule the antigen was abundant in the mucoid and scirrhous cancers (Special Plate, Fig. 5), and less prominent in the adenocarcinomas ; it was also found in the lymph node metastases. Staining of tumour for gastric antigen in the stomach (Special Plate, Fig. 6) and lymph node metastases showed it to be less abundantly present than the intestinal antigen. Commonly, both the gastric and intestinal antigens were depleted in large areas of the cancer ; such depletion was never observed in normal mucosa or in areas of intestinal metaplasia.

DISCUSSION

In this study we have demonstrated two antigens in the superficial mucous epithelium of human foetal stomach. One of these, designated gastric antigen, occurs only in the stomach and persists here throughout normal adult life ; the other, designated intestinal, is also present in intestine, but it disappears from the stomach some weeks after birth, though it persists in normal adult intestine. Failure to manufacture the intestinal antigen in normal adult stomach is apparently a consequence of phenotypic suppression in the mucous cells because the antigen may re-emerge during senescence or in association with certain pathological conditions. Parallel observations have been made by Häkkinen *et al.* (1968), who in their studies of the distribution of alimentary canal sulphoglycoprotein antigens noticed similarities between the foetal condition and pathological states in the adult gastric mucosa.

Wherever the intestinal antigen is re-expressed in the stomach there has been a variable reduction of the gastric antigen with sometimes complete depletion. The loss of an adult antigen and the re-expression of a normal constituent of foetal gastric mucosa in senescence and disease are presumably due to functional change in the cell genome, caused either by endogenous or by exogenous factors. The re-emergence of the intestinal antigen in both metaplasia and neoplasia suggests that these two conditions are related. The observation by Yasin and Bergel (1965) and Leese (1965) of similar isoenzyme patterns in intestinal metaplasia and stomach cancer is further evidence for such a relationship. The metaplasia presumably reflects cytogenetic instability, which under certain circumstances is manifested as frank neoplasia. The antigen depletion in neoplasia is probably the result of further dedifferentiation of cancer cells.

It is of interest that the gastric and intestinal antigens could also be found in some of the cells of the lymph node metastases. This indicates that a proportion of the metastatic cancer cells have retained some ability to differentiate ; whether preservation of this ability is a favourable prognostic sign remains to be seen.

This research programme has been supported by grants from the Anti-Cancer Council of Victoria and the Australian Research Grants Committee. We are indebted to Dr. A. V. Jackson, Dr. Elsie L. Abrahams, Dr. A. L. Williams, and Dr. Joan C. Booth for providing biopsy and necropsy specimens.

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Fluorescence photomicrographs of sections stained by the "sandwich" immunofluorescence method with absorbed anti-intestinal or antigastric rabbit serum followed by fluorescein-conjugated goat anti-rabbit-globulin.

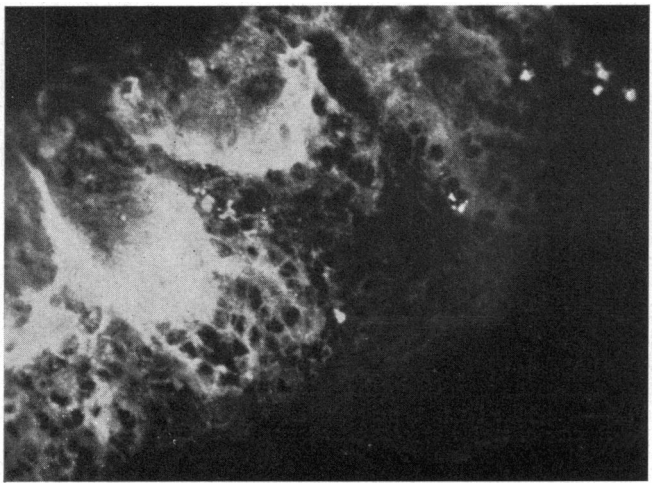

FIG. 1.—Fresh frozen section of 15-week human foetal stomach treated with anti-intestinal serum. Specific fluorescent staining of superficial mucosal cells. (×150.)

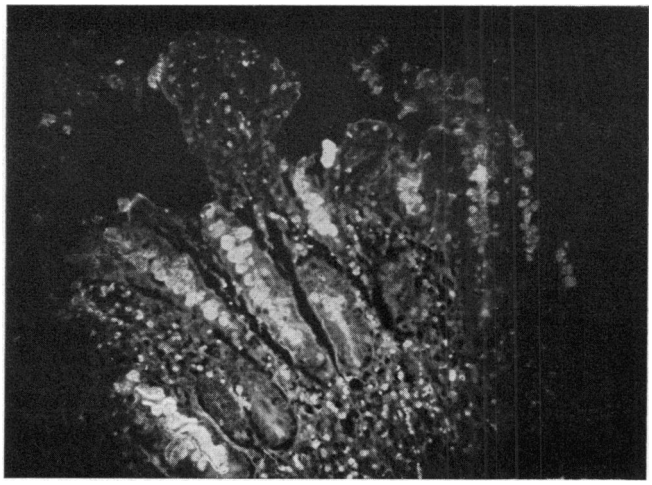

FIG. 2.—Paraffin section of gastric biopsy (Case 14, Table II) treated with anti-intestinal serum. Specific fluorescent staining of areas of intestinal metaplasia. (×75.)

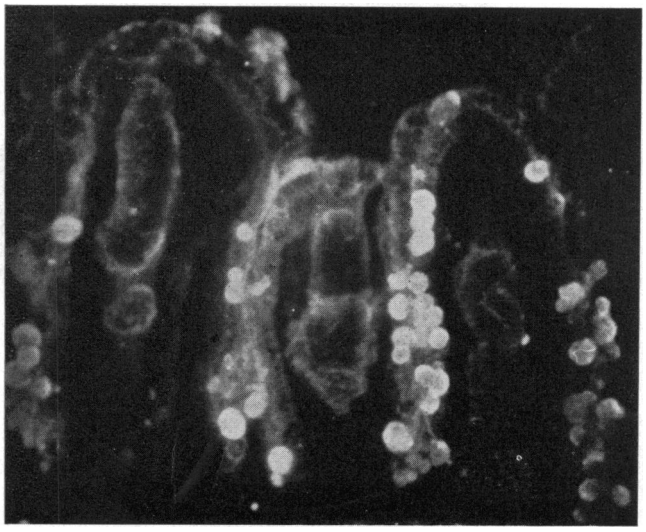

FIG. 3.—Fresh frozen section of non-cancerous areas of stomach of patient with gastric cancer (Case 34, Table III). Anti-intestinal serum. Specific fluorescent staining of areas of intestinal metaplasia. (×90.)

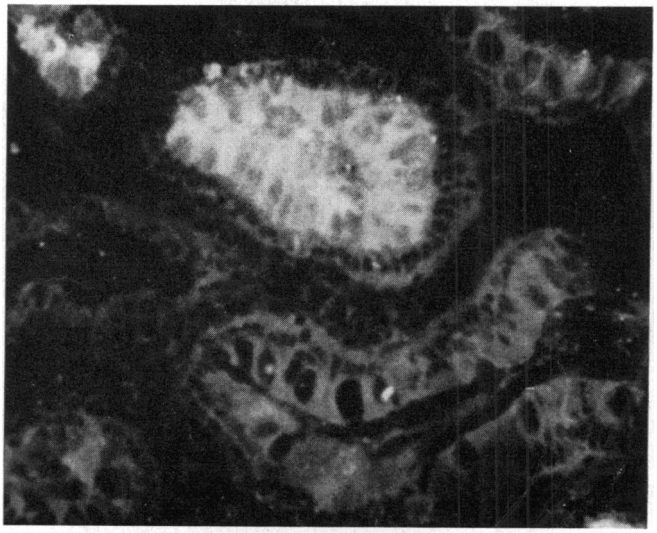

FIG. 4.—Fresh frozen section of non-cancerous area of stomach of same patient as in Fig. 3. Antigastric serum. Specific fluorescent staining of gastric mucous epithelium and depletion of the gastric antigen in areas of intestinal metaplasia. (×150.)

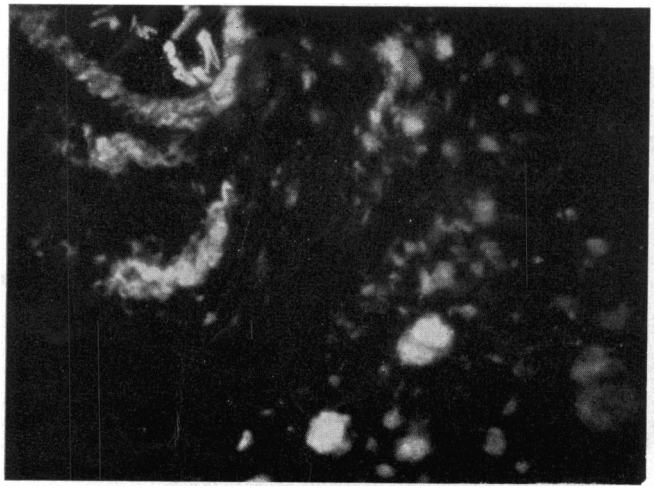

FIG. 5.—Fresh frozen section of cancerous area of stomach of same patient as in Fig. 3. Anti-intestinal serum. Specific fluorescent staining of cancer cells which have invaded stomach wall. Bright autofluorescence of elastic tissue in artery, top left. (×150.)

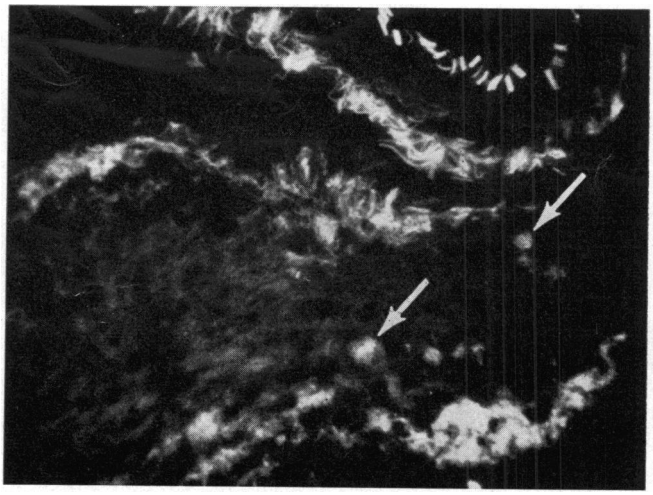

FIG. 6.—Fresh frozen section of stomach of same patient as in Fig. 3. Antigastric serum. Cancer has invaded vein in stomach wall. Only a few cells (arrows) show fluorescent staining; the majority are depleted of gastric antigen. Bright autofluorescence of elastic tissue in artery, top right. (×225.)

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