# STUDY OF STROMA
# IN SCIRRHOUS GASTRIC CARCINOMA

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## ABSTRACT

Rabbits were immunized with the supernatant of a homogenate of non-cancerous gastric mucosa to obtain an antiserum. This antiserum, after absorption by human jejunal mucosa and liver tissue, produced precipitin lines with the supernatants of homogenates of gastric carcinoma and gastric mucosa. Fluorescent staining was attempted using this antiserum. Well-stained cytoplasms were observed in cells of tissue imprints of normal gastric mucosa and gastric carcinoma. When frozen sections of peroperatively extirpated materials were likewise stained, the cells and the lumen of the glands were stained to a large extent in the non-cancerous gastric mucosa and tubulary adenocarcinoma of the non-scirrhous type. In scirrhous gastric carcinoma fluorescence-stained substances were scantly in the lumen of the glands but observable to some degree in the tumor stroma. It is suggested that infiltration of the interstitium by glandular antigens induces proliferation of stroma in gastric carcinoma.

Keywords: Scirrhous carcinoma, Tumor stroma

## INTRODUCTION

There is a variety of proliferating modes of gastric carcinoma.[1,2,3,4] Among these, the General Rules for the Gastric Cancer Study in Surgery and Pathology prepared by the Japanese Research Society for Gastric Cancer includes so-called linitis plastica and type IV Bormann classification of the scirrhous type.[5] This scirrhous type, therefore, is assumed to be the general category for all carcinoma showing histologically rich interstitia.[5,6]

The etiology of the proliferation of interstitial connective tissue in scirrhous carcinoma is unknown,[1,4,7] but the connective tissue of the tumor stroma seemingly influences the tumor growth.[8] We made a hypothesis that carcinoma cell products infiltrating to the interstitia may induce stromal reaction such as scirrhous type whether the products are specific or non-specific to malignant cells. We used an immunofluorescent staining procedure in an attempt at clarification. The antiserum we used was presumed to be reactive against products commonly secreted from both cancerous and non-cancerous gastric epithelial cells. Substances which relate to scirrhous carcinoma were discussed with reference to the observation of interactions between such cellular secretions and the interstitial connective tissue.

## MATERIALS AND METHODS

*Preparation of antigen*

Gastric mucosa which seemed to be normal and which had been obtained during surgery for duodenal ulcer was used for the preparation of the antigen. The specimen was finely cut with a pair of scissors and homogenized in a micro-homogenizer at a ratio of 2 g. of tissue to 5 ml. of phosphate-buffered saline. After centrifugation at 1800 g for 15 minutes, the supernatant was used as the antigen.[9] Similar preparations were made from the tissue of gastric carcinoma and jejunal mucosa. One g. of lyophilized extract was reconstituted to 10 ml. in phosphate-buffered saline before use as an antigen.

*Preparation of antibody*

The antigen preparation from gastric mucosa was then emulsified with Freund's complete adjuvant. The antigen (0.25 ml) was injected at each of 4 sites in rabbit foot-pads and subcutaneous tissue, and the same antigen emulsion was injected repeatedly at 2-week intervals for 2 months. The rabbit blood was collected 1 week after the booster shot to obtain the antiserum. This antiserum was absorbed by the human blood cells (AB type), the human liver tissue, and the human jejunal mucosa, and finally saturated with a 30% ammonium sulfate solution to obtain a γ-globulin-rich preparation in PBS. The preparation was dialized in phosphate buffered saline and applied to 2 X 30 cm. columns containing Sephadex G-25.[11] Final protein concentration was adjusted to 30 mg/ml, and the protein preparation was stored at −80°.

*Immunofluorescent technique*

The gastric carcinoma tissue and the non-cancerous gastric mucosa extracted peroperatively were immediately frozen at −20°C. Tissue sections 5 μm in thickness were prepared by the use of a cryostat and submitted for indirect immunofluorescence staining as follows. Anti-gastric mucosa protein fraction (diluted 10 times in phosphate buffered saline) was placed on the acetone-fixed sections. After incubation for 1 hour at 37°C in 100% humidity, the sections were rinsed for 20 minutes in phosphate buffered saline. They were then stained by isothiocyanate-labeled goat immunoglobulin fraction against rabbit γ-globulins (available from Hoechst A.G.) by incubation for 1 hour at 37°C. After rinsing for 20 minutes in phosphate buffered saline, they were studied under a fluorescent microscope (Chiyoda Kogaku, Tokyo).

All specimens obtained from 28 patients were stained by the above antiserum. Of the 28 patients, 4 were cases of gastric ulcer, 10 of non-scirrhous gastric carcinoma, 8 of scirrhous gastric carcinoma, 3 of early stage gastric carcinoma with ulceration of the epithelium, and the remaining 3 of colon carcinoma.

## RESULTS

As illustrated in Fig. 1, the antiserum to gastric mucosa, before absorption by jejunal mucosa and red blood cell, produced multiple precipitin lines against preparations from gastric carcinoma, gastric mucosa, jejunal mucosa, gastric juice and human serum. Precipitin lines of gastric carcinoma (in the top well) were contiguous with those of gastric mucosa (in the right upper well). After absorption by jejunal mucosa, the antiserum produced two precipitin lines each, with gastric mucosa and gastric carcinoma (Fig. 2).

Fig. 3 illustrates a fluorescence-stained specimen of gastric carcinoma cells and non-cancerous gastric epithelial cells. The cytoplasm is well stained in both types of cells.
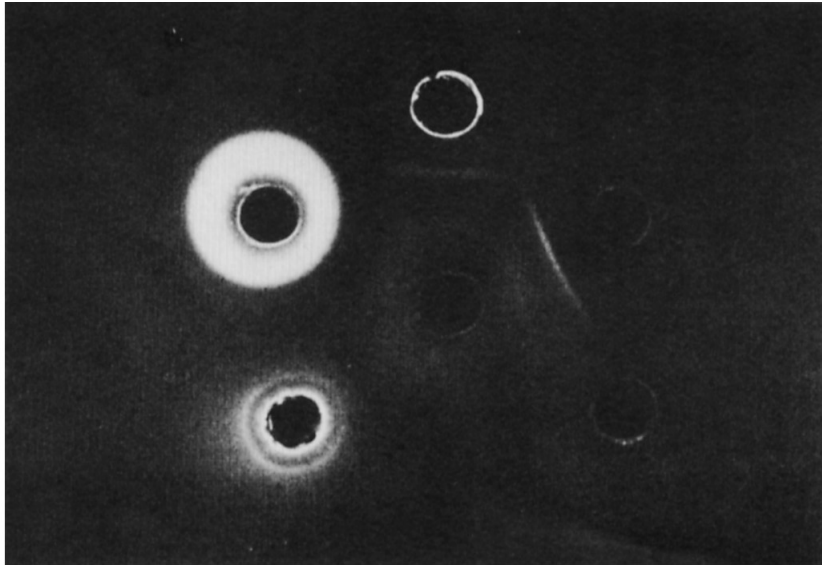
Fig. 1  In the central well, antiserum against gastric mucosa after absorption by human liver tissue was placed. The preparation from gastric carcinoma was placed in the top well, that from gastric mucosa in the right upper well, that from jejunal mucosa in the right lower well, that from gastric juice in the bottom and left lower wells and that from human 0 type serum in the left upper well.
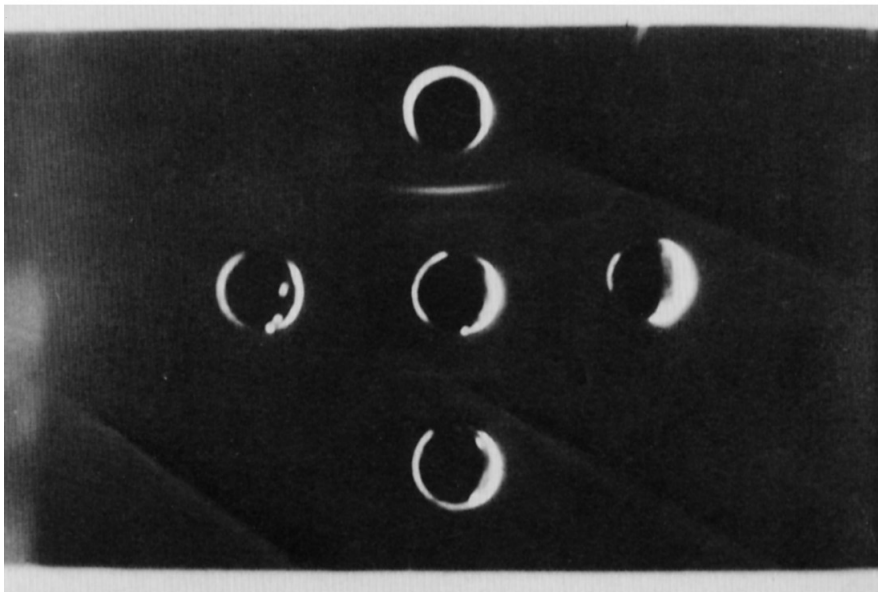


Fig. 2  In the central well, antiserum against gastric mucosa after absorption by human liver tissue, AB type red blood cells and jejunal mucosa was placed. The preparation from gastric mucosa was placed in the top well, that from jejunal mucosa in the right well, that from gastric carcinoma in the bottom well and that from gastric juice in the left well.
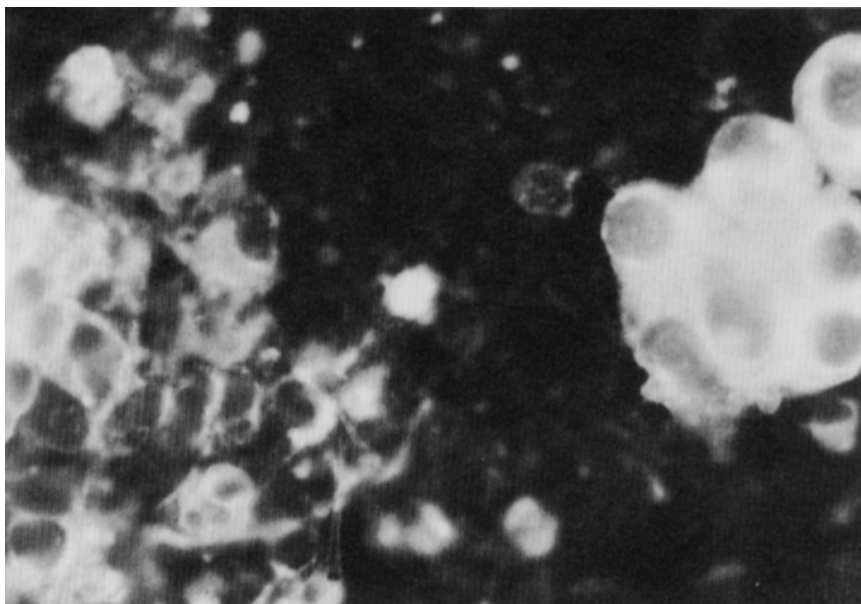
Fig. 3    Fluorescence-stained tissue imprint of gastric carcinoma (right side) and non-malignant gastric
          epithelial cells (left side), ×1000.

Fig. 4 depicts a fluorescence-stained specimen from the gastric mucosal epithelium. The epithelial cells are moderately stained; and the lumens of the glands are well stained. Fig. 5 is fluorescence-stained non-malignant epithelium showing metaplasia. All of the tested non-malignant epithelial cells and glandular lumens were well stained by this immunoglobulin fraction.

Fig. 6 illustrates a fluorescence-stained specimen of non-scirrhous tubulary adenocarcinoma. Stained substances are dominant in the cytoplasm and in the lumen of the glands.

Fig. 7 represents a hematoxylin-eosin-stained specimen of interstitium-rich carcinoma with dissociated glandular formation. Fig. 8 illustrates the same specimen and almost the same area as Fig. 7. The tumor stroma shows a large amount of hyalinized connective tissue. The stained substances are visible in the cytoplasms and to some degree in the stroma as well. Fig. 9 is a fluorescence-stained specimen from the same material, but the area illustrated represents a rather small amount of tumor stroma with less hyalinization taken from the lesion adjacent to that shown in Figs. 7 & 8. This is characterized not only by the stained cells but also the lumen of the glands. Fig. 10 illustrates an ulcerated area of early stage gastric carcinoma. Scattered malignant cells and, seemingly, their products are stained. No such stainability was observed in the benign ulcer nor in non-scirrhous carcinoma without ulceration.

As controls, non-scirrhous gastric carcinoma was stained only by fluorescence-labeled goat anti-rabbit γ-globulin serum as illustrated in Fig. 11. No stainability was observed in the cytoplasm or in the lumen of the glands, which contrasted well with Fig. 6. Scirrhous carcinoma (Figs. 8 & 9) was stained by fluorescence-labeled anti-rabbit immunoglobulin after staining with non-labeled anti-gastric mucosa immunoglobulin fraction which was absorbed by gastric mucosa preparation (Fig. 12), or by fluorescence-labeled anti-gastric mucosa γ-globulin after absorption by anti-gastric mucosa γ-globulin (Fig. 13). The interstitia were not stained as shown in Fig. 12 & 13.
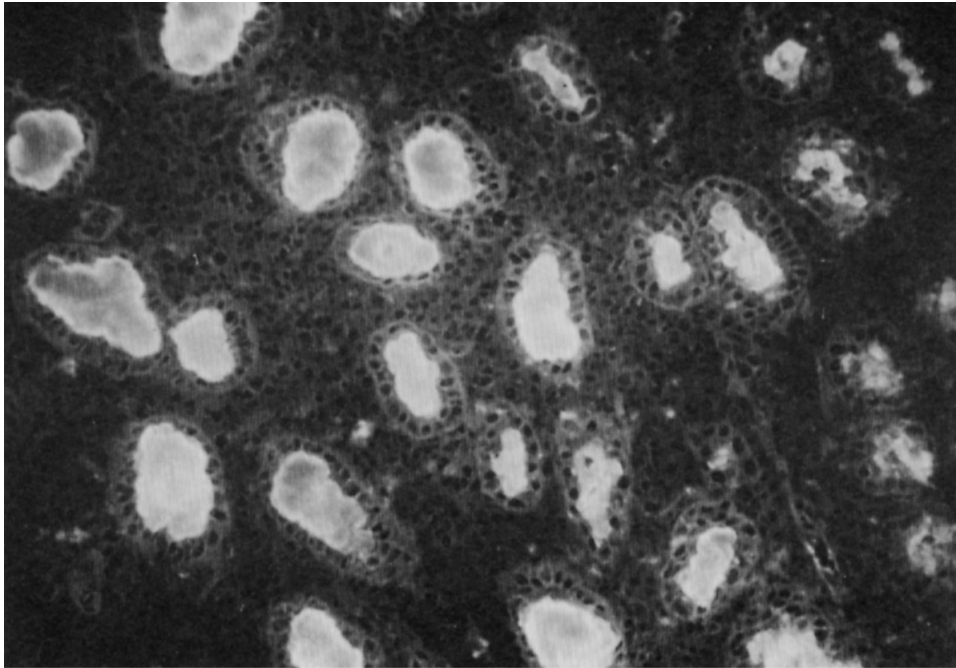
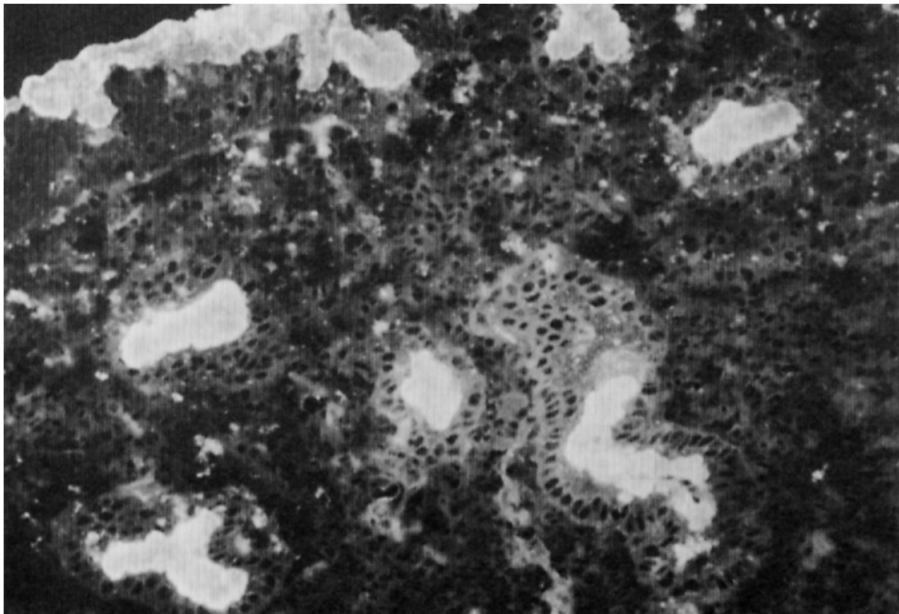Fig. 4  Fluorescence-stained gastric mucosa taken from the non-malignant patients, ×250.



Fig. 5  Fluorescence-stained non-malignant gastric mucosa with metaplasia, specimen taken from epithelium adjacent to carcinoma lesion, ×250.
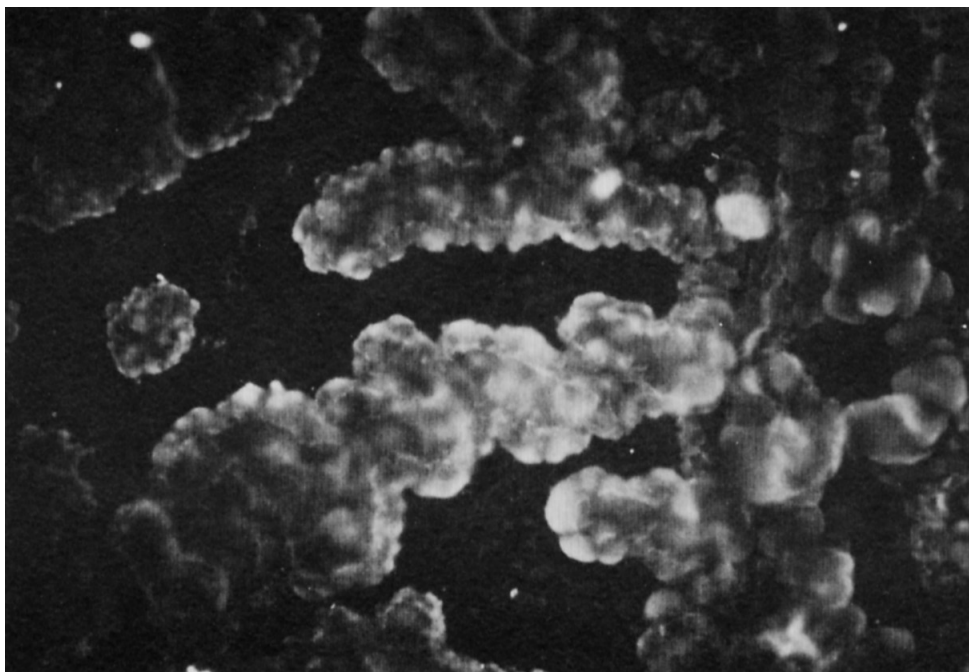
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Fig. 6    Fluorescence-stained non-scirrhous tubular adenocarcinoma, ×500.
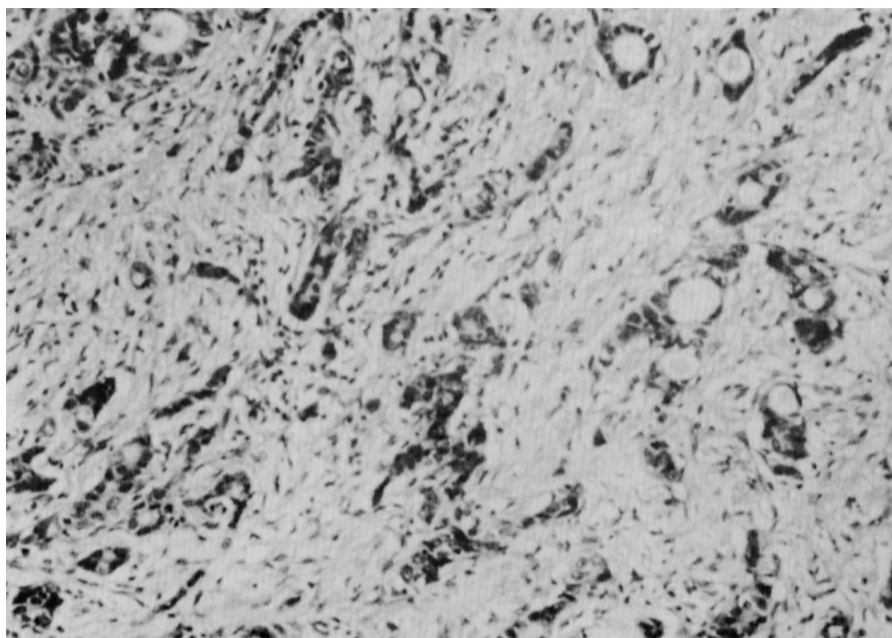


Fig. 7    Markedly dissociated adenocarcinoma with rich interstitium, H.E. stain, ×250.
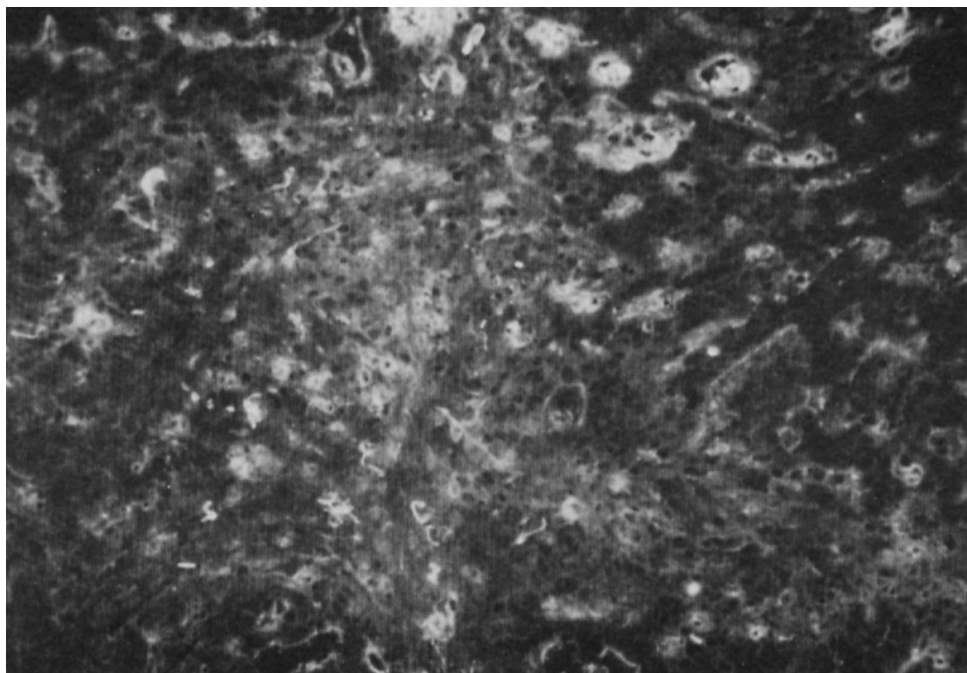
Fig. 8 Fluorescence staining of a lesion similar to that shown in Fig. 7, ×250.



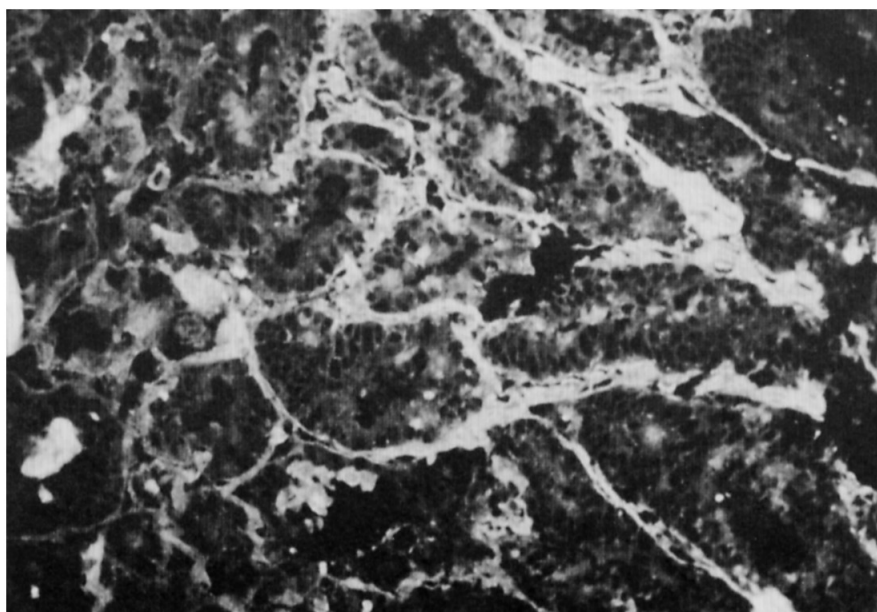Fig. 9 Fluorescence staining of the lesion adjacent to the area of Figs. 7 & 8. The glands are moderately dissociated with rather small amount of interstitium, ×250.
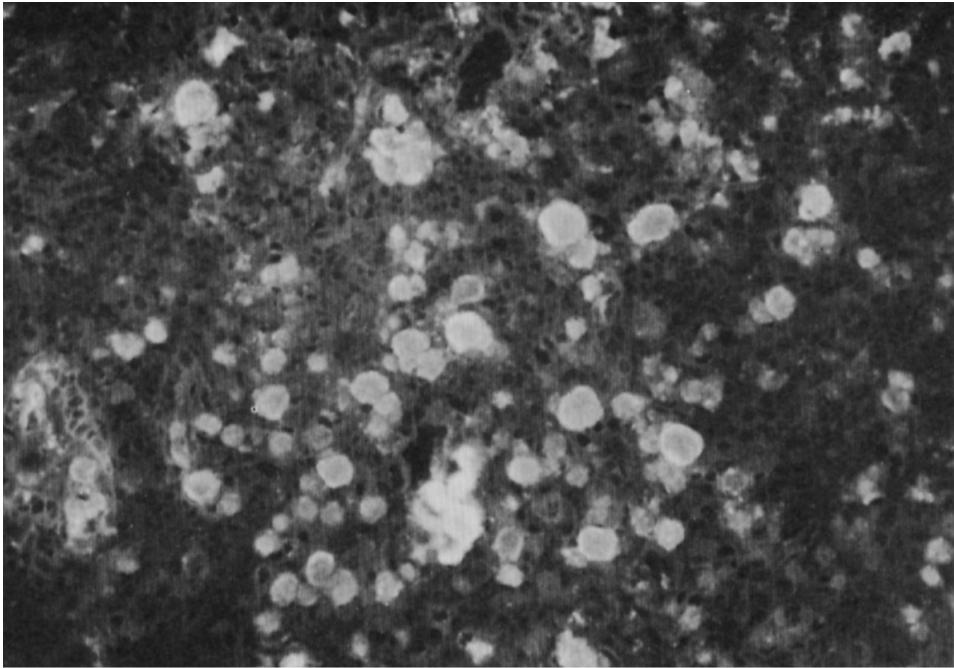
Fig. 10    Superficial ulcer of early gastric carcinoma. Scattered cancer cells and their products in the stroma were visible. ×500.
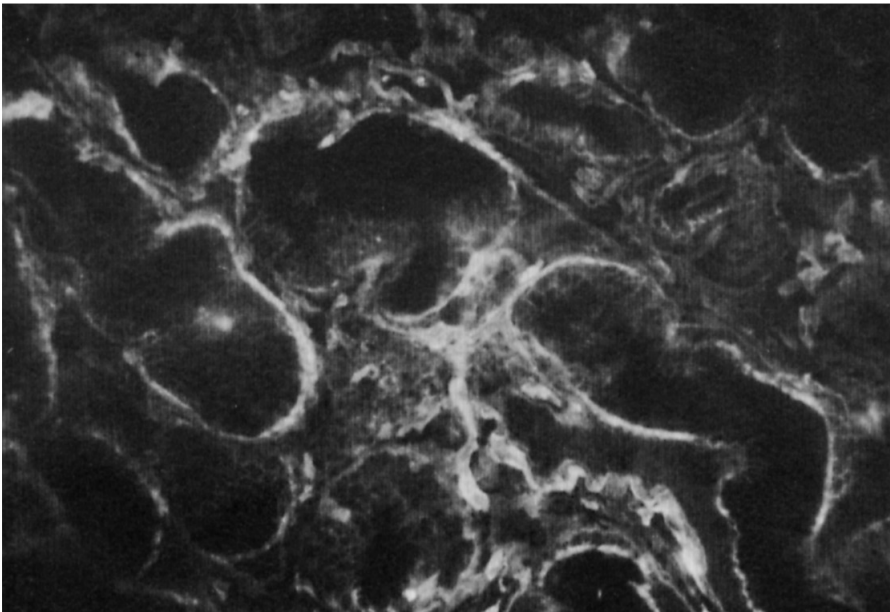


Fig. 11    Non-scirrhous gastric carcinoma stained directly by fluorescence-labeled goat anti-rabbit γ-globulin serum, ×500.
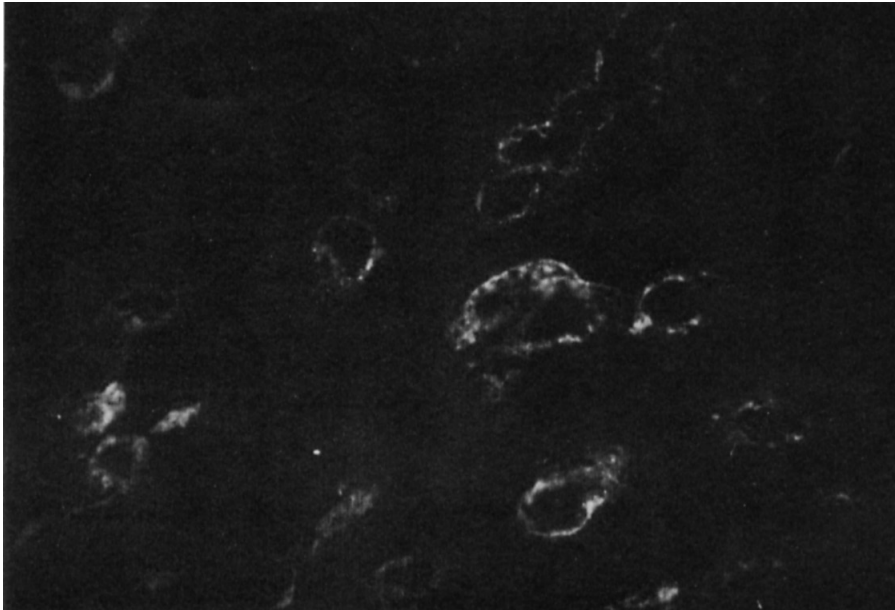
Fig. 12    Scirrhous type gastric carcinoma stained by immuno-fluorescence-labeled anti-rabbit immuno-
globulin after staining with non-labeled antigastric mucosa immunoglobulin fraction which
was absorbed by gastric mucosa preparation, ×250.



Fig. 13    Stained by immunofluorescence-labeled anti-gastric mucosa immunoglobulin fraction after
the absorption by non-labeled anti-gastric mucosa serum, ×250 (similar area with Fig. 9)

As shown in table 1, metastatic lesions of gastric carcinoma in the lymph nodes and in both malignant and non-malignant colon epithelial cells were positively stained with this antiserum. Other organs tested by this antiserum were negative for staining. Gastric mucosa of mice and rats were also not stainable.
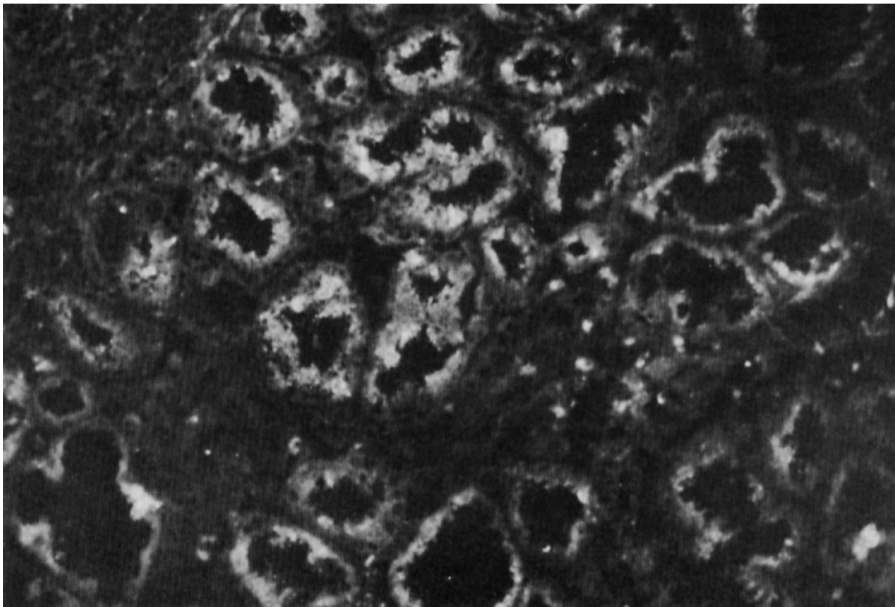
Table 2 shows the result of stainablility of the cells, the lumen of glands and the interstitia in 28 cases by the use of this immunoglobulin fraction. Gastric epithelial cells were mostly stained positive whether they were non-malignant or malignant. Colon epithelium of normal and malignancy were also more or less stainable. Glandular lumens were well stained except for the scirrhous type, while interstitia were stainable in the ulcerated area, where dissociated glandular arrangement was observed even at early stages, and in the scirrhous type. In both cases of medullary gastric carcinoma without glandular arrangement, well-stained substances were observed in the cells but not in the interstitia. In table 2, non-scirrhous gastric carcinoma included 2 cases of early carcinoma without formation of ulcer whose interstitia were not stained by this immunoglobulin fraction.

Table 1. Result of the immunofluorescent staining of various human organs and gastric mucosa of animals by the use of antihuman gastric mucosa γ-globulin fraction.

| Organ | Number of cases | Number showing grade of staining* | | |
|---|---|---|---|---|
| | | − | ± | + |
| esophagus | 3 | 3 | 0 | 0 |
| small intestine | 3 | 3 | 0 | 0 |
| large intestine | 4 | 2 | 2 | 0 |
| metastasis of gastric carcinoma to lymph node | 4 | 0 | 0 | 4 |
| metastasis of colon carcinoma to lymph node | 3 | 0 | 3 | 0 |
| lymph nodes | 4 | 4 | 0 | 0 |
| spleen | 3 | 3 | 0 | 0 |
| liver | 3 | 3 | 0 | 0 |
| pancreas | 2 | 2 | 0 | 0 |
| skin | 2 | 2 | 0 | 0 |
| gastric mucosa of rat | 3 | 3 | 0 | 0 |
| gastric mucosa of mouse | 3 | 3 | 0 | 0 |

* −: negative for stain  ±: positive but trace for stain  +: definitly positive for stain

Tissue from organs was obtained during surgery. Gastric mucosa of rat and mouse was stained shortly after excizing under anesthesia by ether.

Table 2. Grade of stain in various alimental lesions by the use of anti-human gastric mucosa γ-globulin fraction.

| Case | Number of cases | Cell | | | Glandular lumen | | | Stroma | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | − | + | ++ | − | + | ++ | − | + | ++ |
| gastric ulcer (epithelim taken apart from ulcer) | 4 | 0 | 0 | 4 | 0 | 0 | 4 | 4 | 0 | 0 |
| gastric ulcer (lesion of ulcer without epithelization) | 4 | | — | | | — | | | — | |
| non-scirrhous gastric carcinoma | 8 | 0 | 3 | 5 | 0 | 2 | 6 | 7 | 1 | 0 |
| medullary gastric carcinoma without glandular formation | 2 | 0 | 1 | 1 | | — | | 2 | 0 | 0 |
| scirrhous gastric carcinoma | 8 | 0 | 2 | 6 | 3 | 5 | 0 | 0 | 4 | 4 |
| early gastric carcinoma with ulceration | 3 | 0 | 1 | 2 | | — | | 0 | 3 | 0 |
| colon carcinoma | 3 | 2 | 1 | 0 | 1 | 2 | 0 | 3 | 0 | 0 |

−: negative for stain  +: moderately positive  ++: strongly positive

Figure indicates number of cases.

# DISCUSSION

As observed in Figs. 1 & 2, this immunoblobulin fraction, after its absorption by the human liver tissue, produced multiple precipitin lines with the preparations from gastric carcinoma, jejunal mucosa and gastric juice. Since precipitin lines are only observed with the preparations from gastric carcinoma, gastric mucosa and gastric juice after absorption by jejunal mucosa and are contiguous with each other, they are seemingly specific to cell components and products of the gastric epithelium regardless of whether these are normal or malignant. However, other antibodies which are not detectable by the precipitin test but which are sensitive to immunofluorescent staining may be presented in this fraction. This immunoglobulin fraction gave positive immuno-fluorescence staining to cells and their products of gastric origin but not to those of other organs nor to heterologous organs, the only exception being colon epithelium which produced a more or less cross reaction with this fraction. These results strongly suggest that this fraction is specific to gastrointestinal natures. It is well known that malignant cells and their products keep organ-specific antigenicities although these may decrease during malignant transformation.[11] Recently, many monoclonal antibodies have been detected against normal and non-malignant cells, and most of them are against organ or tissue-specific antigens or stage-specific embryonic antigens.[12,13,14,15] Even though these monoclonal antibodies produce positive staining in the stroma, the reacting antigens can not be the inducers of stromal reaction. The immuno-globulin fraction used in this study was not monospecific; however, the object of observation is whether or not infiltration of cell products induces the proliferation of interstitial connective tissue in gastric carcinoma.

It is characteristic that fluorescence-stained location in scirrhous carcinoma was specific compared to other types of gastric carcinoma and benign epithelium. In scirrhous carcinoma, there was fluorescent staining in the stroma and slight staining in the lumen of the glands. This suggested that infiltration of the interstitium by glandular antigens occurred. This may be explained by the fact that dissociated glands no longer communicate with the viscus cavity.[4,8] In scirrhous gastric carcinoma, signet cells are frequently observed at the mucosal layer. However, the type of cell at the mucosal layer is not always the same as that observed in the submucosal layer. This

seemingly explains why the microscopic cell type is not specific to scirrhous carcinoma. In early gastric carcinoma, on the other hand, signet ring cells are frequently observed in case of the ulcerforming type.[5,16] There is speculation that early gastric carcinoma of ulcer-forming type may develop into scirrhous gastric carcinoma.[16] The results showing that immunofluorescence staining was well visualized in the stroma of scirrhous carcinoma and in the ulcerating area of early gastric carcinoma strongly suggested that these infiltrating substances induce scirrhous type gastric carcinoma.

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