Distribution of Gastrin in Human Digestive Organs Demonstrated by Direct Immunofluorescence*

Takayoshi Tobe, Kimio Henmi, Kozo Fukuchi, Shin-Tse Chen1) and Shinro Tachibana2)

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Summary. 1. Antiserum was prepared in the rabbit against synthetic human gastrin I conjugated with bovine serum albumin. The IgG fraction of this specific antiserum was labelled with FITC (fluoresceine isothiocyanate). With a direct immunofluorescence technique the distribution of gastrin in the human digestive tract and associated glands was investigated with special reference to extragastric gastrin.

2. Gastrin-containing cells were numerous in the antral mucosa of the human stomach especially in the prepyloric region (2-4 cm proximal to the pylorus). In the body and cardia of the stomach no gastrin-containing cells were seen.

3. Some gastrin-containing cells were seen in the duodenum but fewer than in the antrum and they decreased distally.

4. No gastrin-containing cells were seen in the esophagus, jejunum, ileum and colon.

5. In the pancreas numerous autofluorescent granules were seen, but no specific gastrin-containing cells. Nor could these cells be found in the parotid and submandibular glands.

6. The release of gastrin after immersion in 0.5 M glycine solution was investigated. A slight decrease of the specifically fluorescent substance could be seen. A complete release of the substance followed freezing in an isopentane bath in aqueous nitrogen.

The existence of gastrin was first reported in 1906 by Edkins, about 7 decades ago. Occurrence and structures of two gastrins, gastrin I and II were elucidated by Gregory and Tracy in the early 1960’s. More recently, gastrin, along with other hormones of the gastro-entero-pancreatic (GEP) endocrine system (Fujita and Kobayashi, 1973) has been attracting attention for the following reasons:

1. As gastrin (1964) and secretin (1967) came to be synthesized and other polypeptides could be purified, the physiological effects of these polypeptide hormones as pure or nearly pure substances came to be tested.

2. About 10 kinds of GEP endocrine cells were found electron microscopically (for reference see Solcia et al., 1973; Sasagawa et al., 1973), one of which was named G cell and proposed to be the source of gastrin (Solcia, Vassallo and Capella, 1969), whereas others were connected with secretin and other hormones.

3. Specific antiserum to gastrin (pure synthetic human gastrin) was obtained by McGuigan (1968a), and the serum gastrin level became measurable by radioimmunoassay (McGuigan and Trudeau, 1970). The distribution of gastrin in the human gastro-intestinal tract then could be visualized at the cellular level by immunohistochemical techniques (McGuigan, 1968b; McGuigan and Greider, 1971; Pearse and Buussolati, 1970).

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Although it has been established both by immunofluorescence and electron microscopy that the gastrin is produced by G cells which are found concentrated in the gastric antrum and pylorus, the occurrence of gastrin and the G cell in other parts of the digestive tract and in some digestive glands has caused much controversy. It is widely acknowledged that gastrin is contained in the duodenum (Elwin and Uvnäs, 1966; Grossman, 1970) but our knowledge of the extragastric distribution of the G cells has been very poor. The occurrence of gastrin in the D cells in the pancreas has been once widely accepted (see for instance the review by Hellman and Taljedal, 1972) but recently disputed (Bussolati and Canese, 1972; Solcia et al., 1973). Some authors proposed that salivary glands contain gastrin (Takeuchi et al., 1973) but it has neither been confirmed nor connected with a cytological study.

This paper describes immunofluorescence studies on the distribution of gastrin in different portions of the human digestive tract, pancreas and salivary glands.

**Material and Method**

Stomach tissue was obtained from 15 patients with gastric carcinoma, 15 with peptic ulcer and 4 with benign adenoma or polyp, who were treated by gastrectomy.

Sections of the antrum were taken just after gastrectomy and distribution of gastrin in the tissue was examined by direct immunofluorescence.

The distribution of gastrin in the normal stomach (antrum, body, cardia), duodenum, jejunum, ileum, colon, rectum, pancreas, parotid gland and submandibular gland was studied by the same method in patients without gastric diseases.

To determine the release of gastrin by glycine, a piece of the antrum, which had been immersed in 0.5 M glycine solution for 30 min, was also studied by direct immunofluorescence. Frozen section was studied by the same technique, too.

**Immunofluorescence**

Direct immunofluorescence technique was applied to formalin-fixed and paraffin-embedded tissues. Specific antiserum to synthetic human gastrin I was obtained by a modification of McGuigan's method. The IgG fraction of this antiserum was separated and labelled with FITC by the following procedure:

**Preparation of antigen**

Synthetic human gastrin I (SHG) (Imperial Chemical Industries Ltd.) was coupled with bovine serum albumin (Sigma Chemical Co.) by a modification of McGuigan's (1968a) method. Two mg of bovine serum albumin were dissolved in 2 ml of 0.1 M potassium phosphate buffer, pH 7.4, containing 1 ml of N, N-dimethylformamide. To this solution 2 mg of synthetic human gastrin were added. After cooling to 0°C, 2 mg of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide were added three times at intervals of 30 min.

If necessary, the pH was adjusted to 7.4 with 0.01 N hydrochloric acid, then the mixture was allowed to stand overnight at 0°C. To this reaction mixture 5 mg more of carbodiimide were added and stirred at room temperature for 4 hrs, after which it was dialyzed at 4°C for 24 hrs against 5l of 0.01 M potassium phosphate buffer containing 0.15 M sodium chloride. The resultant slightly opalescent solution was dialyzed against 5l of distilled water at 4°C for 12 hrs two times. Following dialysis, it yielded 3.2 mg after lyophilization.
**Immunization of the rabbit**

Two mg of SHG I-bovine serum albumin conjugate in phosphate buffer solution (pH 7.4) was mixed well with complete adjuvant (2 ml) until it was completely emulsified. This emulsion was injected into the footpad of the rabbit. Two months later SHG-bovine serum albumin conjugate (1 mg) in phosphate buffer solution was injected intravenously as a booster.

Fourteen days after this booster injection a small amount of serum was evaluated by radioimmunoassay and the Ouchterlony agar gel double diffusion test. After the test evidenced the formation of a specific antibody to SHG (Fig. 1), whole blood was drawn by cardiac puncture.

**Preparation of FITC labeled anti-gastrin antibody**

Anti-serum to SHG I was precipitated at 4°C by dropwise addition of saturated ammonium sulfate. Repeatedly dialyzed precipitate in buffer was conjugated with FITC (Baltimore Biological Laboratories) at 4°C for 6 hrs during constant stirring with a magnetic stirrer. After dialysis, it was applied to a DEAE cellulose column, eluted with 0.05 M phosphate buffer (pH 6.3) and collected as fraction No. 1. Its protein concentration was 4.8 mg/ml and its FP ratio was 1.4.

**Direct immunofluorescence technique**

Antral mucosa and other tissues were taken immediately after surgery, and small pieces were fixed for 12 hrs in cold 10% formaldehyde to be embedded in paraffin.

Paraffin-embedded blocks were sliced and after deparaffinization the sections were washed well with a staining buffer solution (pH 7.4).

Specific anti-gastrin antibody containing globulin labeled with FITC obtained by the above method was layered over the sections for 12 hrs. After thorough washing with staining buffer solution, the sections were covered with a cover slide and studied by fluorescence microscopy (Carl Zeiss).

The specificity of the staining was examined by the blocking test with SHG solution.
Fig. 2. Gastrin-containing cells in the antrum of the human stomach. ×160
Fig. 3. Gastrin-containing cells in the prepyloric region of the human stomach, where their population is highest. A case of cancer patient. ×160
Results

Distribution of Gastrin-Containing Cells

Stomach

Gastrin-containing cells were numerous in the antrum of the human stomach (Fig. 2, 3) as McGUIGAN and other authors have already noted (see Discussion). They were dispersed in the cryptal epithelium and situated in the middle zone of the mucosal thickness.

They were most heavily concentrated in the middle portion (2-4 cm proximal to the pylorus) of the antrum and became sparser in the pylorus and beyond. They were pyramidal in shape, extending a long process to the gastric lumen. Gastrin fluorescence generally was more intense in the basal than in the apical cell part.

In the cardia or the body of the stomach there were no gastrin-containing cells.

Fig. 4. Gastrin-containing cells in the second portion of the human duodenum. ×160
**Duodenum**

In the human duodenum gastrin-containing cells could be found as shown in Figures 4 and 5.

The number of gastrin-containing cells in the human duodenum is definitely lower than in the antrum, and it becomes increasingly lower as the distance from the pylorus. In the third portion of the duodenum their population was very low (Fig. 5).

**Esophagus, jejunum, ileum, colon and rectum**

No gastrin-containing cells could be identified in the esophagus (Fig. 6), jejunum (Fig. 7), ileum, colon or rectum.
Pancreas, parotid and submandibular glands

Careful studies were done on fresh specimens of surgically removed pancreas, parotid gland and submandibular gland. Though numerous non-specific autofluorescent granules were demonstrated by fluorescence microscopy, gastrin-containing cells showing specific reaction could not be seen in our study (Fig. 8–10).

The distribution of the gastrin-containing cells in the human digestive tract and glands as revealed by this study is summarized in Figure 11.

Release of Gastrin by Freezing or by 0.5 M Glycine Solution

Glycine solution (0.5 or 0.3 M) is often used to evaluate the response of endogenous gastrin release (McGuigan and Trudeau, 1970; Matsuo, 1973). A piece of antral mucosa taken just after gastrectomy was immersed in 0.5 M glycine solution for 30 min and then fixed in routine formalin solution.

Gastrin fluorescence in antral tissue tended to decrease after immersion in glycine
solution, though not remarkably. On the other hand, complete release of gastrin from gastrin-containing cells of the antral mucosa was noted after freezing in an isopentane flask in an aqueous nitrogen bath or in an acetone dry ice bath (−30° to −70°C).

**Discussion**

The polypeptide and aminic hormones in the GEP endocrine system play important roles in the secretion and motility of the mammalian digestive system. Gastrin is one of the most important endocrine hormones in relation to stomach surgery. The roles of gastrin in the pathophysiological conditions before and after gastrectomy have gradually been elucidated. The distribution of gastrin in the human gastrointestinal tract has been described by McGUIGAN (1968) and by PEARSE and BUSSOLATI (1970a) using the immunofluorescence method.

There are three difficult problems in the immunofluorescence demonstration of
68 T. TOBE et al.: gastrin: 1) The molecular weight of gastrin is so low (gastrin I, 2096; gastrin II, 2176) that anti-gastrin antiserum can be obtained only when synthetic gastrin is conjugated with bovine serum albumin—a chemical problem. 2) Rabbits can be immunized only after long treatment with this gastrin-serum albumin conjugate—an immunological problem. 3) As the anti-gastrin antiserum thus obtained is expensive and small in amount, many investigators have used an indirect immunofluorescence technique. To avoid non-specific staining caused by this method, a direct immunofluorescence technique should be used—an immunohistochemical problem.

In this study a direct immunofluorescence technique was used with the IgG fraction of the specific anti-SHG I antiserum.

In the human stomach gastrin-containing cells are numerous in the antral mucosa as has been demonstrated by previous immunofluorescence studies (McGUIGAN, 1968; PEARSE and BUSSOLATI, 1970a; McGUIGAN and GREIDER, 1971). The shape and location of these cells are as described in those papers. The richest distribution was shown

Fig. 8. Pancreas. No gastrin-containing cells can be seen in the human pancreas. ×160
in the present paper to be in the antral mucosa 2-4 cm from the pylorus. In the
stomach; their number decreased distally. These cells are believed to correspond to
the extragastric gastrin (see Korman et al., 1972) which have been known to occur in
the duodenum (Elwin and Uvnäs, 1966), especially in its first portion (Grossman, 1970).
Intestinal gastrin-containing cells have been shown in a few of the previous immuno-
histochemical and electron microscopic studies.

There are conflicting opinions about the existence of gastrin-containing cells in
the pancreas. In the Zollinger-Ellison syndrome a non B-cell islet tumor produces
gastrin or a gastrin-like substance. OmoLe et al. (1972) demonstrated gastrin activity
from the extracts of the mammalian pancreas, though many other researchers failed
to extract gastrin from a normal pancreas (see Fujita and Kobayashi, 1973). Greider
and McGuigan (1971), using immunofluorescence, reported the presence of gastrin-containing cells in the human pancreas. Lomsky et al. (1969) using an indirect immunofluorescence technique, also reported gastrin in mammalian islet cells. These authors believed that the gastrin cell corresponded to the D-cell, but evidence meanwhile has accumulated that the pancreatic D cell represents a cell type quite different from the antral G cell.

In the pancreas numerous autofluorescent granules were seen by fluorescence microscopy, but these could be eliminated by a blocking test. The specific direct immunofluorescence technique demonstrated no gastrin-containing cells in many fresh specimens removed by laparotomy in our study. The view reached by Solcia and others in a recent symposium held in Bologna (Solcia et al., 1973) agrees with our results in respect to the non-occurrence of gastrin cells in the pancreas.

In the parotid and submandibular glands occurrence of immunoreactive gastrin and immunofluorescence for gastrin were reported by Takeuchi et al. (1973). In these
salivary glands the same auto-
fluorescent granules as noted in
the pancreas were numerous
and could be eliminated by
the blocking test, whereas no
gastrin-containing cells could
be recognized. This finding
was also confirmed in our study
in dog salivary glands, which
will be reported elsewhere.

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Fig. 11. Distribution of gastrin in human digestive tract
demonstrated by direct immunofluorescence. ×160
5. グリシン（0.5 M）はガストリン遊離物質として知られているが、組織学的根拠では、ガストリン遊離は著明には認められない。一方液体チッソで冷却したインベンタン中で組織片を凍結させると、ガストリンの完全な遊離が認められる。

References


戸部隆吉
〒606 京都市左京区聖護院川原町
京都大学附属病院
第二外科教室

Dr. Takayoshi Tobe
2nd Surgical Department
Kyoto University Medical School
Kyoto, 606 Japan