ON PLASMA GASTRIN LEVELS, pH IN GASTRIC SECRETIONS, 
AND ULTRASTRUCTURAL OBSERVATIONS OF THE G CELLS 
OF THE PYLORIC MUCOSA UNDER SEVERAL 
EXPERIMENTAL CONDITIONS

T. Wada, A. Yachi, S. Sakamoto, H. Abe and T. Yabana  
1st Department of Internal Medicine (Director: Prof. T. Wada)  
Sapporo Medical College

Summary

Changes of plasma gastrin level following acetylcholine stimulation were determined using two-antibody technique in rat with and without atropine pre-treatment, and the results were compared with fluctuations of the gastric acid secretion and the ultrastructural findings of the antral G cell, simultaneously. Relations among these thus confirmed biochemically and electronmicroscopically were discussed.

The mechanism by which the release of gastrin takes place has been explained by the stimulus to the gastric mucosal receptor by ingested proteins, amino acids and alcohol, and by the distention of the stomach due to diet, which is then conveyed via vagal nerve to the gastrin producing cells. In relation to the above mechanism, it was reported that stimulation of the antral mucosa with acetylcholine (Ach) resulted in the release of gastrin\(^{1-3}\), and on the contrary, the blockade of gastrin release by atropine administration has also been reported\(^4\). These studies are, however, concerned with the indirect assumption of the mechanism of gastrin release through changes observable by determinations of gastric acid output in various conditions. The method of direct determination of blood gastrin level was not available until recently, when Walsh, Yalow and Berson\(^5\) for the first time established a radioimmunoassay system and reported a clinical observation of atropine effect upon blood gastrin levels. On the other hand, the nature of gastrin producing cells is extensively studied by Forssmann and Orci\(^6,7\), Solcia et al.\(^8\), Pearse and co-workers\(^9\), Fujita and co-workers\(^10\), and by Ogata\(^11\). These studies indicate that among four types of endocrine cells found in pyloric antrum, "G" cells (Solcia\(^8\)) definitely stand for the gastrin producing cells. Forssmann et al.\(^9\) studied the secretory cycle of this type of cells in cats through ultrastructural changes of these cells under fasting and under atropine and alcohol effects. However, none of these studies simultaneously dealt with blood gastrin levels, ultrastructural changes of G cells and gastric acid output. The present authors have determined the blood gastrin levels with the radioimmunoassay technique, and compared with the changes of gastric acid secretion, and at the same time observed the ultrastructural changes concurrent with the gastrin release and its blockade. These were studied in rats with stimulation with Ach and blockade with atropine. For the identification of endocrine cells in the pyloric antral region,

Key words: Plasma gastrin, Pyloric G cell, Gastric secretion.
the method reported by Sasagawa\textsuperscript{10} was followed, although the classifications of Solcia et al.\textsuperscript{8} and by Pearse et al.\textsuperscript{9} were also taken into consideration.

**Materials and methods**

**Animals:** Wister strain male rats weighing from 250 to 350 g were divided into four groups, each group consisting of two or three rats. After fasting for 18 hours, duodenal fistula was built up in each rat with the method reported by Ghosh\textsuperscript{12}. The Group I rats received Ach solution (1\%, 2 ml) through stomach tubes placed at antral stomach mucosa, and antral mucosal tissue materials were obtained at 5 minutes and 30 minutes, respectively, after the ingestion. The Group II rats received subcutaneous injections of atropine sulfate (0.1 mg/kg), and antral mucosal tissue materials were obtained after 1, 4 and 7 hours, respectively. The Group III rats received injections of atropine sulfate, and 4 minutes later, they received ingestions of Ach solution (1\%, 1.5 ml) into the antral region. Pyloric antral mucosal tissues were obtained at 15 minutes after the ingestion. Group IV consisted of untreated control rats.

**Electron microscopic observations:** Tissue materials were cut into ca. 1 mm cubes in cold 2.5\% glutaraldehyde-0.1 M cacodylate buffer, pH 7.2, and fixed in cold (4℃) 4\% glutaraldehyde-0.1 M cacodylate buffer for 1 hour. The fixed materials were then rinsed in cold 0.1 M cacodylate buffer containing 0.14 M sucrose for 30 to 60 minutes, and fixed in cold (4℃) 2\% OsO\textsubscript{4}-0.1 M cacodylate buffer, pH 7.2, for 3 to 8 hours, followed by embedding in Epon 812.

**Radioimmunoassay of serum gastrin levels:** Guinea pig antiserum against synthetic human gastrin (Wilson Lab., 25,000X) was used in the radioimmunoassay as reported elsewhere\textsuperscript{10}, and blood gastrin levels were assayed with the two-antibody method.

**Determination of gastric acid secretion:** Gastric content of the rat was collected through the duodenal fistula while physiologic saline maintained at 37℃ was dripped at a constant rate through a small tube placed into the stomach via the esophagus. The acidity of the gastric content was continuously recorded with an automatic pH meter. The pH of the gastric irrigation fluid before stimulation with Ach was around 6.8.

**Results**

1) **Changes of blood gastrin levels in rats after stimulation with Ach.**

Results in 3 rats are shown in Table 1. In all animals, there was a rise in blood gastrin levels at 5 minutes after the administration of Ach. These elevated levels returned to the pre-treatment levels by 30 minutes after the stimulation. One of the

<table>
<thead>
<tr>
<th>Rat No.</th>
<th>Before</th>
<th>Post-Ach 2 min.</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>10</th>
<th>30</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>630 pg/ml</td>
<td>=</td>
<td>=</td>
<td>980</td>
<td>=</td>
<td>=</td>
<td>530</td>
</tr>
<tr>
<td>2</td>
<td>&lt;100</td>
<td>=</td>
<td>=</td>
<td>240</td>
<td>=</td>
<td>=</td>
<td>290</td>
</tr>
<tr>
<td>3</td>
<td>250</td>
<td>=</td>
<td>=</td>
<td>290</td>
<td>=</td>
<td>=</td>
<td>230</td>
</tr>
<tr>
<td>4</td>
<td>290</td>
<td>640</td>
<td>280</td>
<td>=</td>
<td>410</td>
<td>430</td>
<td>340</td>
</tr>
</tbody>
</table>

Table 1. Changes of blood gastrin levels before and after stimulation with Ach