Gastric Secretion in the Mouse

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ABSTRACT

OGAWA, T. AND H. NECHELES. Gastric secretion in the mouse. Am. J. Physiol. 194(2): 303-307. 1958.—The basal and stimulated gastric secretion of unanesthetized Holtzman substrain of Rockland RAP white Swiss male mice was investigated. Intubation of the stomach with a plastic tube yielded low values of secretion and acidity due to irritation and mucus production in the stomach. Simple ligation of the pylorus demonstrated that the mouse has a constant basal secretion. Histamine stimulated acid production only when employed in very large doses. Best results were obtained when 0.7 mg of histamine dihydrochloride/gm of body weight (70 mg/kg) was injected subcutaneously. Experiments with ligated pylorus of more than 6-hour duration demonstrated lower acidities, and 24-hour experiments showed no free acid, low total acidity and pH near neutrality.

In experiments with pyloric ligation in mice (the 'Shay mouse'), we have observed considerably lower incidence of ulcer formation (1) compared with that in the 'Shay rat' (2). This fact may be attributable to the rate of volume and acid secretion in the mouse, to effects of tension of the stretched stomach wall, or to technical difficulties. Since we were not able to find data in the literature on in vivo gastric secretion in this animal, the present study was undertaken. In preliminary experiments we encountered complications, such as considerable weight loss during preoperative fasting (especially by dehydration), coprophagy, trichophagy, sensitiveness to changes of room temperature and possible psychological effects. However, our final results indicate the degree and some of the mechanisms of gastric secretion in the mouse.

PROCEDURE

Unanesthetized adult, white male mice of the Holtzman substrain of the Rockland RAP Swiss strain were used, with preoperative weights ranging from 22 to 37 gm. The samples of gastric secretion were refrigerated overnight so that solids sedimented; then they were left at room temperature for about 1 hour, the supernatant was removed, and free and total acidity were determined, using dimethylaminoazobenzole and phenolphthalein as indicators and N/100 NaOH solution (in addition, pH was checked with Hydrion indicator). Pepsin was determined by the Anson and Mirsky method in specimens with free acidity. Histamine dihydrochloride or Mecholyl chloride was given subcutaneously, dissolved in 0.9% NaCl solution, 1 or 2 mg/ml.

Our first attempt was to collect gastric juice continuously during a 24-hour period by cannulation of the stomach. However, in these experiments the gastric juice showed surprisingly low acidity or even alkalinity. The presence of considerable amounts of mucus in most samples made us suspicious that the plastic tube might have irritated the gastric mucosa, thereby causing increased secretion of mucus and neutralization of acidity; large doses of histamine or Mecholyl failed to raise acid secretion. On the basis of this idea, the use of intubation was abandoned (suggested also by Dr. Horace Davenport).

A total of 141 mice were used, of which 82...
Table 1. Analysis of gastric juice obtained by pyloric ligation

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Hours of Secretion</th>
<th>Total Doses Histamine S.C.</th>
<th>No. of Animals</th>
<th>Body Weights* Preoperative</th>
<th>Volume*</th>
<th>pH*</th>
<th>Free HCl*</th>
<th>Total Acidity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>3</td>
<td>3</td>
<td>27</td>
<td>20.3 (23-36)</td>
<td>1.2</td>
<td>2.5</td>
<td>13.0</td>
<td>68.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.01-0.05</td>
<td>12</td>
<td>28.7 (27-34)</td>
<td>1.2</td>
<td>3.0</td>
<td>25.7</td>
<td>73.7</td>
</tr>
<tr>
<td>II</td>
<td>3</td>
<td>0.1-0.2</td>
<td>15</td>
<td>29.0 (25-37)</td>
<td>0.9</td>
<td>1.7</td>
<td>28.5</td>
<td>89.2</td>
</tr>
<tr>
<td>III</td>
<td>3</td>
<td>0.5</td>
<td>13</td>
<td>29.0 (27-36)</td>
<td>1.0</td>
<td>2.0</td>
<td>38.5</td>
<td>116.8</td>
</tr>
<tr>
<td>IV</td>
<td>3</td>
<td>1.0</td>
<td>15</td>
<td>20.2 (25-36)</td>
<td>1.1</td>
<td>4.4</td>
<td>66.3</td>
<td>116.8</td>
</tr>
<tr>
<td>V</td>
<td>3</td>
<td>1.0</td>
<td>13</td>
<td>28.4 (23 35)</td>
<td>0.8</td>
<td>1.6</td>
<td>58.9</td>
<td>104.8</td>
</tr>
<tr>
<td>VI</td>
<td>3</td>
<td>2.0</td>
<td>15</td>
<td>28.8 (25-33)</td>
<td>0.6</td>
<td>0.8</td>
<td>(17-116)</td>
<td>(56-160)</td>
</tr>
<tr>
<td>VII</td>
<td>3</td>
<td>3.0</td>
<td>15</td>
<td>28.8 (24-35)</td>
<td>1.0</td>
<td>2.4</td>
<td>(14-118)</td>
<td>(55-150)</td>
</tr>
<tr>
<td>VIII</td>
<td>6</td>
<td>0</td>
<td>12</td>
<td>20.1 (25-33)</td>
<td>1.5</td>
<td>2.1</td>
<td>40.7</td>
<td>70.6</td>
</tr>
<tr>
<td>IX</td>
<td>6</td>
<td>1.0</td>
<td>11</td>
<td>28.9 (24-35)</td>
<td>1.6</td>
<td>2.4</td>
<td>35.0</td>
<td>81.0</td>
</tr>
<tr>
<td>X</td>
<td>6</td>
<td>2.0</td>
<td>12</td>
<td>20.1 (25-33)</td>
<td>1.5</td>
<td>2.1</td>
<td>40.7</td>
<td>70.6</td>
</tr>
<tr>
<td>XI</td>
<td>6</td>
<td>3.0</td>
<td>10</td>
<td>20.1 (24-33)</td>
<td>1.8</td>
<td>1.4</td>
<td>68.9</td>
<td>108.6</td>
</tr>
</tbody>
</table>

* Averages. Variance in parentheses.

were fasted for 4 hours preoperatively and 59 for 15 hours. Under ether anesthesia, the pylorus was ligated without injuring its vessels, i.e. the same operation was performed as the one for the Shay mouse (1). The duration of the operation was less than 3 minutes and the mice were awake at the end of the operation. One milliliter of normal saline was given subcutaneously at the end of the operation. The animals were not restrained, but kept in separate cages at a room temperature of 28°C. Three hours after operation, the mice were killed with ether, the lower esophagus clamped, the stomach removed, and gastric contents collected by aspiration with a syringe. When the stomach contained excessive solids or blood, the sample was discarded. Satisfactory samples from 64 of 82 mice with 4-hour preoperative fasting and 44 of 59 with 15 hour fasting were collected. By this technique, acid gastric juice was obtained in most cases.

In order to study the effects of total pyloric obstruction on secretion, particularly of acid, in the presence of increasing distention of the stomach, experiments were done in the same manner as above, but with longer experimental periods: 6 hours in 65 cases and 24 hours in 49. In the latter group, only 23 mice survived for 24 hours, in 11 of which the samples were satisfactory; in the former, 45 satisfactory samples were obtained.

RESULTS

While in our preliminary experiments with intubation of the stomach very few animals had any free gastric acidity, the experiments with pyloric ligation and 3- and 6-hour secretion periods showed free acidity in most cases (see groups I and VIII in table 1). Histamine was effective in these experiments but only in large doses; with a total single dose of histamine up to 0.2 mg, no change in either volume or acidity was elicited (groups II, III); with doses of 0.5-1.0 mg of histamine, a slight increase in acidity was noted (groups IV, V). Increase in acidity of gastric juice, especially in concentration of free HCl, was definite when 2.0 or 3.0 mg of histamine was administered, 2.0 mg being most effective (groups VI, VII). These doses mean 0.07-0.1 mg/gm of body weight, or 70-100 mg/kg, which are extremely high compared with effective doses in the dog and even in the rat (3). Thus, histamine defi-
nitely increased acidity of gastric juice of the mouse, with the most effective dose of 0.07 mg/gm of body weight, whereas the volume of gastric secretion is not affected significantly (group VI).

Results of the 6-hour secretion period show some increase in volume, but no distinct change in acidity (group VIII) compared with the samples of the 3-hour study (group I). Administration of large doses of histamine (0.07-0.1 mg/gm of body weight) increased acidity of gastric juice but had no distinct effect on the volume of secretion (group X, XI). In one case of the 6-hour study, ulcers of the pro-stomach with perforation were observed.

Of 49 mice used for the 24-hour study period, 26 died before the period ended and in these, erosions or ulcers in the pro-stomach were found in 9 (in 2 of which perforation had occurred) and petechiae in 7. Of the 17 animals that lived until the end of the period, 8 had ulcers in the pro-stomach (4 of which had perforated) and 4 had multiple petechiae in the mucosa of the glandular stomach. We obtained only 11 satisfactory specimens in this study; most of the results were discarded because of mechanical break of the stomach at the pyloric ligature due to overdistention of the stomach. For these reasons, the results of the 24-hour secretion studies are not included in the table. Compared with the results obtained in shorter secretion periods, volumes were not appreciably greater, and acidities were considerably lower. Free acid was present in none of the samples, and no evidence of histamine effect was found. The same was seen in the samples from mice which died before the 24-hour collection period (12-20 hr.).

The above results suggest that secretion of the mouse stomach slows down and acid is neutralized when it is overdistended, probably a little over 6 hours after pyloric ligation, with 2-4 ml of gastric juice accumulated in it. In a few cases of the 6-hour study, alkaline gastric juice was obtained after adequate doses of histamine. These results were omitted from table 1, because we felt that in these animals a more rapid neutralization process had occurred than in the majority of the animals. We do not believe that reabsorption of acid by the gastric mucosa played a role in the production of low acidity.

In order to learn whether duration of preoperative fasting influences gastric secretion, results obtained from two groups of mice with 4- and 15-hour preoperative fasting periods, respectively, are compared in table 2. The animals received subcutaneous injections of saline solution after operation. No remarkable differences are seen, except a little larger volume of gastric juice in the mice with 15-hour than in those with 4-hour preoperative fasting periods (groups I, II). Group I lost little or no weight during the preoperative fasting period, but group II had lost several grams, due largely to dehydration. Effective doses of histamine decreased the volume of secretion slightly and increased acidity markedly. However, volume of secretion was similar in the 4- and 15-hour histamine groups (groups III, IV), although the 15-hour group had lost considerable weight during fasting. It may be presumed that in the mouse the rate of gastric secretion is not noticeably influenced by a mild degree of preoperative dehydration, differing from the dog (4) and the rat (5).

The concentration of pepsin was determined

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**Table 1.**

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Hours of Preoperative Fasting</th>
<th>Total Dose Histamine s.c.</th>
<th>No. of Animals</th>
<th>Body Weights, Preoperative</th>
<th>Volume*</th>
<th>pH*</th>
<th>Free HCl*</th>
<th>Total Acidity*</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>4</td>
<td>0</td>
<td>14</td>
<td>20.0 (27-33)</td>
<td>1.0 (0.3-1.8)</td>
<td>2.7 (1.3-5.0)</td>
<td>16.0 (0-52)</td>
<td>75.0 (14-130)</td>
</tr>
<tr>
<td>II</td>
<td>15</td>
<td>0</td>
<td>13</td>
<td>9.5 (23-36)</td>
<td>1.4 (0.4-2.8)</td>
<td>2.7 (1.0-6.3)</td>
<td>9.0 (0-58)</td>
<td>59.0 (18-100)</td>
</tr>
<tr>
<td>III</td>
<td>4</td>
<td>2-3</td>
<td>16</td>
<td>32.1 (24-35)</td>
<td>0.9 (0.5-1.4)</td>
<td>1.5 (0.8-2.7)</td>
<td>66.6 (14-118)</td>
<td>110.9 (55-150)</td>
</tr>
<tr>
<td>IV</td>
<td>15</td>
<td>2-3</td>
<td>10</td>
<td>26.5 (22-30)</td>
<td>0.9 (0.5-1.7)</td>
<td>1.6 (1.0-2.4)</td>
<td>56.3 (17-110)</td>
<td>105.7 (70-160)</td>
</tr>
</tbody>
</table>

* Averages. Variance in parentheses.
A direct relationship between the weight of the mouse and the volume of gastric juice was observed as Madden et al. (7) noted in the rat. Nelson (8) pointed out that rats deprived of food for 24 hours will drink less and become dehydrated. The same behavior was observed in our study on mice, in which water intake appeared to be reduced during fasting; they lost approximately 26% of their body weight in 24 hours, and 10% in 15 hours.

Friedman has described decrease in the rate of secretion with dehydration in the rat (5), but in the mouse this may not occur, at least if dehydration is not too severe. Mice seem to continue secreting gastric juice at the same rate after 15 hours of preliminary fasting as without preliminary fasting.

Wolf and Wolff (9) observed that in man, vigorous rubbing of the gastric mucosa increased the production of mucus but did not increase gastric secretion. On the other hand, Donald and Code (10) found approximately the same acidity in gastric juice of rats obtained by continuous drainage through a polyethylene tube introduced into the stomach as after pyloric ligation (5 and 7 hr.). Our attempt with the same technic in mice was unsuccessful because of apparent irritation and considerable increase in mucus secretion and neutralization of gastric acidity. The plastic tube may have been too much of an irritant for the delicate stomach of the mouse.

The only animals that have been found refractory to histamine are the elasmobranch fishes, the lower amphibia and the rat (3, 5, 6). According to Friedman (5), the rat does not respond to doses of histamine effective in the dog; only with large doses (3.5-5.5 mg/kg) acid secretion is increased and the response is not uniform. We have found that the mouse is even more refractory to histamine than the rat. Significantly increased concentration of gastric acid was seen only with very large doses (70-100 mg/kg) of histamine. Davenport and Jensen (11, 12) obtained similar results with _in vitro_ studies of mouse stomach. Friedman (5) assumes that the low degree of effectiveness of histamine in the rat may perhaps be related to the rapid rate of disappearance of injected histamine from the blood and relatively small amount retained by the stomach. This also seems to explain the fact that the rat has a high tolerance for histamine, and
GASTRIC SECRETION IN THE MOUSE 307

may be applicable even more in the case of the mouse. The rapidity of disappearance of histamine in these animals may possibly be accounted for by action of histaminase. Rose and Browne (13) determined concentration of histamine in various organs and blood of the rat after intravenous injection of histamine, and noted its rapid disappearance from the blood, relatively low concentration in the stomach, and long lasting high concentration in the kidney which contains no histaminase.

A slight tendency toward a decrease in concentration of acid as the duration of the tests increased was noted in the gastric juice of rats taken 3, 5 and 7 hours after pyloric ligation (10). In our study on the mouse, gastric juice continues to be secreted with steady acid concentration up to 6 hours after pyloric ligation, and thereafter acid is neutralized, and acid secretion appears to be suppressed.

In view of decreasing acidity in the stomach of the mouse beginning 6 hours after pyloric ligation, we wonder whether acid and pepsin are responsible for gastric ulceration in the Shay mouse, in which the optimal period of time for the production of ulcers (22 hr., see 1) does not coincide with high secretion of acid. Production of ulcer in the Shay mouse appears to correlate better with distention of the stomach which may interfere with circulation; consequently, acid secretion falls, acidity is neutralized, and necrobiosis by intracellular enzymes may produce ulceration in sufficiently devitalized areas.

REFERENCES