Experimental Production of Duodenal Ulcers in Rats with Corresponding Changes in Gastric Secretory Patterns

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EXPERIMENTAL PRODUCTION OF DUODENAL
ULCERS IN RATS WITH CORRESPONDING CHANGES
IN GASTRIC SECRETORY PATTERNS

by

James Ellis Dale

A Thesis
Submitted to the
Faculty of The Graduate College
in partial fulfillment
of the
Degree of Master of Arts

Western Michigan University
Kalamazoo, Michigan
August 1972
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My thanks go to Dr. André Robert of The Upjohn Company for all his time, assistance, encouragement and advice throughout my stay in his laboratory. My thanks also go to Dr. Jack Wood and Dr. Jean Lawrence, Department of Biology, Western Michigan University, and to Dr. Thomas M. Parkinson, The Upjohn Company, without whose assistance the opportunity to do research at The Upjohn Company would not have been made available. I would also like to express my appreciation for the many hours Mr. James E. Nezamis and Mr. J. Paul Phillips spent helping me complete my program of study.

James Ellis Dale
MASTERS THESIS

DALE, James Ellis
EXPERIMENTAL PRODUCTION OF DUODENAL ULCERS IN RATS WITH CORRESPONDING CHANGES IN GASTRIC SECRETORY PATTERNS.

Western Michigan University, M.A., 1972
Biology

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CHAPTER I
GENERAL BACKGROUND AND PURPOSE

Background

Since the first part of the Twentieth Century, much time and effort has been spent in learning the causative factors of gastric and duodenal ulcerations. Two main areas have been extensively studied, and have been found to play significant roles in the etiology of ulcerations. Psychological factors are primarily responsible for ulcerations in man, although physiological malfunctions such as in the Zollinger-Ellison syndrome do account for a small percentage.

Ulcerations are often acute, and may be chronic. Chronic ulcers are serious due to the many complications which may result (perforation, hemorrhage, or obstruction.) Portions of the alimentary tract which are in contact with gastric secretion (esophagus, stomach, duodenum and jejunum) are the sites of ulcer formation. The role of acid and pepsin secretions in ulcer formation has been widely studied, and yet it is known that, although the majority of people do secrete acid and pepsin, only a very small percentage (10%) ever develop ulcers. Clearly then, this acid-pepsin complex by itself is not the only factor in ulceration.

Until 1970, a satisfactory model for the production of duodenal ulcers in small laboratory animals did not exist, although a review of the literature revealed a method involving feeding certain strains of rats and mice a diet deficient in pantothenic acid (Berg, Zucker,
and Zucker, 1949). However, this method has proven unsatisfactory, since only certain strains of rats or mice are sensitive to the treatment, and the incidence of ulceration never exceeds 60%. Although these duodenal ulcers could be correlated to gastric hyperacidity, they appeared to be a result of the selective effect of this particular avitaminosis, and did not appear to represent a model which could be readily correlated to the human disease. Injection of a histamine-beeswax preparation has been effective in the dog, guinea pig and cat (Hay, Varco, Code and Wangensteen, 1942). Infusion of gastrin has been effective in dogs, cats and guinea pigs (Dragstedt, Oberhelman and Smith, 1951, Emas and Grossman, 1957, and Gobbels and Adkins, 1967).

A suitable model for the production of duodenal ulcers in the rat would be extremely useful, since in man, duodenal ulcerations are four to five times more frequent than gastric ulcerations. The rat has been used for experimentation, and is known to develop gastric ulcers under a variety of conditions: administration of corticoids (steroid induced ulcers), exposure to acute stress (exertion, restraint, cold), serotonin, reserpine, or caffeine ulcers, and ligation of the pylorus (Shay ulcers). However, under the conditions listed above, only the stomach is affected, while the duodenum appears resistant to ulcer formation.

Robert and Stout (1970) have shown that infusion of secretagogues could produce ulcers in the duodenum. Their method involved the constant subcutaneous infusion of various secretagogues either
alone or in combination. There are several distinct advantages of this new technique: the ulcers which develop are primarily located in the duodenum, the stomach remaining unaffected; the incidence of duodenal ulceration is 90 - 100%; and the time needed for the development of these duodenal ulcers is only 24 - 48 hours. The fact, that the rat is particularly resistant to duodenal ulcer formation, made the technique even more useful. Conditions capable of breaking this resistance could prove profitable in yielding information of the pathogenesis of duodenal ulcers.

In Robert and Stout's studies, three secretagogues; histamine dihydrochloride, carbachol and pentagastrin, alone or in combination, were infused subcutaneously into the rat for a 48 hour period.

The Purpose

The present studies were undertaken to determine at what time during the 48 hours of constant infusion duodenal ulcer formation begins, at what time the incidence and severity of ulceration reach maximal levels, and then to correlate this to a corresponding change in the composition and/or rate of gastric secretions. Using Robert and Stout's model, the following projects were investigated: the time sequence of ulcer formation, and the effect of time on changes in the rate and/or composition of gastric secretions; the influence of feeding vs. fasting, and the importance of physical contact of the gastric juice with the duodenal mucosa for development of duodenal ulcers.
CHAPTER II
PRELIMINARY CONSIDERATIONS

Pharmacological Agents

In the studies by Robert and Stout (1970), it was noted that the combination of histamine and carbachol was especially effective in inducing duodenal ulceration while the mortality rate remained at appropriate low levels (Figures 1 – 3). Consequently, a dosage of 0.1 mg/kg per minute histamine dihydrochloride plus 0.3 µg/kg per minute carbachol was selected and exclusively used in these studies.

Histamine dihydrochloride is a decarboxylation product of histidine, and is a potent vasodilator (Grollman, 1962). Carbachol is a synthetic compound with parasympathomimetic activity. As has been reported in the literature, histamine and carbachol each induce gastric secretion, but when combined, the synergism which develops is capable of inducing duodenal ulcers in the rat at relatively low doses.

Experimental Animals

Female Upjohn rats, [Upj:tuc (SD) Spf] formerly from the Sprague Dawley strain, were used throughout these studies. The weight differences which occurred within each experiment are taken into consideration in calculating the dosages used, and these individual weight differences are noted in the chapter on Methods.
Figure 1. Ulcer incidence. (Robert and Stout, 1970).
Histamine and carbachol in combination

Figure 2. Perforation incidence. (Robert and Stout, 1970).

Figure 3. Ulcer incidence. (Robert and Stout, 1970).
Figure 1

![Graph showing the incidence of ulcer per time of Carbachol and Histamine administration.]

0.05mg/kg per min
Histamine

0.1mg/kg per min
Histamine

0.25mg/kg per min
Histamine

Figure 2

![Graph showing the incidence of perforation per time of Carbachol and Histamine administration.]

Figure 3

![Graph showing the incidence of ulcer per time of Carbachol and Histamine administration.]

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Surgical Technique: Chronic Fistula Preparation

Two months prior to experimental use, a stainless steel cannula was placed in the stomach of rats weighing between 200-215 grams after an overnight fast (Komarov, Bralow and Boyd, 1963). The rats were anesthetized with ether throughout surgery. After implantation of the cannula, a one inch square of Marlex mesh was fitted around the cannula to add support to the abdominal wall. During the two month recuperative period, the animals were placed in cages fitted with wide mesh screen bottoms. The wide mesh was designed so as to protect the seal between the cannula and the stomach. Animals in which a leakage was noted were not used for any subsequent experimentation. During the recuperative period the rats were also placed in stainless steel restraining tubes which were designed by Dr. Robert to allow the rats limited movement, but afford protection to the infusion line by preventing the rat from turning around and chewing through the lines during the infusion period. The restraining tubes varied in diameter so each individual rat could be properly fitted. The tubes were perforated allowing for adequate ventilation and were not considered a causative factor in ulcer formation. The rats were placed in the restraining tubes, first for short periods of time, and later for periods of the same duration as those used under experimental conditions. It was felt that if the rats were allowed time to adjust to the tubes, the probability of damaging the cannula would be significantly reduced.
Variables Studied

At autopsy, the appearance of the abdominal organs, the intestinal wall, and the intestinal contents in the experimental groups was compared with that of control animals. The stomach and 30 to 40 mm. of the duodenum were then removed, cut along the mesenteric attachment for the duodenum and the greater curvature of the stomach, and examined for ulcerations. A rating scale was developed for all observed ulcerations: ranging from 0.5 plus, for barely visible ulcers or traces, to 3.0 plus, for extensive ulcerations or perforations. The site (stomach or duodenum) where the ulcerations occurred, the number of ulcerations per area, and the distance from the ulceration to the pyloric sphincter were noted. In the stomach, three separate sites were considered; the forestomach, the corpus, and the antrum including the area of the pylorus.

Characteristics of Ulcers

Forestomach ulcers were generally 1.0 mm. or smaller, and blister-like in appearance. They were usually superficial, and often appeared to be hemorrhagic.

Corpus ulcers were generally 0.5 mm. in diameter or smaller, and appeared as tiny black spots. The corpus ulcers were usually at the top of a mucosal fold, occasionally numerous and often in the 2.0 to 2.5 plus range. Of the experimental animals which died early in treatment, approximately 90% had numerous severe corpus ulcers which appeared black and hemorrhagic.
Antral ulcers often extended past the pyloric sphincter into the duodenum. In cases where this occurred, a determination was made by the examiner as to which of the areas, or if both areas, were affected. Antral ulcers were usually black in appearance and hemorrhagic.

Duodenal ulcers varied greatly in severity. Occasionally, perforations extended for a distance of 20-23 mm. and were 2-4 mm. in diameter. Mild duodenal ulcers, 1.0 plus to 1.5 plus, had craters which appeared red, while ulcers in the 2.0 to 2.5 range were black and hemorrhagic. In the severest cases, the duodenum appeared as one necrotic region for a distance of 10-20 mm., and was graded as only one ulceration. The majority of duodenal ulcers were located on the side of the duodenum opposite the mesenteric attachment. Great care was used in the removal of the stomach and duodenum so as to minimize possible damage to the site of ulceration.

Associated Changes

Several changes associated with the onset and formation of duodenal ulcers were noted. In the pre-ulcerative stage, there occurs a dilatation of the duodenum, followed in most cases by the formation of a red area or a red line, usually marking the site of an ulcer. Red lines may be indicative of mild ulcer formation usually in the 0.5 to 1.0 plus range. With increasing severity, 1.5 to 2.0 plus ulcers become black and blister-like in appearance. Changes associated with perforations include adhesions (mainly involving the liver and spleen), the release of gastric juice.
into the peritoneal cavity, resulting in peritonitis with eventual death.

General Infusion Technique

The secretogogues were infused subcutaneously at a rate of .54 ml/hr. (13 ml/24 hours). For each experimental group tested, a group of control animals, infused with saline, were run under identical experimental conditions. Female Upjohn rats, weighing between 205 and 215 grams after an overnight fast, were used. The average fasted weight was used to calculate the doses of secretogogues. All doses were calculated as weight of compound per kilogram of rat per minute, weighed on an analytical balance, and dissolved in saline. After appropriate dilution, the solution was put into 13 ml syringes, which were fitted with Tygon® tubing (Transflex, Minnesota Mining and Manufacturing, St. Paul, Minnesota.) to which an 18 gauge needle was attached. With the syringes in the infusion pump and the appropriate rate set, the machines were turned on and allowed to run until the solution flowed at a constant rate from the needles. The tubing was then attached to the dorsal region of the rat with Michel wound clips, and the rats, with tubing, were placed in the stainless steel restraining tubes. After 24 hours of continuous infusion, the empty syringes were replaced. At the end of 48 hours of infusion, the rats were removed, the needles taken out and the survivors killed with chloroform. The stomach and duodenum were removed, coded to prevent identification by the examiner, and examined with a binocular
magnifier. All ulcerations were graded on a 0.0 to 3.0 plus rating scale.
CHAPTER III
METHODS

Time Intervals and Ulcer Formation

Rats, weighing between 205 and 215 grams after an overnight fast, were used. Selected time periods of infusion were chosen, and the rats were divided into groups, one group per time period. The periods were: 4, 6, 8, 10, 12, 18, 24, 30, 36, 42 and 48 hours. A control group was infused with saline for 48 hours.

At the end of each time period, the surviving rats in each group were removed from the restraining tubes, killed with chloroform, their stomachs and duodenums (extending down to about 4 cm. from the pylorus) were dissected out. The percentage of animals with gastric and/or duodenal ulcers was noted, and the distance of duodenal ulcers from the pylorus was measured.

Effect of Feeding on Duodenal Ulcer Formation

This study was performed on animals which had been fasted overnight prior to infusion. What possible role prolonged fasting played in the formation of duodenal ulcers was not known, although control fasts fasted overnight and infused with saline developed no ulcers. Clinical studies in man have shown that feeding alleviates the symptoms of duodenal ulceration, and this experiment provided an adequate test of the new rat model. Rats, weighing 210 grams, were divided into four groups: Group I was infused with saline and
fed; Group II infused with saline and fasted, Group III infused with histamine plus carbachol and fed, and Group IV infused with histamine and carbachol and fasted. The animals were placed in individual restraining tubes and infused subcutaneously with either saline or histamine dihydrochloride plus carbachol. Groups I and III were given water ad libitum, while Groups II and IV had no water for the entire 48 hour period. At the end of 48 hours, the animals were killed with chloroform, and their stomachs and duodenums were removed, dissected, and examined with a binocular magnifier for ulcerations.

Gastric Juice Analysis

Rats weighing 240 grams after a 24 hour fast were used. Selected time intervals closely corresponding to those used in the constant infusion studies were chosen. For each time interval, the rats were infused with the ulcerogenic combination of 0.1 mg/kg/per minute histamine dihydrochloride plus 0.3 μg/kg per minute carbachol. For each time interval in which histamine and carbachol were infused, the rats were infused with saline for an identical time period. Each infusion period consisted of two parts: An initial period of infusion with the cannula closed preventing drainage, followed by two hours of infusion with collection each hour (Figure 4). Acid determination by titration with .01 N NaOH to pH 7 (glass electrode) as well as a pepsin determination by the hemoglobin method were done (Anson, 1938). The rats were then returned to their cages and allowed to recuperate for one week. Following extended infusion
periods with histamine and carbachol, control experiments using saline were performed in order to ascertain that the rats had returned to a normal secretory pattern.

Cannula Open Vs. Cannula Closed

Rats weighing 240 grams were used. A stainless steel cannula had been implanted two months earlier. The animals were divided into two groups; Group I was placed in infusion tubes with the cannula opened to allow continuous drainage, and infused with histamine and carbachol for a 24 hour period. Gastric secretion was collected at two hour intervals and analysed for acid output and concentration. Group II was subjected to identical experimental conditions, the exception being that the cannula remained closed, preventing drainage for the entire 24 hours. At the end of 24 hours, both groups were killed with chloroform, the stomachs and duodenums removed and examined for ulcerations.

Histamine in Gelatin

Rats weighing 200 to 205 grams after an overnight fast were used. A gelatin solution (prepared by The Upjohn Company) was used as the vehicle for the histamine. Each ml. contained 160 mg. of gelatin and 5 mg. of phenol in water. The gelatin was heated in an oven until it became liquid. Histamine dihydrochloride in doses of 100, 200, 250 and 300 mg. per 2 ml. based on rat body weight was dissolved in the gelatin. The preparation was mixed with magnetic stirrers for fifteen minutes and poured into 2 ml. syringes. Each
Figure 4

Periods of Infusion and Collection
(Histamine plus Carbachol or Saline)
Figure 4

2 hours of Infusion with collection
.... 2 hours of infusion with cannulas closed (no collection)

A. ___ 2
B. ..?.. 2
C. ...4.... 2
D. ....6.... 2
E. ....8....... 2
F. ...10........... 2
G. ...12............... 2
H. ................. 2
syringe was fitted with an 18 gauge needle and allowed to stand at room temperature for at least 30 minutes. Each rat was then injected with 2 ml. of the histamine-gelatin preparation, placed in individual cages with water given ad libitum, but without food. At the end of 72 hours, the surviving rats were killed with chloroform and their stomachs and duodenums were removed and examined for ulcerations.
CHAPTER IV
RESULTS

Production of Duodenal Ulcers

**Constant infusion**

Controls: Of the 72 rats which were infused subcutaneously for 48 hours with saline, no duodenal ulcerations were recorded. Examination of the stomachs revealed 6/72 (9%) had developed corpus ulcers, 4/72 (5%) antral ulcers, with no forestomach ulcers having been recorded (Figure 8). These ulcerations were not severe, and were in the 0.5 plus range, with antral ulcers usually surrounding the pyloric sphincter.

Treated Animals: Constant subcutaneous infusion with a combination of 0.1 mg/kg per minute histamine dihydrochloride plus 0.3 μg/kg per minute carbachol for a period of 48 hours resulted in an 86% (43/51) incidence of duodenal ulceration, with 33% (17/51) having died. Of the dead animals, 100% had duodenal perforations, and death was due to peritonitis developing after perforation.

As the period of infusion increased from 4 to 48 hours, the resulting incidences, perforations and severity of ulcerations increased in a time dependent fashion (Figures 5 - 7). After four hours of infusion, 3% (1/39) had developed mild (0.5 plus) duodenal ulcers. After 6 hours of infusion, 19% (6/32) had duodenal ulcerations. Between 6 and 8 hours, the greatest rise in ulceration took place, and at 8 hours, 73% (28/39) had duodenal ulcerations ranging
in severity from 0.5 to 1.5 plus. Perforations and mortalities, on the other hand, reached maximal levels at approximately 36 hours of infusion, and remained at that level for the duration of the infusion period. As incidence of ulceration increased, so did the number of ulcerations per duodenum (Figure 9). Severity of ulceration reached maximal levels only after 48 hours of infusion, increasing as each successive time interval was examined (Figure 10).

**Fasting vs. feeding**

Of the fasted, treated rats, a 92% incidence of duodenal ulcers was recorded; 38% of the rats had died and 19 of 36 had perforated duodenums (Table I). Of the dead rats, 100% had perforated duodenums. On the other hand, there were no deaths among fed rats, and only 14% had developed duodenal ulcers (Table I). Groups I and II, infused with saline, showed no ulcerations. The groups which were given food pellets (Groups I and III) ate much of the food, and at autopsy, 20% had visible food remaining in the stomach. Examination of the stomachs revealed no corpus, antral, or forestomach ulcers in either fasted or fed groups.
Figure 5. Duodenal ulcer incidence for 48 hours of constant infusion with 0.1 mg/kg per minute histamine dihydrochloride plus 0.3 μg/kg per minute carbachol.

Figure 6. Perforation incidence for 48 hours of constant infusion with 0.1 mg/kg per minute histamine dihydrochloride plus 0.3 μg/kg per minute carbachol.
Mortality incidence in rats infused with 0.1 mg/kg per minute histamine dihydrochloride plus 0.3 μg/kg per minute carbachol.
Gastric ulcerations in rats infused with histamine plus carbachol.
Figure 3

Corpus ulcers .......
Antral ulcers -------

%  
100  
75  
50  
25  
0  

6 12 18 24 30 36 42 48  
Time Hrs.
Figure 9

Number of ulcers per duodenum. Rats infused with 0.1 mg/kg per minute histamine dihydrochloride plus 0.3 μg/kg per minute carbachol.
Figure 10

Severity of duodenal ulcerations, graded on a zero to three scale.
Table I

Effect of Feeding on Duodenal Ulcer Formation

<table>
<thead>
<tr>
<th></th>
<th>Saline</th>
<th>Histamine plus Carbachol*</th>
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<tbody>
<tr>
<td></td>
<td>I Fed</td>
<td>II Fasted</td>
</tr>
<tr>
<td>No. of Animals</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Ulcer Incidence %</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Perforations %</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mortality %</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>No. per Duodenum</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Histamine 0.1 mg/kg per minute plus carbachol 0.3 μg/kg per minute.
Histamine in gelatin

Histamine dihydrochloride in gelatin was injected in doses of 100, 200, 250 and 300 mg. per 2 ml. injection. Examination of rats at 48 hours revealed no ulceration, however at the end of 72 hours 60% (36/60) of the rats injected with the 300 mg. dose had duodenal ulcers, 33% (20/60) had perforated, and 6% (4/60) had died. The results are shown in Table II. Doses greater than 300 mg. greatly increased the incidence of mortality so that efforts in these directions were curtailed. At the 300 mg. level, 3% (2/60) developed antral ulcers, 3% (2/60) corpus ulcers, with no forestomach ulcers being recorded.

Skin lesions, at or about the site of injection, developed between 24 and 36 hours, appearing as areas of necrotic tissue. Shortly after injection, the rats began to salivate excessively and remained in a prone position for 3 to 5 hours. Several animals died during this initial period, and examination of the stomachs revealed severe corpus and antral ulcerations. Control injections of 2 ml. gelatin resulted in no ulceration in either stomach or duodenum.

Gastric Juice Analysis

Analysis of gastric secretion collected over the selected time periods indicated that in both control (saline) and treated (histamine and carbachol) rats, there was an increase in volume during the first four hours of infusion. Following the initial rise, the volume of
<table>
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<th>Histamine in Gelatin</th>
<th>Dosages in mg/2 ml</th>
<th>100</th>
<th>200</th>
<th>250</th>
<th>300</th>
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<tbody>
<tr>
<td>No. of Animals</td>
<td></td>
<td>20</td>
<td>20</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>Ulcer Incidence %</td>
<td></td>
<td>15</td>
<td>10</td>
<td>20</td>
<td>70</td>
</tr>
<tr>
<td>Perforations %</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>33</td>
</tr>
<tr>
<td>Mortality %</td>
<td></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>6</td>
</tr>
</tbody>
</table>
juice collected in treated animals remained below that of controls for the remainder of the infusion periods. Control volumes increased between 6 and 12 hours and then approached the values for treated groups.

All secretions collected were analysed for both acid and pepsin output and concentration. Analysis for acid output and concentration showed a gradual increase in output for control groups to 12 hours of infusion, followed by a decline, so that the 24 hour readings were approximately equal to those at 2 hours of infusion. Acid output in treated animals reached maximal levels at 4 to 6 hours and then declined to values below that of controls at the 24 hour periods. Acid concentrations in control groups remained constant throughout all intervals, while readings for treated groups declined steadily after an initial rise at 3 hours (Figures 11 - 15).

Cannulated rats infused constantly, with cannulas allowed to drain for the entire period, were compared to those with cannulas closed to prevent drainage. In the group with the cannula closed, 83% developed duodenal ulcers; 17% had perforations, and an average of 1.2 duodenal ulcers per animal was recorded. The group with the cannula open had no perforations, and 33% duodenal ulcerations with no gastric ulcers recorded (Table III). Duodenal ulcers in this group were very mild; 0.5 plus as compared to those with the cannula closed in which the severity was in the 2.0 to 2.5 plus range. The volume of juice collected over the 24 hours, and collected at two hour intervals, reached a peak between 4 and 6 hours. Acid
concentration and output followed similar patterns, the highest readings being between 4 and 6 hours (Figures 11 - 15).
Figure 11

Gastric juice analysis: Volume (ml) for selected time intervals.
Figure 11

[Graph showing volume of gastric juice collected per 2-hour period over time in hours (0-24)]

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Figure 12

Gastric juice analysis: Acid concentration (mEq/L) for selected time intervals.
Figure 12

Saline...........
Histamine
Carbachol

Time Hrs

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Gastric juice analysis: Acid output (mEq/2 hrs) for selected time intervals.
Figure 13

Saline
Histamine
carbachol

Time Hrs

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Figure 14

Gastric juice analysis: Pepsin concentration for selected time intervals.
Saline

Histamine

Carbachol

Time Hrs

Figure 1b

20

15

10

5

2 4 12 24

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Figure 15

Gastric juice analysis: Pepsin output (uEq/2 hrs) for selected time intervals.

43

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Figure 15

Gastric juice analysis

Saline

Histamine

carbachol

Time Hrs

Gastric juice analysis

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Table III

Duodenal Ulcer Formation in Fistula Rats with CannulasOpened or Closed. Continuous Infusion for 24 Hours.

<table>
<thead>
<tr>
<th></th>
<th>I* Cannula Open</th>
<th>II* Cannula Closed</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of Animals</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Mortality %</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Perforations %</td>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>Ulcer Incidence %</td>
<td>33</td>
<td>83</td>
</tr>
<tr>
<td>Average No. Ulcers per Duodenum</td>
<td>0.4</td>
<td>1.2</td>
</tr>
<tr>
<td>Severity of Ulceration</td>
<td>0.4</td>
<td>2.1</td>
</tr>
</tbody>
</table>

*Histamine 0.1 mg/kg per minute plus carbachol 0.3 μg/kg per minute.
Table IV

Duodenal Ulcer Formation Following Continuous Infusion with 0.1 mg/kg per minute Histamine Dihydrochloride plus 0.3 μg/kg per minute Carbachol for Selected Time Intervals, Followed by Infusion with Saline for the Remainder of the 48 Hour Infusion Period.

<table>
<thead>
<tr>
<th>Infusion Periods Hours</th>
<th>6</th>
<th>12</th>
<th>24</th>
<th>48</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulcer Incidence %</td>
<td>10</td>
<td>45</td>
<td>64</td>
<td>80</td>
</tr>
<tr>
<td>Perforations %</td>
<td>0</td>
<td>12</td>
<td>27</td>
<td>31</td>
</tr>
<tr>
<td>Mortality %</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>Average No. of Ulcers per Duodenum</td>
<td>0.3</td>
<td>.75</td>
<td>1.70</td>
<td>1.34</td>
</tr>
</tbody>
</table>
CHAPTER V

DISCUSSION

General Discussion

It has been shown by Robert and Stout (1970) that the rat is not inherently resistant to duodenal ulcer formation, and that if a duodenal barrier does exist, it can be broken by constant infusion of secretagogues. The ulcerogenic combination of histamine dihydrochloride plus carbachol as shown by Robert and Stout was chosen in these studies on the basis of its ulcer producing capacity. Rats treated with the combination developed duodenal ulcerations with accompanying perforation, peritonitis and eventual death. Histological studies done on treated animals indicated that in all likelihood, the developing ulcers proceed from the outer duodenal mucosa down to and through the serosa, as in the case of perforation. This method of producing ulcers in rats represents a potential workable model for the study of the corresponding human disease. The fact that the technique is capable of inducing duodenal ulcers is of particular interest in that the incidence of duodenal ulcers to peptic ulcers in man is 4 to 1. The technique of Robert and Stout affords a tool with which the researcher can study development of, and causative factors surrounding ulcer formation in man. Not only are the ulcers selectively produced in the duodenum, but they develop along the same lines as in the human condition, i.e., they bleed and perforate.
Why the stomach remains unaffected by the treatment is not completely explained. Quite likely, the gastric juice with its high digestive powers cannot be sufficiently neutralized before it reaches the duodenum, and the result is duodenal mucosal damage. When treated rats were allowed to ingest food during the ulcerogenic treatment, the percentage of rats which developed duodenal ulcers was reduced to 14% as compared to 92% in fasted treated animals. The food acted as a buffer for the acidic juice thus neutralizing its effects sufficiently to prevent duodenal ulcer formation in the majority of the animals. On examining the stomach and duodenum of fed treated rats, it was noted that 7 of the 20 still had food present. This supports the hypothesis that food when present in the stomach may act as a buffer for acidic gastric secretion. Gastric ulcers, when present, were associated with duodenal perforations and may be due to the stress of peritonitis.

The duodenal ulcers which develop appear to be correlated to a corresponding rise in acid concentration and output. Acid secretion reaches maximal levels between 3 and 8 hours which closely corresponds to the onset of duodenal ulcer formation. After this initial hyper-acidic phase, acid levels remain at, or slightly below, that of controls treated with saline. This suggests that the initial hyper-acidic secretion initiated duodenal ulcer formation at the mucosal level and that the continued flow of acidic gastric secretion at control levels are sufficient to perpetuate continued ulcer development. This assumption is substantiated by the studies in which fistula rats were infused with the ulcerogenic combination and in
one group the gastric secretions were permitted to flow over the duodenum, while in the second group gastric juice was drained from the stomach through a cannula. When gastric secretion was permitted to flow over the duodenum, 83% developed duodenal ulceration while in the group in which it was collected only 33% had duodenal ulcers.

The mechanism responsible for the rise in acid output and concentration following infusion with histamine plus carbachol remains to be answered. Carbachol by itself is a powerful secretagogue, as is histamine dihydrochloride. However, it is doubtful that carbachol itself can stimulate gastric secretion at the cellular level, but whether or not histamine acts as the final common mediator in acid secretion remains to be clarified.

All stimuli may act by releasing histamine as their final common agent in the vicinity of the oxyntic cells. The theory that histamine is the final common mediator when acid secretion is stimulated is substantiated by the following evidence: (1) Histamine can stimulate secretion by a mucosa stripped of all nerves, and therefore it can act directly on the cells distal to the action of any nerve. (2) Most cells whose granules contain histamine and heparin are present in the gastric mucosa near the oxyntic cells, whereas in all other areas of the digestive tract they are closer to the submucosa. (3) The gastric mucosa contains histidine decarboxylase which converts histidine to histamine but not diamine oxidase which destroys it. (4) Radioactive histamine is formed in the mucosa when C14-histidine is administered. (5) Histamine is present in acid gastric juice in amounts nearly paralleling acid
output, regardless of the mode of stimulation. Compounds which inhibit the enzyme diamine oxidase, protecting histamine from destruction, increase the secretory response to feeding. (6) There appears to be a parallelism between urinary excretion of free histamine and gastric secretion following a meat meal. Since a constant proportion (10%) of injected histamine is excreted in the urine, excretion of histamine during digestion most likely reflects histamine liberation (Davenport, 1966). In spite of this large amount of evidence, recent studies indicate that perhaps the relationship between histamine metabolism and gastric secretion may be an indirect one. When 24 hour acid secretion is increased 50 times by transplantation of the antrum to the colon, urinary excretion of histamine increases only 13%. Also, when meat is placed in the jejunum, histamine excretion increases ten times or more, but acid secretion remains low; and later, when acid secretion increases in response to the intestinal phase of stimulation, histamine excretion has fallen back to its original level.

In this particular case the histamine excretion is probably the result of histamine formation by bacterial reaction on histidine in the meat. Histamine excretion is greatly reduced following meat feeding when the intestine has been sterilized. When meat is placed in the antrum which is surgically isolated from the intestine, it evokes acid secretion, but no increases in histamine secretion. Antihistaminic drugs in concentrations which abolish the actions of histamine elsewhere in the body, have no effect in inhibiting gastric secretion. It has been shown that the topical effect of
antihistaminic drugs on the gastric mucosa is to reduce gastric secretion, but this may be attributed to the possible destructive effect on the mucosa as well as the atropine-like properties (Davenport, 1966). It appears that the action of histamine may be an indirect one, although no theories as to the possible mechanism(s) have been proposed.

Should the mechanism be indirect, release of additional substance(s) triggered by histamine could stimulate gastric secretion. If the substance(s) were released in the same area as histamine release, i.e., in the area of the oxyntic cells, it is possible that gastric secretion could continue in the absence of histamine.

Histamine in Gelatin

Injections of histamine dihydrochloride in gelatin induce the formation of duodenal ulcers in rats. Resulting ulcers appear to follow much the same course of development as those produced by constant infusion. The mechanism involved in the formation of these ulcers cannot be completely ascertained, in as much as analysis of the gastric secretion in treated animals was not undertaken. Any correlation between ulcer formation and changes in acid output and/or concentration would at this time be purely speculative. However, from visual observation of the ulcers, it appears that they develop in the same manner as those observed during constant infusion. It is likely that the histamine released from the gelatin causes an initial rise in acid output and concentration which in turn
initiates duodenal mucosal damage ending with the development of duodenal ulcers.

Experiments in which the dosage of histamine per 2 ml. injection was increased were undertaken. However studies in this area were unsuccessful, probably due to the immediate release of histamine following injection. With injections higher than 300 mg. per 2 ml. an increase in the mortality rate was so high as to be prohibitive. Likewise if the duration of treatment was reduced to 48 hours, the resultant ulcer formation was greatly reduced. Skin lesions which developed at or about the site of injection are a direct result of immediate histamine release, as histamine is known to be a local irritant. Investigations are being undertaken to perfect this new technique, and it is hoped that a deviation from the normal secretory patterns will be observed in treated animals.
SUMMARY

Duodenal ulcer formation in rats treated with the ulcerogenic combination of histamine plus carbachol in selected dosage over various time intervals appears to result from hyperacidic secretions. Ulcer incidence, severity and perforations increase with time, which tends to indicate progressive mucosal damage.

Acid concentration and output show a significant increase between 4 and 12 hours of infusion, which corresponds to the initial rise in ulcer incidence. It appears that the initial hypersecretion is sufficient to damage the mucosal barrier with resultant ulcer formation; following this initial lesion, normal acidic secretory patterns are sufficient to continue attacking the duodenal mucosa.
LITERATURE CITED


