Figure 25e. Dog DD5. The histological changes along the posterior wall of the greater curvature of the antrum.

In summary, 2 of the 5 dogs in this study developed mucosal abnormalities after cholecystectomy. It is interesting to note that these 2 dogs had much higher post-cholecystectomy amount of bile reflux than the other 3 dogs. A detailed discussion is presented in section 5.3.3.
5.3 DISCUSSION

5.3.1 Discussion of the methods used

In this study the amount of reflux was measured by estimating the concentration of lecithin and lysolecithin in the gastric contents (section 5.1.2). By using both phospholipids the picture of reflux was more accurate. In various reported studies lysolecithin concentration alone was used to measure reflux (24,27). However, lysolecithin may be formed from lecithin in vitro after the collection of gastric juice. Considering that lecithin is converted to lysolecithin (section 3.5.1) and that the ratio of lecithin to lysolecithin varied even in the same experimental animal from time to time (sections 5.2.5 and 5.2.6), and depended on the experimental conditions (eg. secretin stimulation or vagotomy), it is clear that the use of only one of these phospholipids may be misleading in measuring reflux. A detailed discussion of this problem is given in section 5.1.11.2.

When studying reflux, long collection periods of time should be used because of the varying pattern of reflux. During the experiments it became evident from the colour of gastric juice that reflux was occurring intermittently during the same test. This observation is in agreement with the findings of Sorgi et al (255). The motility pattern of the antroduodenal junction (an important factor in D/G reflux) is influenced by the secretory activity of the upper gastrointestinal tract which occurs in
alternating cycles of activity and quiescence (30). The amount of reflux varied from time to time in the same animal, both before and after cholecystectomy (sections 5.2.5 and 5.2.6). From this observation it becomes clear that a number of tests should be carried out. Single measurements, especially over a short period of time, could be misleading, with respect to the amount and pattern of reflux. A more detailed discussion of this problem has been made in section 5.1.11.1.

For the collection of gastric juice a permanent gastrostomy cannula was used. Problems which may be associated with the presence of the cannula in the stomach have been discussed in section 5.1.13.1. The cannula was inserted 8 cm proximal to the pylorus, and thus any disturbance of antroduodenal function was unlikely. Furthermore, the same cannula was present in each dog during the control studies.

There has been much discussion as to whether the concentration of duodenal markers in the gastric juice is a reliable index of reflux (233). The main argument against this method is that the concentration of duodenal markers in the stomach is determined by many factors. These include the rate of gastric emptying and gastric secretion. Although this is true, it is unlikely to pose any serious problem in this study: the rate of gastric emptying and gastric secretion most likely remained the same, before and after cholecystectomy. The reason for this statement is the fact that the volumes of the collected gastric contents
were the same, before and after cholecystectomy. Furthermore, it is the concentration of cytotoxic contents in the stomach that finally determines the mucosal damages regardless of what factors determined the concentration (234). The advantages and disadvantages of this technique are presented in section 4.2.2.

The importance of the 'Swiss roll' technique, used in the histological assessment, has been discussed in section 4.1.13.3.

5.3.2 Discussion of biochemical results

All control animals showed a small but consistent amount of reflux. This suggested that a small amount of reflux might be a normal phenomenon. However, alternatively, it might be due to the presence of the gastrostomy cannula in the stomach.

After cholecystectomy all 5 dogs showed increased concentration of lecithin and lysolecithin in the stomach, although in 2 this change was transient. Since cholecystectomy alone is very unlikely to have changed the rate of gastric secretion or the rate of gastric emptying (section 5.3.1), the increased concentration of bile in the stomach is most likely to be the result of increased bile reflux into the stomach. The increased post-cholecystectomy bile reflux may be the result of mechanical or hormonal changes. After cholecystectomy, since there is no gall bladder reservoir to store bile during fasting, hepatic bile flows continuously into the duodenum. As a result, bile is
always present in the duodenum and if local conditions permit, bile reflux occurs. Earlam's suggestion \( (47, 48) \) that after cholecystectomy bile reflux should be less because of the continuous flow of unconcentrated bile into the duodenum, does not seem to be supported, at least in dogs.

Hormonal changes might also play a role in bile reflux. It has been shown that cholecystokinin (CCK) plasma levels rise after cholecystectomy \( (257) \). CCK affects the pressure gradient across the pylorus \( (45, 258) \) and this might have some effect on reflux. Another hormone which may play a role is secretin. As early as 1911, Cathcart suggested this possibility \( (259) \). It has been shown that secretin causes certain changes of pressures at the gastroduodenal junction \( (section \, 3.6) \), and this might affect reflux. In this study secretin \( (1 \, \text{unit per Kg of weight}) \) was shown to promote reflux. However, it is not known whether secretin plasma levels change after cholecystectomy and if they do whether this change is enough to affect reflux.

Of all these theories, that of continuous bile flow into the duodenum after cholecystectomy is probably the most likely explanation. It appears from the present study that after cholecystectomy the volume of D/G reflux does not increase, despite the higher concentration of bile in the stomach. The factor which seems to change after cholecystectomy is the concentration of bile in the duodenum at all times. This is suggested by the fact that the volumes of gastric contents under
basal fasting conditions, were the same before and after cholecystectomy (section 5.2.10). As estimations of the rate of gastric emptying and gastric secretion were not done in this study, the unlikely possibility that after cholecystectomy there was increased volume of refluxed duodenal contents, with faster gastric emptying, or an increased volume of reflux with decreased gastric secretion, cannot be excluded. These conditions can give a picture where post-cholecystectomy intragastric bile concentration would be increased, while the volume of gastric collections would be the same as before cholecystectomy.

In 2 dogs (DD\textsubscript{2} and DD\textsubscript{3}) the post-cholecystectomy increase in bile reflux was transient (10 and 8 weeks respectively), and then returned to pre-cholecystectomy levels. This change may be the result of mechanical or hormonal re-adjustments. It has been shown that in dogs the common bile duct (CBD) dilates up to 3 times the normal size, after cholecystectomy (114-117). A dilated CBD perhaps takes over part of the gall bladder function. It cannot be assumed that a similar situation would occur in humans. Embryologically, anatomically and functionally, the intraduodenal part of the CBD in humans is different from dogs (112,113). Details are given in section 3.2.1. The generally accepted concept is that the CBD in humans does not change after cholecystectomy (121-130). Besides the suggested role of mechanical re-adjustment of the CBD, hormonal or duodenal motility re-adjustments may be of some importance. Why the increased reflux was transient in only 2 dogs is not clear. Further studies
on hormonal and motility changes, and CBD changes are needed. During the 6 month period after cholecystectomy of the present investigation, there was a persistent increase in bile reflux in 3 dogs. What occurs after 6 months is unknown.

Opinions as to the effect of secretin on bile reflux vary considerably, possibly due to differences in species or to the hormone preparations used in experiments. Some authors reported no effect on bile reflux (35). Others reported that it prevented reflux (37,78) and others that it increased reflux (244). A detailed discussion of these reports is presented in section 4.3.

Two events of particular interest occurred after the administration of secretin (Boots) at a dose of 1 unit per Kg weight.

a) It consistently and significantly promoted reflux into the stomach in all 5 dogs before cholecystectomy (section 5.2.7). However, after cholecystectomy secretin did not cause any significant change in the amount of reflux (section 5.2.8).

In theory there is a possibility that the increased concentration of lecithin and lysolecithin in the stomach of all pre-cholecystectomy dogs and some post-cholecystectomy tests after secretin infusion, might be the result of a decrease in gastric acid secretion due to the inhibitory effect of secretin (79,82,83). This possibility can reasonably be excluded as a few minutes after secretin infusion started, the colour of the gastric juice
was seen to change abruptly from clear to bright yellow. This is very suggestive of a sudden large increase of reflux, and not an increased concentration of bile products in the stomach due to decreased gastric secretion.

Various explanations may be offered to explain the effects of secretin on bile reflux: (i) secretin stimulates the flow of hepatic bile into the duodenum (182-184) and, as a result, more bile is available for reflux into the stomach; (ii) secretin itself has a minimal effect on the gall bladder (section 3.6). However, certain contaminants such as CCK may promote contraction of the gall bladder and therefore more bile is available in the duodenum for reflux into the stomach. After cholecystectomy this factor is abolished and as a result secretin will not have any effect on bile reflux; (iii) secretin changes the pressure pattern across the pylorus. It causes contraction of the pylorus and at the same time it decreases the intragastric and intraduodenal pressures (38,67,78,179,180). These changes in pressures may promote reflux. Pathophysiologically the pressure gradient on either side of the pylorus is a more important factor in the control of reflux, than is the diameter of the pyloric canal itself (73,74). This explains the sudden change of colour of the gastric juice from clear to bright yellow soon after secretin infusion. This abrupt change is suggestive of a sudden large reflux.
From the post-cholecystectomy results it seems that if bile reflux is already increased, secretin stimulation does not cause any further increase.

b) Secretin changed the ratio of lecithin to lysolecithin in favour of lysolecithin, both before and after cholecystectomy. This might be due to the fact that secretin increases the flow of hepatic bile into the duodenum (182-184), promotes the secretion of pancreatic enzymes, including trypsin (79,81) and increases the pH of the duodenal contents. These factors would favour the production of lysolecithin (section 3.5.1).

It is important to stress that the above results were obtained with Boots secretin. Romanski (260) and Ratishauser (261) compared the effects of Boots secretin and Kabi secretin (G.I.H. Laboratories) which is considered to be highly purified. They reported an increase in bile flow and bile lipid content after Boots secretin stimulation, unlike the more purified Kabi secretin which had little or no effect. The authors suggested that the stimulating properties of Boots secretin were most probably due to contaminants of this preparation.

Secretin consistently increased the pH of gastric contents, both before and after cholecystectomy (section 5.2.13). This is explained by the fact that secretin inhibits gastric acid secretion (79,82,83) and promotes the flow of hepatic bile and pancreatic juice, which is rich in HCO$_3^-$ (79,182-184). It also increases duodenogastric reflux (section 5.2.7).
5.3.3 Discussion of histological results

No histological mucosal abnormalities were found in the 2 dogs (DD\textsubscript{2} and DD\textsubscript{3}) with transiently increased bile reflux after cholecystectomy. One possibility is that the increased reflux was not of long enough duration to produce mucosal changes. Another possibility is that gastritis might have developed but then settled after the increased reflux returned to normal levels. The second possibility cannot be excluded since no biopsies were taken during the post-cholecystectomy period.

In dog DD\textsubscript{2} there was a period of 5 weeks and in DD\textsubscript{3} a period of 4 weeks before the animals were sacrificed, when reflux was not increased.

Of the other 3 dogs who had a persistently increased bile reflux after cholecystectomy, 2 developed gastric mucosal changes (DD\textsubscript{4} and DD\textsubscript{5}). The dog with the least increased post-cholecystectomy bile reflux (DD\textsubscript{6}) had no mucosal damage. As indicated previously, the damaging effect of the cytotoxic agents depends on the concentration of the agents in the stomach and the time of contact with the gastric mucosa (33, 75, 76). The concentration of lysolecithin needed to cause mucosal damage in chronic exposures is not known. Davenport (26), in a study with short exposures to cytotoxic agents, found that lysolecithin caused mucosal damage at concentrations higher than 20 mg\%. Johnson et al (25) measured the concentration of lysolecithin in gastric aspirates of patients with gastric ulcer and found a mean concentration of
20 mg%. (In controls it was 1.8 mg%). In a similar study, Burgi (175) found this concentration in patients with gastric ulcer to be 66.1 mg%. In the present study the post-cholecystectomy concentration of lysoleithin in the dogs with mucosal damage was: 11.435 ± 2.531 mg% (mean ± standard error of the means) in dog DD5, and 12.389 ± 1.700 mg% in DD4. Dog DD6, which did not develop any mucosal changes had a concentration of 3.403 ± 0.922 mg%.

Both dogs which developed mucosal changes after cholecystectomy had distal gastritis, involving only the antrum. The fundus was completely normal. The most severely affected area of the antrum was the lesser curvature in both dogs. Many reports support these findings. Lawson (11) found antral gastritis in dogs 3 to 6 months after diverting pure bile, pure pancreatic juice, and total duodenal contents into the stomach. In another study (23) the same author reported that the gastritis caused by bile was more extensive in the lesser curvature. Other authors agree that involvement of the stomach is distal in cases of bile gastritis (9,33,243,263). However, Buxman (248), in a series of 107 patients with bile gastritis, reported a generalized distribution of the lesions in 57%, involvement of the fundus only, in 16%, and of the antrum only in 27%. Many patients in this study had previously undergone gastric surgery for peptic ulcer and perhaps other factors besides bile reflux might have been involved in the pathogenesis of the gastritis.
In the present study the distribution of gastritis in the antrum was patchy. Similar findings were reported by Lawson (211).

The patchy nature of the gastritis means that biopsy specimens may not be representative. The use of the 'Swiss roll' technique eliminates this problem. If this technique cannot be performed, multiple biopsies should be taken.

The most common type of mucosal abnormality observed in the two dogs with gastritis was foveolar hyperplasia (epithelial proliferation), although occasional patches of mild or moderate atrophic gastritis were recorded. Many authors have reported similar histological findings with bile reflux. Lawson (11) found foveolar hyperplasia after biliary reflux procedures in dogs. Harri et al. (263) and Mosimann et al. (264) reported similar results in dogs and humans respectively. It has been suggested that foveolar hyperplasia is an histological marker of bile reflux (263,264).

In the present study the animals were sacrificed from 3 to 6 months after cholecystectomy (Table 1). These periods are considered long enough for histological changes to occur. Lawson, in two separate studies (11, 211), found that histological changes occurred within 3 months after bile reflux procedures.

The length of the gastric mucosa as measured on the 'Swiss roll' does not correspond with the actual size of the strip in the intact stomach (254). When the 'Swiss roll' is prepared the
muscle layer is removed (section 5.1.7) and during the preparation of the roll the mucosal folds are straightened, thus increasing the length of the strip.

5.3.4 Clinical significance of the results of the present study

To the best of the author's knowledge this is the first reported controlled experimental study in which an investigation was made into the possible relationship between cholecystectomy and bile reflux. Previously only uncontrolled clinical studies have been reported and results have been conflicting (section 4.5). The clinical significance of bile reflux into the stomach has been discussed in section 4.1. It is generally accepted that bile in the stomach might produce a syndrome of 'bile reflux gastritis' in some patients. The syndrome is characterized by epigastric pain, nausea, vomiting and weight loss (42,195). It is known that after cholecystectomy many patients develop the so-called 'post-cholecystectomy syndrome' the incidence of which has been estimated to be between 10 and 40% (42,132,195).

The object of the present investigation was to examine the hypothesis that one of the factors responsible for the 'post-cholecystectomy syndrome' may be gastritis caused by abnormal amounts of bile refluxing into the stomach. While recognizing the danger of extrapolation of experimental results to the human situation, it does appear that cholecystectomy in dogs led to an
increase in bile reflux. In 2 dogs abnormal reflux was transient and was not associated with histological changes. In a further 3 animals however, reflux of statistically significant increased amounts of bile occurred and persisted for the whole 6 month period after cholecystectomy of the present study. Two of these dogs developed histological gastritis. The experimental findings therefore lend support to the suggestion that at least in some cases of the 'post-cholecystectomy syndrome' seen in human subjects, the cause could be gastritis caused by abnormal amounts of bile refluxing into the stomach. The therapeutic implication is that in the appropriate case, substances such as cholestyramine which binds bile salts may be beneficial. Again in the appropriate case, a surgical procedure designed to prevent reflux could be considered.

5.4 CONCLUSION

An experimental study was designed to investigate the question of bile reflux after cholecystectomy and after secretin infusion. Dogs were used as experimental models and each one acted as its own control. Duodeno-gastric reflux is a periodic phenomenon, varying from time to time in the same dog. In order to obtain meaningful data a large number of experiments, over long periods of time, were performed on each dog. In all dogs cholecystectomy was found to promote bile reflux into the stomach, although in 2 of them this change lasted for a period of 8 to 10 weeks only.

Increased bile reflux into the stomach is probably the result of
the continuous flow of bile into the duodenal contents which follows cholecystectomy. Two of the dogs which had the greatest amount of post-cholecystectomy bile reflux developed foveolar hyperplasia of the gastric mucosa, which is considered by some authors to be a marker of bile reflux.

Secretin promoted bile reflux in dogs with an intact gall bladder probably by changing the pressure pattern at the antoduodenal region. In cholecystectomized dogs, where bile reflux was already increased, secretin infusion did not cause any additional significant change in the amount of bile reflux. However, in all dogs, irrespective of the amount of reflux, secretin changed the ratio of lecithin to lysolecithin in favour of lysolecithin.

The possible clinical significance of this experimental study is that it supports the view that in some cases of the so-called 'post-cholecystectomy syndrome' in humans, the clinical features may be the result of gastritis induced by reflux of bile into the stomach. If this is correct, medical or surgical methods of treatment in the appropriate case, may be effective.
6. EFFECTS OF TV-P AND CHOLECYSTECTOMY
ON D/G REFLUX
6. EFFECTS OF TV+P AND CHOLECYSTECTOMY ON D/G REFLUX

6.1 Materials and Methods

6.1.1 Experimental animals

In this experiment 4 mongrel dogs each trained to stand quietly in a Pavlov stand were used. Each dog acted as its own control. Their pre-operative weights ranged between 11.2 Kg and 21 Kg.

6.1.2 Preparation of the experimental animal

6.1.2.1 Anaesthesia

As described in section 5.1.1.2.

6.1.2.2 Insertion of gastrostomy cannula

As described in section 5.1.1.3.

6.1.2.3 Cholecystectomy

As described in section 5.1.1.4.

6.1.2.4 Truncal vagotomy with pyloroplasty

Truncal vagotomy was performed using a standard surgical technique. The abdomen was entered by means of a right paramedian incision. The anterior and posterior vagus nerves were identified at the oesophago-gastric junction by incising the peritoneum in front of the abdominal oesophagus. The nerves were mobilized for a length of at least 2.5 cm along the oesophagus, to ensure that all branches to the stomach were identified. A segment 2.5 cm in
length was excised from each nerve, and the ends tied with silk 2/0. If the chest cavity was entered, the lungs were inflated before closing the cavity with a continuous suture. The phreno-oesophageal ligament was reconstructed. A 4 cm long Heineke-Miculitz pyloroplasty was performed using one layer of interrupted chronic 3/0. The abdomen was closed in 3 layers.

6.1.2.5 Post-operative management

As described in section 5.1.1.5.

6.1.2.6 Assessment of adequacy of surgical vagotomy

The completeness of vagotomy was assessed in all 4 dogs subjected to this procedure. The Hollander test as described in section 3.9 was used: basal gastric secretions were collected via the gastrostomy cannula every 15 minutes for one hour. These four 15-minute samples were titrated to a pH of 7.0 with 0.1 molar sodium hydroxide (NaOH) and the results were expressed as millimoles of acid per litre. After taking the four basal collections, soluble insulin in a dose of 0.3 units per kilogram weight was administered intravenously and 8 collections of gastric juice were performed at 15-minute intervals. These samples were titrated to a pH of 7.0 as described above. Blood sugar levels were checked once before insulin injection and 8 times after insulin injection, at 15-minute intervals.
6.1.3  **Collection of gastric contents**

The technique of collection of gastric contents under basal fasting conditions and during secretin stimulation has been described in sections 5.1.3.1 and 5.1.3.2.

6.1.4  **Technique of bile reflux estimation**

The technique of extraction and quantitative measurement of lecithin and lysolecithin in the gastric juice has been described in section 5.1.4.

6.1.5  **Measurement of pH of gastric contents**

The method has been described in section 5.1.5.

6.1.6  **Assessment of the physical condition of the experimental animals**

As described in section 5.1.8.

6.1.7  **Histological assessment**

As described in section 5.1.7.

6.1.8  **Postmortem examination**

As described in section 5.1.9.

6.1.9  **Design of the experimental study**

Four mongrel dogs were used in this study, and each dog acted as its own control. In the first stage of the experiment a truncal vagotomy with Miculitz pyloroplasty was performed as described in section 6.1.2.4, a gastrostomy cannula was inserted (section
5.1.1.3) and mucosal biopsies were taken from the gastric fundus and antrum (section 5.1.1.3). The dogs were allowed a period of 3 weeks to recover from the procedure before any tests were conducted. Each dog was subjected to a minimum of 7, and a maximum of 9, six-hour collections of gastric juice under basal fasting conditions (section 5.1.3.1). Each test under basal fasting conditions was followed by a two-hour test under continuous intravenous infusion of secretin (section 5.1.3.2.). The period between two consecutive tests ranged from 5 to 10 days. The volume of the collections was measured and aliquots of the specimens were stored at -20°C. The specimens were later analyzed at random for lecithin and lysolecithin concentration (section 5.1.4) and the pH was measured (section 5.1.5).

When the initial control stage of the study had been completed, a cholecystectomy with gastric mucosal biopsies was performed on all 4 dogs as described in section 5.1.1.4. After a recovery period of 3 weeks, tests with or without secretin stimulation were performed (sections 5.1.3.1 and 5.1.3.2). Fifteen to 24 six-hour collections of gastric juice under basal fasting conditions were performed on each dog at intervals ranging from 1 to 3 weeks. Some of the collections were followed by 2-hour collections during secretin stimulation. Between 7 and 8 secretin tests were performed on each dog. The specimens were stored at -20°C and were later analyzed at random as described previously. The experimental animals were sacrificed 5 to 6.5 months after cholecystectomy and the stomach was removed and examined histologically as described in section 5.1.7.
6.2 RESULTS

6.2.1 Introduction

This study investigated the effect on bile reflux of truncal vagotomy with pyloroplasty and cholecystectomy, with or without secretin stimulation. D/G reflux from each dog is shown on a separate graph, and results are statistically analysed individually and collectively. All raw data obtained in the study is presented in Appendices E, F and G. Histological data is presented in bar diagrams.

6.2.2 Experimental animals

Four mongrel dogs, each one acting as its own control, were used in this part of the study (DD₀, DD₁₀, DD₁₂ and DD₁₄). One of the dogs (DD₁₂) developed diarrhoea and rapid gastric emptying, as confirmed by a barium meal, soon after the operation which persisted until the end of the experiments. Despite this, the dog had lost very little weight by the end of the experiments. The other 3 dogs remained healthy throughout the duration of the experiments and gained weight. A constant complication in all dogs was minor skin sepsis at the site of exit of the gastrostomy cannula. Details of the animals used are shown in Table 13.
TABLE 13: DETAILS OF DOGS IN THE STUDY

<table>
<thead>
<tr>
<th>Dog</th>
<th>Sex</th>
<th>Weight (Kg)</th>
<th>Total duration of experiments (months)</th>
<th>Duration of experiments after cholecystectomy (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DD_8</td>
<td>M</td>
<td>14.1</td>
<td>8.5</td>
<td>6</td>
</tr>
<tr>
<td>DD_10</td>
<td>M</td>
<td>11.2</td>
<td>7.5</td>
<td>5.5</td>
</tr>
<tr>
<td>DD_12</td>
<td>M</td>
<td>14.2</td>
<td>6.5</td>
<td>5</td>
</tr>
<tr>
<td>DD_14</td>
<td>F</td>
<td>21</td>
<td>8</td>
<td>6.5</td>
</tr>
</tbody>
</table>

* At the time of truncal vagotomy with pyloroplasty
** At the time of cholecystectomy
*** At the end of the experiments

6.2.3 Number of experiments carried out

A total of 162 tests were performed on 4 dogs. There were 30 tests under basal fasting conditions before cholecystectomy, 30 with secretin stimulation before cholecystectomy, 73 under basal fasting conditions after cholecystectomy and 29 with secretin after cholecystectomy. A detailed breakdown of the number of tests is presented in Table 14.
Author: Demetriades D  
Name of thesis: The effect of cholecystectomy on duodenogastric reflux an experimental study 1984

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