7.2.8.2 After cholecystectomy

Secretin stimulation of dogs with HSV and cholecystectomy significantly increased the pH of the gastric contents in all of them (Table 37). Raw data is given in Appendix J.

<table>
<thead>
<tr>
<th>Dog</th>
<th>$\bar{x} \pm$ SEM (basal conditions)</th>
<th>$\bar{x} \pm$ SEM (secretin infusion)</th>
<th>Statistical significance (t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DD$_7$</td>
<td>3,539 $\pm$ 0,078 (13)</td>
<td>4,503 $\pm$ 0,332 (8)</td>
<td>p 0,05</td>
</tr>
<tr>
<td>DD$_9$</td>
<td>3,372 $\pm$ 0,084 (20)</td>
<td>5,270 $\pm$ 0,376 (7)</td>
<td>p 0,01</td>
</tr>
<tr>
<td>DD$_{13}$</td>
<td>3,430 $\pm$ 0,254 (21)</td>
<td>5,901 $\pm$ 0,469 (8)</td>
<td>p 0,01</td>
</tr>
</tbody>
</table>

$x =$ mean value of pH  
$\text{SEM} =$ standard error of the mean  
( ) = numbers in parentheses represent the number of tests

7.2.8.3 Secretin infusion before cholecystectomy compared with infusion after cholecystectomy

The pH of gastric contents during secretin stimulation in dogs with HSV and an intact gall bladder was not statistically different ($p$ 0,05) from the pH during secretin stimulation in dogs with HSV and a cholecystectomy (Table 38).
7.2.8.2 After cholecystectomy

Secretin stimulation of dogs with HSV and cholecystectomy significantly increased the pH of the gastric contents in all of them (Table 37). Raw data is given in Appendix J.

TABLE 37: pH OF GASTRIC CONTENTS UNDER BASAL FASTING CONDITIONS AND DURING SECRETIN STIMULATION IN DOGS WITH HSV AND CHOLECYSTECTOMY

<table>
<thead>
<tr>
<th>Dog</th>
<th>( \bar{x} \pm \text{SEM} ) (basal conditions)</th>
<th>( \bar{x} \pm \text{SEM} ) (secretin infusion)</th>
<th>Statistical significance (t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DD_7</td>
<td>3,539 ± 0.078 (13)</td>
<td>4,503 ± 0.332 (8)</td>
<td>p 0.05</td>
</tr>
<tr>
<td>DD_9</td>
<td>3,372 ± 0.084 (20)</td>
<td>5,270 ± 0.376 (7)</td>
<td>p 0.01</td>
</tr>
<tr>
<td>DD_13</td>
<td>3,430 ± 0.254 (21)</td>
<td>5,901 ± 0.469 (8)</td>
<td>p 0.01</td>
</tr>
</tbody>
</table>

\( \bar{x} \) = mean value of pH
SEM = standard error of the mean
() = numbers in parentheses represent the number of tests

7.2.8.3 Secretin infusion before cholecystectomy compared with infusion after cholecystectomy

The pH of gastric contents during secretin stimulation in dogs with HSV and an intact gall bladder was not statistically different (p 0.05) from the pH during secretin stimulation in dogs with HSV and a cholecystectomy (Table 38)
### TABLE 38: pH OF GASTRIC CONTENTS DURING SECRETIN STIMULATION BEFORE AND AFTER CHOLECYSTECTOMY

<table>
<thead>
<tr>
<th>Dog</th>
<th>$\bar{x} \pm SEM$ (secretin before cholecystectomy)</th>
<th>$\bar{x} \pm SEM$ (secretin after cholecystectomy)</th>
<th>Statistical significance (t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$DD_7$</td>
<td>4.109 ± 0.356 (7)</td>
<td>4.503 ± 0.332 (8)</td>
<td>$p &gt; 0.05$</td>
</tr>
<tr>
<td>$DD_9$</td>
<td>5.630 ± 0.456 (8)</td>
<td>5.270 ± 0.376 (7)</td>
<td>$p &gt; 0.05$</td>
</tr>
<tr>
<td>$DD_{13}$</td>
<td>5.693 ± 0.139 (8)</td>
<td>5.901 ± 0.469 (8)</td>
<td>$p &gt; 0.05$</td>
</tr>
</tbody>
</table>

- $\bar{x}$ = mean value of pH
- SEM = standard error of the means
- () = numbers in parentheses represent the number of tests

7.2.9 Post-mortem findings

7.2.9.1 Macroscopic findings

Post-mortem examinations were carried out in all 3 dogs after they had been sacrificed by intravenous injection of sodium pentobarbitone (section 5.1.3). Besides minor skin sepsis around the gastrostomy cannula exit site, no other abnormality was found.

The stomach, duodenum, liver and spleen were all macroscopically normal. No macroscopic lesions were seen on the gastric mucosa.
Control mucosal biopsies were taken from the greater and lesser curvature of the antrum and fundus, when the HSV was performed and again at cholecystectomy (section 7.1.10). No mucosal abnormality was found in any of the biopsies.

After the dogs had been sacrificed, the gastric mucosa was examined using the 'Swiss roll' technique (section 5.1.7). No abnormalities were found in any of the dogs.
7.2.10 Comparison of dogs with HSV with: (a) dogs with an intact stomach, and (b) dogs with TV+P.

In a previous section, 6.2.15, dogs with IV+P were compared with dogs with an intact stomach. In this section a comparison will be made between dogs with HSV (group C) and (a) dogs with an intact stomach (group A), and (b) dogs with TV+P (group B).

7.2.10.1 Bile reflux under basal fasting conditions

The amount of bile reflux in 3 dogs (25 tests) with HSV was compared with the reflux in 5 dogs (35 tests) with intact vagi and an intact pylorus. There was no significant difference between the two groups (Mann-Whitney, p > 0.05). However, when cholecystectomy was added to all dogs, bile reflux was significantly higher in the group with intact vagi and an intact pylorus (54 tests in group C compared with 71 tests in group A, p < 0.01).

The amount of bile reflux in 3 dogs (25 tests) with HSV was compared with the reflux in 3 dogs (23 tests) with TV+P and no significant difference was found (p > 0.05). When cholecystectomy was added to both groups, the dogs with TV+P had significantly higher reflux than the dogs with HSV (54 tests in dogs of group C compared with 73 tests in group B, p < 0.01).
7.2.10.2  Bile reflux during secretin stimulation

The amount of bile reflux during secretin stimulation in dogs with HSV (23 tests) was compared with reflux in dogs with intact vagi and an intact pylorus (35 tests) and no significant difference was found ($p>0.05$). When cholecystectomy was added to both groups of dogs there was again no significant difference (23 tests in group C and 43 tests in group A, $p>0.05$).

The amount of bile reflux during secretin stimulation in dogs with TV+P (30 tests) was significantly higher than in dogs with HSV (23 tests) ($p<0.05$). This difference remained so when cholecystectomy was added to both groups (29 tests in group B and 23 tests in group C, $p<0.05$).

7.2.10.3  Ratios of lecithin to lysolecithin

7.2.10.3.1  Under basal fasting conditions

The ratios of lecithin to lysolecithin in 3 dogs with HSV (25 tests) were compared with the ratios in 5 dogs with intact vagi and an intact pylorus (35 tests). In dogs with HSV, the values of these ratios were significantly higher than in the dogs with intact vagi and an intact pylorus ($p<0.01$). When cholecystectomy was added to each group, again the ratios of lecithin to lysolecithin in dogs with HSV (54 tests) were significantly higher than the ratios in dogs of group A (80 tests) ($p<0.01$).
The ratios of lecithin to lysolecithin in 3 dogs (25 tests) with HSV were compared with the ratios in 4 dogs (30 tests) with TV+P. In dogs with HSV, these ratios were significantly higher ($p<0.01$) and remained so when cholecystectomy was added to both groups (group B 73 tests, group C 54 tests, $p<0.01$).

7.2.10.3.2 During secretin stimulation

The ratios of lecithin to lysolecithin during secretin stimulation in dogs with HSV (23 tests) were significantly higher ($p<0.01$) than in dogs with intact vagi and an intact pylorus (35 tests). This difference remained significant after cholecystectomy was performed on both groups of dogs (36 tests in group A, 23 in group C, $p<0.01$).

The ratios of lecithin to lysolecithin during secretin stimulation in dogs with HSV (23 tests) were significantly higher ($p<0.01$) than in dogs with TV+P and secretin stimulation (30 tests). This difference remained significant after cholecystectomy was added to both groups (30 tests in group B and 23 in group C, $p<0.01$).

7.2.10.4 Volumes of gastric contents

7.2.10.4.1 Under basal fasting conditions

The pre-cholecystectomy volumes of gastric contents collected under basal fasting conditions in dogs with HSV (25 tests) were
significantly smaller (p<0.01) than in dogs with intact vagi and an intact pylorus (35 tests). This difference remained significant after a cholecystectomy was added to both groups (80 tests in group A, 54 in group C, p<0.01).

The pre-cholecystectomy volumes of gastric contents in dogs with HSV (25 tests) were significantly smaller (p<0.01) than in dogs with TV+P (30 tests). After a cholecystectomy was added to both groups, this difference remained significant (73 tests in group B, 54 in group C, p<0.01).

7.2.10.4.2 During secretin stimulation

The pre-cholecystectomy volumes of gastric contents during secretin stimulation in dogs with HSV (23 tests) were significantly smaller (p<0.01) than in dogs with intact vagi and an intact pylorus (35 tests). After cholecystectomy was performed on both groups of dogs, secretin stimulation in dogs with HSV (23 tests) was again associated with smaller volumes (p<0.01) than those in dogs of group A (36 tests).

The pre-cholecystectomy volumes of gastric contents during secretin stimulation in dogs with HSV (23 tests) were significantly smaller (p<0.01) than in dogs with TV+P (30 secretin tests). This difference remained significant after cholecystectomy was carried out on all dogs (29 secretin tests in group B, 23 in group C, p<0.01).
7.2.10.5  pH of gastric contents

7.2.10.5.1 Under basal fasting conditions

The pre-cholecystectomy pH of gastric contents in dogs with HSV (25 tests) was significantly higher ($p<0.01$) than in dogs with intact vagi and an intact pylorus (35 tests). This difference remained significant after cholecystectomy was performed on all dogs (80 tests in group A, 54 in group C, $p<0.01$).

The pre-cholecystectomy pH of gastric contents in dogs with HSV (25 tests) was not significantly different from the pH in dogs with TV+P (30 tests, $p>0.05$). The pH in dogs with TV+P post-cholecystectomy (73 tests) was significantly higher ($p<0.05$) than that in dogs with HSV (54 tests).

7.2.10.5.2 During secretin stimulation

The pre-cholecystectomy pH during secretin stimulation in dogs with HSV (23 tests) was significantly higher ($p<0.01$) than that in dogs with intact vagi and an intact pylorus (35 tests). This difference remained significant after a cholecystectomy was added to all dogs (23 secretin tests in group C and 43 in group A).

The pre-cholecystectomy pH during secretin stimulation in dogs with HSV (23 tests) was significantly lower ($p<0.01$) than in dogs with TV+P (30 secretin tests). This difference remained unchanged after cholecystectomy (23 secretin tests in group C).
and 30 in group B, p<0.01).

7.2.10.6 Histological findings

None of the dogs with HSV developed any mucosal abnormality, whilst 2 of the 5 dogs with an intact stomach and 2 of the 4 dogs with TV+P developed gastritis by the end of the experiments.

7.3 DISCUSSION

7.3.1 Discussion of materials and methods

In this part of the study, 4 dogs were used. One of them (DD) was excluded due to a positive Hollander test, therefore tests were performed on only 3 dogs. While the number of dogs used was relatively small, each dog acted as its own control and a large number of tests were carried out on each animal. A total of 79 tests on the 3 animals were carried out under basal fasting conditions, and 46 during secretin stimulation.

7.3.2 Discussion of biochemical findings

The amount of bile reflux in the 3 dogs with HSV did not differ significantly from the reflux that occurred in the 5 dogs in group A (intact vagi and an intact pylorus) nor from that which was seen in 3 of the 4 dogs of group B (TV+P) (section 7.2.10.1). However, the nature of the refluxed bile was different: the ratios of lecithin to lysolecithin in dogs with
TV+P or HSV were significantly higher than in dogs with intact vagi (section 7.2.10.3.1). It may be that vagotomy inhibits the production of lysolecithin from lecithin. In the case of TV+P the possible mechanisms have been discussed in section 6.3.1. A possible explanation of the effect of HSV on lysolecithin production is that HSV may indirectly inhibit secretin release by inhibiting gastric acid secretion. It is known that hydrochloric acid stimulates the release of secretin (81). In the present study, secretin was found to promote lysolecithin production.

When cholecystectomy was performed on all dogs with HSV, reflux did not increase. In contrast with this observation, reflux did increase in cholecystectomy-mized dogs with TV+P or with intact vagi (sections 5.2.6 and 6.2.6). It seems that HSV prevents D/G reflux. Dewar et al (245,247,266) reported that the concentration of bile acids and lysolecithin in the stomach of patients with gastric or duodenal ulcers decreased significantly after a HSV was performed. The authors suggested that after HSV, the receptive relaxation of the gastric fundus was lost, resulting in increased intragastric pressures. These high pressures combined with an intact antropyloroduodenal segment may prevent reflux from the duodenum into the stomach. Furthermore, the increased rate of gastric emptying of liquids which follows HSV might discourage reflux. An alternative explanation in human patients with duodenal ulcer may be that HSV allowed the ulcer
to heal, so that a normal physiological state was restored.

Results in the present study suggest that HSV is associated with faster emptying of liquids than occurs with TV+P: the volumes of gastric contents in dogs with HSV were significantly smaller than in dogs with TV+P (section 7.2.10.4.1). The volume of gastric contents in a fasting state are determined mainly by three factors: gastric secretion, gastric emptying, and duodeno-gastric reflux. It is unlikely that the rate of gastric secretion was different in dogs with HSV or TV, since in both groups the same acid secreting areas were denervated. Duodeno-gastric reflux would be expected to be the same in both groups since all the dogs with HSV and 3 dogs with TV+P had the same concentrations of lecithin and lysolecithin in the stomach. If the gastric secretion rate and D/G reflux can be disregarded the most probable cause of smaller volumes of gastric contents in dogs with HSV, is a faster gastric emptying of liquids. If this is so it could be an explanation as to why cholecystectomy combined with HSV was not associated with increased bile reflux: a fast gastric emptying of liquids may discourage D/G reflux and empty any refluxed material before it mixes with gastric contents. However this hypothesis is presented with reservations since previous studies have not shown a faster emptying of liquids in HSV than TV+P. A detailed review of the relevant literature is presented in section 3.1.2.3.
Secretin stimulation in dogs with HSV promoted bile reflux into the stomach, both before and after cholecystectomy. A possible explanation might be the effect of secretin on the pressure gradient across the pylorus. A detailed discussion has been presented in section 5.3.2.

As in dogs of groups A and B, secretin favoured lysolecithin production both before and after cholecystectomy. Possible explanations have been given in section 5.3.2.

Secretin stimulation in dogs with HSV resulted in significantly lesser amounts of bile reflux than in dogs with TV+P, both before and after cholecystectomy (section 7.2.10.2). It seems that the pressure changes induced by secretin at the antroduodenal region and the presence of pyloroplasty make local conditions more favourable for reflux.

7.3.3 Clinical significance of the results of the present study

The results of the present study suggest that HSV might protect the stomach against D/G reflux in two ways: firstly by discouraging D/G reflux and secondly by inhibiting production of lysolecithin from lecithin. Pathophysiologically, lysolecithin is a cytotoxic agent which damages gastric mucosa. The present experiments showed that when cholecystectomy was added to HSV there was no increased reflux as was the case in cholecystectomy-mized dogs with intact vagi or with TV+P. Dewar et al (245,
(247, 266) showed that HSV decreased bile reflux in humans with gastric or duodenal ulcers. However, it still remains to be shown if increased bile reflux in the absence of peptic ulcer will reverse after HSV. This could be studied by performing an HSV on dogs with increased bile reflux after cholecystectomy. However this was not undertaken in the present study because of the technical difficulties associated with HSV in the presence of a permanent gastrostomy cannula. This matter will be the subject of a future study.

7.4 CONCLUSION

The object of this part of the present investigation was to examine the relationship between bile reflux into the stomach and cholecystectomy under different experimental conditions. The amount of bile reflux in dogs with HSV before and after cholecystectomy, and after secretin stimulation was measured. HSV alone was associated with the same amount of bile reflux that occurred both in dogs with intact vagi, and in most of the animals who had undergone TV+P.

When a cholecystectomy was added to the HSV, reflux did not increase. However, when a cholecystectomy was carried out in dogs with intact vagi and an intact pylorus (group A) or in dogs with TV+P (group B), there was a significant increase in the amount of bile reflux. A possible explanation for the post-
cholecystectomy increased bile reflux in groups A and B has been offered in sections 5.3.2 and 6.3.1.

A possible explanation as to why HSV combined with cholecystectomy was not associated with increased reflux, is that the increased intragastric pressures which follow HSV, combined with an intact antropyloroduodenal segment, might discourage reflux. A more rapid gastric emptying following HSV may clear any refluxed bile from the stomach rapidly, and thus prevents it from mixing with gastric contents. The mucosa would therefore be exposed to the effect of bile for a period that would be insufficient to allow damage to occur. In addition, HSV may inhibit the production of lysolecithin, a substance known to be cytotoxic, and possibly of importance in the pathogenesis of gastritis.

Under the experimental conditions described, it was established that an increase of bile reflux into the stomach follows cholecystectomy in dogs with an intact stomach or TV+P. However, cholecystectomy in dogs with HSV did not increase bile reflux. While again the danger of extrapolation from the animal to the human situation is well recognized, it would seem that should a human subject require surgical management for combined duodenal ulceration and gall bladder disease at the same time, cholecystectomy and HSV would be the appropriate procedures.
8. FINAL CONCLUSION
An experimental study was designed to investigate the possible relationship between cholecystectomy and the occurrence of bile reflux into the stomach, under various conditions. Duodenogastric reflux is an intermittent phenomenon which varies from time to time in the same experimental animal. In order to obtain a meaningful assessment of reflux, a large number of experiments, over long periods of time, were performed on each dog. Cholecystectomy alone was found to promote bile reflux into the stomach. This is probably the result of the continuous presence of bile in the duodenal contents which follows cholecystectomy. However, in 2 of the 5 dogs the post-cholecystectomy increase of the amount of bile reflux was transient, lasting for about 2 months, and then returning to pre-cholecystectomy levels. This could be the result of changes of the common bile duct, which, in dogs, dilates after cholecystectomy. A dilated common bile duct may take over part of the reservoir function of the gall bladder. Two of the dogs with consistently increased post-cholecystectomy bile reflux developed histological gastritis.

TV+P alone was not invariably associated with increased bile reflux. However, when cholecystectomy was added, all dogs developed persistently increased amounts of bile reflux. This reflux was not significantly higher than that observed in dogs with cholecystectomy alone. These results suggest that perhaps the pylorus does not play a major role in preventing duodeno-
gastric reflux. Two of the dogs with TV+P and cholecystectomy developed histological gastritis, 6 months after cholecystectomy, when the study was concluded.

HSV alone was associated with the same amount of bile reflux as was observed in dogs with an intact stomach, and in 3 of the 4 dogs with TV+P. However, when cholecystectomy was added there was no increase in reflux. It is possible that HSV may discourage reflux. Alternatively, any refluxed material may empty faster before mixing with gastric contents. None of the dogs with HSV developed histological gastritis.

Both HSV and TV+P seem to inhibit the production of lysolecithin from lecithin. Pathophysiologically, this is important because lysolecithin is a cytotoxic agent which is injurious to gastric mucosa. Secretin stimulation promoted duodenogastric reflux in all groups of dogs, probably by changing the pressures in the antropyloroduodenal segment. Secretin promoted lysolecithin production in all groups of dogs.

While recognizing the danger of extrapolation of experimental results to humans, it would seem from this experimental study that some cases of the so-called 'post-cholecystectomy syndrome' might be due to abnormal amounts of bile refluxing into the stomach. The results of the present study suggest that the amount of reflux that occurs with TV+P and cholecystectomy, is
not more than that which occurs with cholecystectomy alone. Moreover, reflux with HSV and cholecystectomy is less than the reflux observed with cholecystectomy alone, or with TV+P and cholecystectomy.
REFERENCES


Mackie, C.R., Wisbey, M., Cuschieri, A. 


Author  Demetriades D
Name of thesis  The effect of cholecystectomy on duodenogastric reflux an experimental study  1984

PUBLISHER:
University of the Witwatersrand, Johannesburg
©2013

LEGAL NOTICES:

Copyright Notice: All materials on the University of the Witwatersrand, Johannesburg Library website are protected by South African copyright law and may not be distributed, transmitted, displayed, or otherwise published in any format, without the prior written permission of the copyright owner.

Disclaimer and Terms of Use: Provided that you maintain all copyright and other notices contained therein, you may download material (one machine readable copy and one print copy per page) for your personal and/or educational non-commercial use only.

The University of the Witwatersrand, Johannesburg, is not responsible for any errors or omissions and excludes any and all liability for any errors in or omissions from the information on the Library website.