5.2.5 Reflux in the control animals

A total of 35 tests under basal fasting conditions were done (Table 2). There was always evidence of a small amount of bile reflux (lecithin + lysolecithin) in all 5 animals. However, the amount of reflux varied from animal to animal, and from time to time in the same animal. Details of reflux in each individual dog are shown in Figures 3-7. The raw data is presented in Appendix B. The ratio of lecithin: lysolecithin again varied from animal to animal and from time to time in the same animal, although the variation between dogs was not statistically significant (p > 0.05 - t-test) (Table 3). The raw data is shown in appendix B.

5.2.6 The effect of cholecystectomy on bile reflux

A total of 80 tests under basal fasting conditions were performed on 5 dogs after cholecystectomy (13 tests on DD₂, 10 on DD₃, 19 on each of DD₄, DD₅ and DD₆) (Table 2). All 5 dogs had a higher concentration of bile markers (lecithin and lysolecithin) in the stomach after cholecystectomy than pre-operatively. In dogs DD₄, DD₅ and DD₆, this change was statistically very significant (p < 0.01 Mann-Whitney test) and remained so for 6 months after cholecystectomy, until the dogs were sacrificed (Figures 3, 4 and 5). However, in the remaining 2 dogs this change was transient. In DD₂ bile reflux was significantly higher (p < 0.05) than before cholecystectomy for 10 weeks, thereafter returning to pre-
cholecystectomy levels (Figure 6). In DD, the change was significant (p<0.01) for only the first 8 weeks after cholecystectomy (Figure 7). When the results from all 5 dogs (35 tests before cholecystectomy and 80 after cholecystectomy) were analysed collectively, the post-cholecystectomy bile reflux was significantly higher (p<0.01). Both the phospholipids, lecithin and lysolecithin, were significantly and proportionately increased.

The total amount of bile phospholipids as well as the ratio of lecithin to lysolecithin varied from dog to dog and from time to time in the same animal. This ratio did not vary significantly from the pre-cholecystectomy values in any of the dogs (p>0.05, Table 3). All raw data is shown in Appendix B.

TABLE 3: RATIO OF LECITHIN TO LYSOLECITHIN BEFORE AND AFTER CHOLECYSTECTOMY (Basal Fasting Conditions)

<table>
<thead>
<tr>
<th>Dog</th>
<th>$x_R \pm SEM_R$ Before cholecystectomy</th>
<th>$x_R \pm SEM_R$ After cholecystectomy</th>
<th>Statistical Significance (t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DD₂</td>
<td>2.69 ± 0.25 (7)</td>
<td>3.54 ± 0.25 (13)</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>DD₃</td>
<td>2.77 ± 0.45 (7)</td>
<td>3.52 ± 0.28 (10)</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>DD₄</td>
<td>3.72 ± 0.71 (8)</td>
<td>3.87 ± 0.32 (19)</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>DD₅</td>
<td>4.91 ± 0.68 (7)</td>
<td>3.41 ± 0.33 (19)</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>DD₆</td>
<td>5.16 ± 0.55 (6)</td>
<td>4.04 ± 0.41 (19)</td>
<td>p &gt; 0.05</td>
</tr>
</tbody>
</table>

$x_R$ = mean value of the ratio of lecithin to lysolecithin

SEM$_R$ = standard error of the mean of the ratios

() = number in parentheses represents number of tests.
Figure 3: Bile reflux (represented by the sum of lecithin and lysolecithin concentrations) before and after cholecystectomy in Dog DD. After cholecystectomy bile reflux was significantly higher (Mann-Whitney test, p<0.01). The tests were started 3 weeks after operation, and they were performed at an average of 1 per week. (n = number of tests. Each point represents the amount of reflux during a 6-hour test).
Figure 4: Bile reflux before and after cholecystectomy in Dog DD₅. The difference was statistically significant (p < 0.01).

(n = number of tests)
Figure 5: Bile reflux before and after cholecystectomy in Dog DD$_6$. The difference was statistically significant ($p<0.01$). ($n =$ number of tests).
Figure 6: Bile reflux before and after cholecystectomy in dog DD₂. During the first 10 weeks the post-cholecystectomy reflux was significantly higher (p<0.05). After this period reflux returned to pre-cholecystectomy levels. (n = number of tests)
Figure 7: Bile reflux before and after cholecystectomy in dog DD3. During the first 8 weeks the post-cholecystectomy reflux was significantly higher than before cholecystectomy (p<0.01), thereafter returning to pre-cholecystectomy levels.

(n = number of tests)
The effect of secretin on bile reflux before cholecystectomy

A total of 35 secretin-infusion tests were done on 5 dogs (6 tests on DD_6, 7 on each of DD_2, DD_3 and DD_5, 8 on DD_4) (Table 2). Secretin consistently and significantly increased bile reflux (both lecithin and lysolecithin concentrations) in the stomach in all 5 dogs (Mann-Whitney test, p<0.01 in DD_2, DD_3, DD_5 and DD_6 and p<0.05 in DD_4, overall p<0.01). It was of interest to note that within a few minutes of the commencement of secretin infusion, the colour of the collected gastric juice changed abruptly from clear to bright yellow. The amount of reflux varied from dog to dog and from time to time in the same dog, but not statistically significantly (p>0.05). Figures 8-12 show the amount of reflux before and after secretin stimulation in pre-cholecystectomy dogs. Each secretin test was performed immediately after a test under basal fasting conditions.

Secretin infusion increased the proportion of lysolecithin in the lecithin:lysolecithin ratio. This change in favour of lysolecithin was statistically significant in 4 out of 5 dogs (DD_2, DD_3, DD_5, DD_6). In dog DD_4 there was a definite change in favour of lysolecithin, but it was not statistically significant (t-test p>0.005). Overall the change was significant (p<0.01). Details are shown in Table 4. This ratio did not vary significantly between any 2 of the 5 dogs (t-test p>0.05). All raw data of the effect of secretin stimulation on dogs with an intact gall bladder is presented in Appendix C.
### TABLE 4: RATIO OF LECITHIN TO LYSOLECITHIN BEFORE AND AFTER SECRETIN INFUSION IN DOGS WITH INTACT GALL BLADDER

<table>
<thead>
<tr>
<th>Dog</th>
<th>$\bar{x}_R \pm SEM_R$ (basal fasting conditions)</th>
<th>$\bar{x}_R \pm SEM_R$ (secretin)</th>
<th>Statistical Significance (t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DD2</td>
<td>2.693 ± 0.258 (7)</td>
<td>1.524 ± 0.162 (7)</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>DD3</td>
<td>2.775 ± 0.453 (7)</td>
<td>1.238 ± 0.766 (7)</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>DD4</td>
<td>3.722 ± 0.711 (8)</td>
<td>2.475 ± 0.402 (8)</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td>DD4</td>
<td>4.910 ± 0.686 (7)</td>
<td>2.604 ± 0.386 (7)</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>DD6</td>
<td>5.168 ± 0.557 (6)</td>
<td>2.441 ± 0.907 (6)</td>
<td>p &lt; 0.01</td>
</tr>
</tbody>
</table>

$\bar{x}_R$ = mean value of the ratio of lecithin to lysolecithin

$SEM_R$ = standard error of the mean of the ratios

() = numbers in parentheses represent number of tests
Figure 3: Bile reflux in DD_2 - pre-cholecystectomy - before and after secretin stimulation (p<0.01). (n = number of tests)
Figure 9: Bile reflux in DD3 - pre-cholecystectomy - before
and after secretin stimulation (p < 0.01) (n = number of tests)
Dog DD₄ before cholecystectomy
- - - baseline fasting conditions
(n=8)
- - - after secretin
(n=8)

Figure 10: Bile reflux in DD₄ - pre-cholecystectomy - before and after secretin stimulation (p<0.05). (n = number of tests)
Figure 11: Bile reflux in DD₅ before cholecystectomy - before and after secretin stimulation (p<0.01). (n = number of tests)
Figure 12: Bile reflux in DD<sub>6</sub> - pre-cholecystectomy - before and after secretin stimulation (p<0.01). (n = number of tests)
5.2.8 The effect of secretin on bile reflux in cholecystectomized dogs.

A total of 45 tests with secretin stimulation were done on 5 cholecystectomized dogs (8 tests on DD$_2$ and DD$_3$, 11 on DD$_4$, 9 on DD$_5$ and 5 on DD$_6$) (Table 2). The effect of secretin on bile reflux varied considerably. Sometimes secretin was associated with an increase in reflux, sometimes there was no change and sometimes there was a decrease. Overall there was no significant change in the amount of reflux after secretin infusion when compared with the one under basal fasting conditions (Mann-Whitney test, p > 0.05 in all 5 dogs, individually and collectively as a single group). Graphs in Figures 13 to 17 show the bile reflux in individual cholecystectomized dogs, before and after secretin. All raw data is presented in Appendix D.

Secretin stimulation changed the ratio of lecithin to lysolecithin in favour of lysolecithin when compared with ratio under basal fasting conditions (t-test $p < 0.01$ in DD$_2$, DD$_3$ and DD$_6$, $p < 0.05$ in DD$_4$ and DD$_5$) (Table 5). Overall the change was significant at a level of $p < 0.01$. This ratio did not vary significantly between any two of the 5 dogs.
TABLE 5: RATIO OF LECITHIN TO LYSOLECITHIN BEFORE AND AFTER SECRETIN INFUSION IN CHOLECYSTECTOMIZED DOGS

<table>
<thead>
<tr>
<th>Dog</th>
<th>$\bar{x}_B \pm \text{SEM}_B$ (Basal fasting conditions)</th>
<th>$\bar{x}_S \pm \text{SEM}_S$ (Secretin)</th>
<th>Statistical Significance (t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DD2</td>
<td>3.545 ± 0.250 (13)</td>
<td>1.633 ± 0.320 (8)</td>
<td>$p &lt; 0.01$</td>
</tr>
<tr>
<td>DD3</td>
<td>3.525 ± 0.285 (10)</td>
<td>2.012 ± 0.369 (8)</td>
<td>$p &lt; 0.01$</td>
</tr>
<tr>
<td>DD4</td>
<td>3.879 ± 0.324 (19)</td>
<td>2.561 ± 0.389 (11)</td>
<td>$p &lt; 0.05$</td>
</tr>
<tr>
<td>DD5</td>
<td>3.419 ± 0.332 (19)</td>
<td>2.347 ± 0.395 (9)</td>
<td>$p &lt; 0.05$</td>
</tr>
<tr>
<td>DD6</td>
<td>4.044 ± 0.413 (19)</td>
<td>2.149 ± 0.343 (7)</td>
<td>$p &lt; 0.01$</td>
</tr>
</tbody>
</table>

$\bar{x}_R$ = mean value of the ratio lecithin to lysolecithin  
$\text{SEM}_R$ = standard error of the mean of the ratios  
() = numbers in parentheses represent number of tests

5.2.9 Secretin stimulation and bile reflux before and after cholecystectomy

The amount of bile reflux during secretin stimulation before and after cholecystectomy was compared in 5 dogs individually (Figures 18 to 22) and collectively as a single group. There was no significant difference in any of the dogs (Mann-Whitney tests, $p > 0.05$ in each of the 5 dogs, overall $p > 0.05$). The ratio of lecithin to lysolecithin again did not differ significantly ($p > 0.05$) in any of the 5 dogs (Tables 4 and 5).
Figure 13: Bile reflux in DD2 - post-cholecystectomy - before and after secretin stimulation (p > 0.05). (n = number of tests)
Reflux Dcg DC 3 after cholecystectomy > basal fasting conditions (n=10) secretin stimulation (n=8)

Figure 14: Bile reflux in DD3 - post-cholecystectomy - before and after secretin stimulation (p > 0.05).

(n = number of tests)
Figure 15: Bile reflux in DD$_4$ - post-cholecystectomy - before and after secretin stimulation ($p>0.05$).

(n = number of tests)
Figure 16: Bile reflux in DD after cholecystectomy: before and after secretin stimulation (p > 0.05). (n = number of tests)
Figure 17: Bile reflux in DD₆ - post-cholecystectomy - before and after secretin stimulation (p > 0.05). (n = number of tests)
Figure 18: Bile reflux in DD₂ during secretin stimulation before and after cholecystectomy (p > 0.05). (n = number of tests)
Figure 19: Bile reflux in DD3 during secretin stimulation before and after cholecystectomy (p > 0.05).

(n = number of tests)