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The effect of cholecystectomy on duodenogastric reflux : an experimental study

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5. The effect of cholecystectomy on D/G reflux.

6. The effect of TV+P and cholecystectomy on D/G reflux.

7. The effect of HSV and cholecystectomy on D/G reflux.

8. Final conclusion.

5. THE EFFECT OF CHOLECYSTECTOMY ON BILE REFLUX

5.1 Materials and Methods

5.1.1 Preparation of the experimental animal

5.1.1.1 The experimental animal

Six unselected mongrel dogs, trained to stand quietly in a Pavlov dog stand, were used. Each dog acted as its own control. The pre-operative weights ranged between 9.6 and 24 Kg. There were four females and 2 males.

5.1.1.2 Anaesthesia

All operations were performed under general anaesthesia. Intravenous pentobarbitone sodium (60 mg/ml) was administered in a dose of 1 ml/Kg body weight. This gave satisfactory relaxation, while the animal continued to breath spontaneously. An endotracheal tube was used routinely. Pure oxygen was administered during the procedure.

5.1.1.3 Insertion of the gastrostomy cannulae

The abdomen was entered by means of a right paramedian incision. Care was taken in the region of the xiphisternum not to enter the pleural cavity. A 4 cm long incision was made in the anterior wall of the upper part of the gastric fundus. The inner nylon cannula (Figure 1,A) was inserted via the opening into the stomach. The tubular part of the cannula was brought out through another small incision on the anterior wall of the

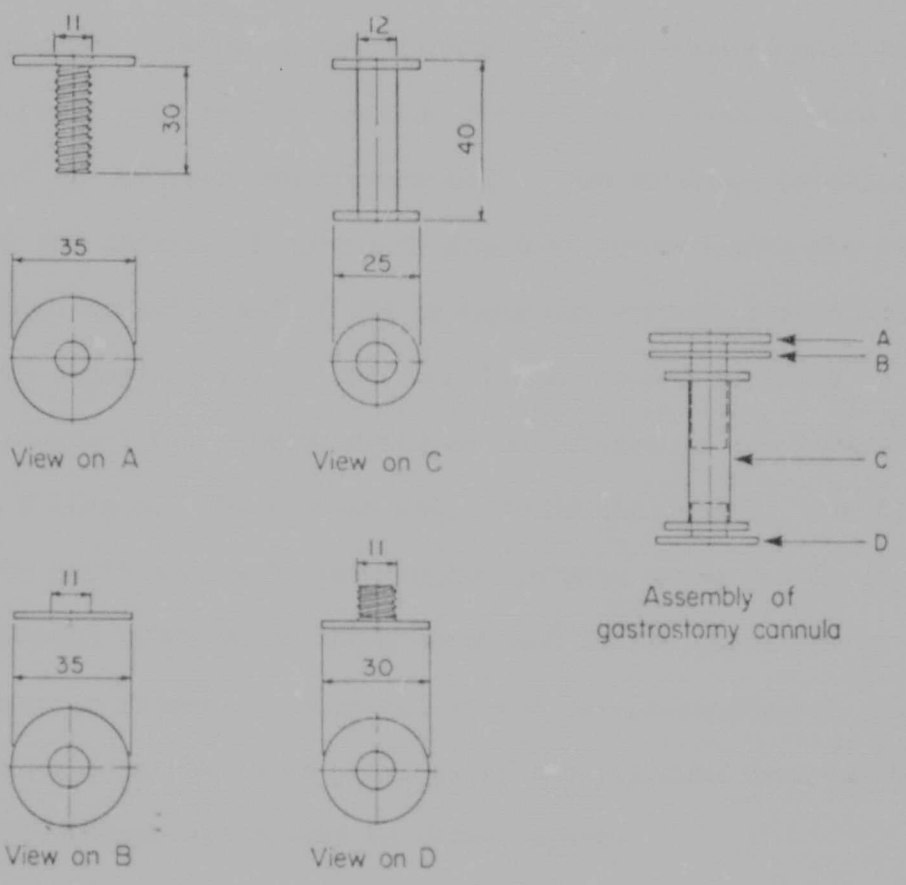


Figure 1. Modified Thomas cannula: Individual parts and a sectional view of an assembled cannula.
The figures given are in mm.

stomach, close to the greater curvature, 8 cm proximal to the pylorus. The nylon nut (Figure 1,B) was mounted onto the tubular part of the cannula, outside the stomach, with care not to compress the stomach wall. The external metallic part of the cannula (Figure 1,C) was then screwed onto the inner nylon cannula, and it was brought out through a stab wound in the abdominal wall. The cannula was sealed by means of a cap (Figure 1,D). The gastrostomy was closed in two layers with 3/0 catgut. The stomach wall around the cannula, was fixed to the peritoneum with interrupted sutures using 2/0 Dexon. Mucosal biopsies of the greater and lesser curvature of the gastric fundus (5 cm distal to the gastro-esophageal junction) and the antrum (3 cm proximal to the pylorus) were taken, and the abdomen was closed in three layers.

5.1.1.4 Cholecystectomy

At a second operation, about 2 to 3 months after the gastric cannula, the abdomen was re-opened through the scar of the previous opeation (5.1.1.3). The cystic duct was ligated and divided about 0,5 cm proximal to its junction with the common bile duct. The cystic artery was ligated and the gall bladder was removed subserosally. Care was taken to ligate any accessory bile ducts in the bed of the gall bladder. Mucosal biopsies were taken again as in 5.1.1.3, and the abdominal wall was closed in three layers.

5.1.1.5 Post-operative management

Post-operatively, the animal was fed with fluids for 2 days, and received antibiotics (Ampicillin, 0.5 gm daily) for 5 days.

5.1.2 Measurement of bile reflux

The estimation of the amount of bile reflux involved the detection and quantification of duodenal content-markers in the stomach (section 4.2.2). In this study lecithin and lysolecithin were used as the markers of reflux. The sum of the concentration of the substances in the stomach represented the index of the amount of reflux. The advantages of using the sum of these two markers rather than one marker only, to represent the reflux, is explained in section 5.1.11.2.

5.1.3 Collection of gastric contents

5.1.3.1 During fasting conditions

The experimental animals received no food but had access to water for 16 hours prior to the scheduled collection. The collection was performed with the dog in a Pavlov stand and no restraint or sedation was used. The cap of the gastrostomy cannula was removed and the lumen was cleaned of any food remnants. A 10 minute interval was allowed and then a urine bag containing 0.1 gm of disodium EDTA (Merck), was connected to the cannula. The EDTA prevented the formation of lysolecithin from lecithin during the collection (3.5.1). The gastric con-

tents were collected over the following 6 hours (8 am to 2 pm). The volume of the collection was then measured and two aliquots were taken and stored at -20°C.

5.1.3.2 During fasting with secretin infusion

This collection was performed immediately after the 6 hour collection described in 5.1.3.1. A urine bag, containing 0,1 gm of disodium EDTA was connected to the gastrostomy cannula, and an intravenous line was established in one of the front legs. Secretin (Boots), dissolved in 30 ml of normal saline, was infused via a pump (Harvard apparatus, model 931 A), at a rate of 1 unit/Kg body weight/hour. The volume of a 2 hour collection was measured and two aliquots were taken and stored at -20°C.

5.1.4 Method of lecithin and lysolecithin measurement

These phospholipids were extracted from gastric contents and separated by means of thin layer chromatography (TLC), and their concentration was estimated by phosphorus determination (250,251).

5.1.4.1 Phospholipid extraction and separation

The specimens collected in 5.1.3.1 and 5.1.3.2 were allowed to liquify at room temperature.

1.6 ml of gastric contents was placed in a 18 ml culture tube. If the gastric content was grossly bile stained, it was diluted with distilled water. (The values were corrected at the end by multiplying by the dilution factor).

Four ml methanol (Analar, Merck) were added and the solution was vortexed for 20 seconds.

Two ml of chloroform (Analar, Merck) were then added and the mixture was shaken in a Griffin flask shaker for 10 minutes.

Two ml $CHCL_3$ were added and the mixture was vortexed for 30 seconds.

After the addition of 2 ml of distilled water and mixing for 30 seconds, the tube was centrifuged at 3000 g for 5 minutes.

The subnatant produced after centrifugation was withdrawn with a syringe and filtered through fat-free Whatman No. 1 filter paper wetted with $CHCl_3$ and containing 1 g anhydrous sodium sulphate.

A second lipid extraction from the supernatant was performed by adding 4 ml of $CHCl_3$, mixing for 20 seconds, centrifuging for 5 minutes and withdrawing the subnatant. The lipid containing subnatants were combined and evaporated at 60°C under nitrogen.

A TLC plate (20 x 20 cm, Machery and Nagel Polygram Sil.G) was pencil-marked. The line of origin was drawn 1,5 cm from the bottom edge of the plate. A 1 cm margin was drawn at each

lateral side of the plate, and vertical lines 3 cm apart marked 5 lanes.

The dried phospholipid extract was redissolved in 0,1 ml $CHCl_3$, mixed for 5 seconds, aspirated with a Hamilton syringe, and applied to the line of origin on the TLC plate. The tube was rinsed with a further 0,03 ml $CHCl_3$ and the solution applied to the same line of origin. A hair dryer was used to speed up drying of the applied spots.

Lecithin and lysolecithin standards (SIGMA) were run with each plate.

The TLC plate was placed for 45 minutes in a Camag tank, lined with Whatman No.1 chromatography paper and containing the developing solution (60 ml $CHCl_3$, 30 ml MeOH, 8,4 ml glacial acetic acid, 3,6 ml distilled water).

The TLC plate was taken out of the tank and dried at 160°C in an oven for 1 minute.

The plate was then exposed to iodine vapour in a closed plastic box for 2 minutes. The lecithin and lysolecithin bands were identified with the help of the respective standards. Areas of equal size were marked around all the lecithin and lysolecithin bands.

At the top edge of the TLC plate, three solvent containing areas of equal size to the lecithin containing areas, and another three equal to the

lysolecithin containing areas, were marked, to act as standards and blanks.

5.1.4.2 Phosphorus determination

All tubes used during the following stages were free of phosphorus contamination. This was achieved by cleaning them with $K_2Cr_2O_7$ solution and rinsing well with glass distilled water.

The phospholipid containing areas and marked areas at the top of the TLC plate (5.1.4.1) were scraped into labelled 22 ml pyrex tubes.

One hundred µl of KH_2PO_4 solution (5% phosphorus) were added to each of 4 tubes containing the scrapings from the TLC areas (5.1.4.1). Two of these tubes acted as lecithin standards and the other two as lysolecithin standards.

0,3 ml of 5M H_2SO_4 (Analar) and 0,1 ml of H_2O_2 30% (Perhydrol, Merck) were added to each of the tubes containing the scraped TLC areas.

The rack with all tubes was placed in a slanting position (to prevent splashing of contents), in a hot oven (210°C) for 30 minutes.

The rack with its tubes was taken out of the oven and 0,1 ml H_2O_2 30% was added to all tubes. The rack with tubes was placed in the oven for a further 30 minutes or until the contents were colourless.

The rack was taken out of the oven and the tubes were left to cool. The contents of each tube were rinsed down by adding 1 ml of distilled water.

Each tube was vortexed in an almost horizontal position for 20 seconds. The contents were poured into correspondingly labelled 10 ml conical centrifuge tubes, and were centrifuged at 3000 g for 5 minutes. 0,5 ml of the supernatant was added to correspondingly labelled tubes containing 0,5 ml of ammonium molybdate tetrahydrate 0,4% (Baker, Analar), and 0,05 ml of freshly prepared L-ascorbic acid 0,6% solution, and vortexed for 1 second. Glass bubbles were put on top of the tubes.

All tubes were placed in Dri-Block (Thehne DB-3) at 100°C for 15 minutes. The tubes were taken out of the Dri-Block, placed in a pre-cooled rack, and left to cool for 5 minutes. Then they were centrifuged for 1 minute at 3000 g.

The colour density of the solution was read at 700 nm on a Unicam SP 500 spectophotometer.

5.1.4.3 Lecithin and lysolecithin estimation

The calculation of the concentration of lecithin and lysolecithin was made using the following formulae:

$$\frac{O.D.S_L}{O.D.St_L} \times 7,4 = \text{mgr \% Lecithin}$$

$$\frac{O.D.S_{LL}}{O.D.St_{LL}} \times 5 = \text{mgr \% lysolecithin}$$

$O.D.S_L$ = optical density of lecithin specimen

$O.D.St_L$ = the average optical density of the two lecithin standards.

$O.D.S_{LL}$ = optical density of lysolecithin specimen

$O.D.St_{LL}$ = the average optical density of the two lysolecithin standards.

(The value '7,4' in the formula for lecithin was calculated as follows:

One mmol of dipalmitoyl lecithin (molecular weight 732) contains 30,97 mgr of phosphorus, therefore the 0,005 mgr of phosphorus in the standard (page 48) is contained in 0,118 mgr of lecithin. This refers to a specimen of 1,6 mls, therefore 100 mls of specimen will be $\frac{0,118 \times 100}{1,16} = 7,4$. The value of '5' for dipalmitoyl lysolecithin (molecular weight 495) formula was estimated in the same way).

5.1.5 Measurement of gastric juice pH

The pH was measured at room temperature using a pH meter. (PHM 82, Radiometer Copenhagen).

5.1.6 Design of the gastrostomy cannula

A modified Thomas cannula (252) was designed by the author and manufactured in the anaesthetic workshop of Baragwanath Hospital.

It consisted of 4 parts (Figure 1): An outer aluminium tube C (internal diameter 12 mm, length 40 mm) with small flanges at both ends (diameter 25 mm), an inner nylon tube A (external diameter 11 mm, length 36 mm) with a flange at one end (diameter 35 mm), a nylon nut B (diameter 35 mm, orifice 12 mm), and an aluminium cap D. The individual parts of the cannula, and a sectional view of an assembled cannula are shown in Figure 1. The insertion of the cannula is described in 5.1.1.3.

5.1.7 Histological assessment

Mucosal biopsies from the greater and lesser curvature of the gastric fundus and antrum were taken when the gastrostomy was made (5.1.1.3) and again at cholecystectomy (5.1.1.4). They were prepared and stained with hematoxylin and eosin, using the standard techniques.

After the experimental animals had been sacrificed the stomach was examined histologically using the 'Swiss roll' method, as described by Stein (253): the stomach was opened along the greater curvature and fixed in 10% formal-saline solution for 2 weeks. It was then cut into strips about 6 mm wide, and the mucosa was excised from the underlying muscle. The strips were rolled without tension, with the proximal end of the stomach inside the roll, and the mucosal side facing inwards (Figure 2). There were 5 'Swiss rolls' from each stomach, representing the anterior wall of the greater curvature, the anterior wall, the lesser curvature, the posterior wall, and the posterior wall of

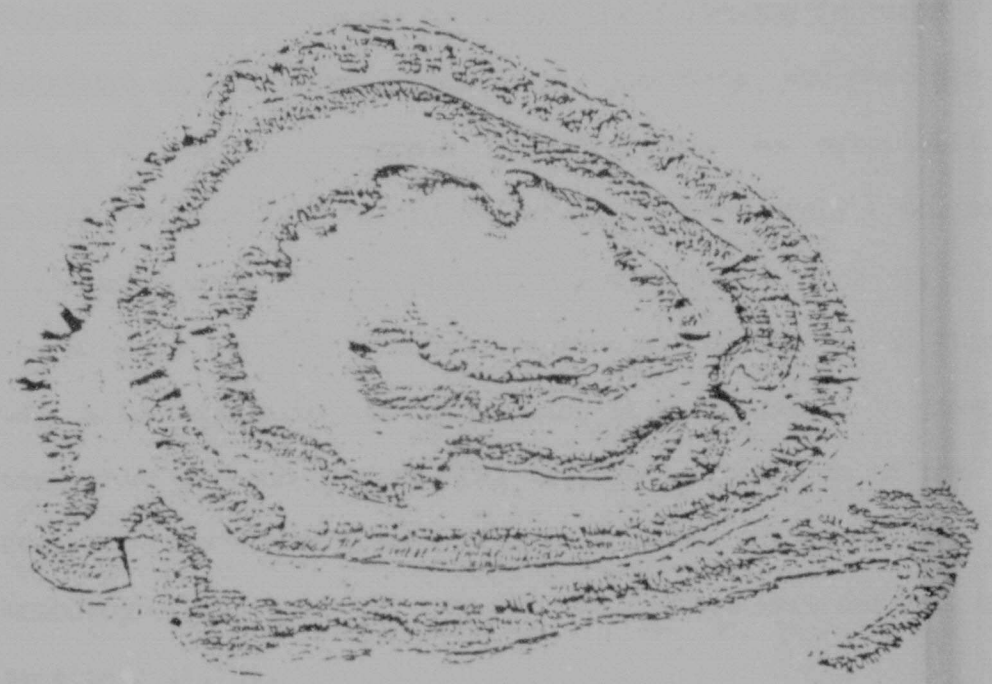


Figure 2: The appearance of a typical 'Swiss roll'.

the greater curvature. All strips were at least 3 cm from the cannula site. A pin was inserted through the roll to prevent it from unrolling. Fixation and staining were performed using conventional techniques (253). The amount and distribution of gastritis was measured using Lawson's method (254). Starting from the distal end of the roll the entire length was examined histologically. At a magnification of 100 x the size of each

field was 2 mm, as measured with a stage and ocular micrometer. In each field the most prominent histological feature was recorded. It was elected to record the following features serially: normal mucosa, superficial gastritis, atrophic gastritis of the mild, moderate or severe types, and metaplasia. It was convenient to record the total findings every 6 mm, as this represented three consecutive 2 mm fields. In every 6 mm the total of each histological feature was estimated. In this way, after examining all five rolls, it was possible to give the amount of each histological feature in each area of the stomach. The findings were presented on bar diagrams. The histological criteria for the abnormal mucosa were defined in section 3.11.2.1.

5.1.8 Assessment of the physical condition of the experimental animals.

The physical condition and the weight of the experimental animals were checked weekly. Mild skin sepsis around the exit of the gastrostomy cannula was a frequent complication. It was treated with application of antiseptic ointment.

5.1.9 Postmortem examination

Postmortem examination was performed on all dogs. They were sacrificed by intravenous injection of 10 ml of Euthanaze (Sodium Pentobarbitone 200 mgm per ml). The abdomen was

examined for any possible pathology, and the stomach was removed and prepared as described in section 5.1.7. Dogs were sacrificed when all the necessary data were obtained, or when the animal was unsuitable for further experiments (eg. weight loss more than 20% of the original).

5.1.10 Design of the experimental study

Six mongrel dogs were used in this study, and each dog acted as its own control. Initially, a gastrostomy cannula was inserted and control biopsies were taken from the gastric fundus and antrum, as described in section 5.1.1.3. The dogs were allowed a period of 2 to 3 weeks to recover before any tests were conducted. Each animal was subjected to a minimum of 6 and a maximum of 8 six-hour collections of gastric juice under fasting conditions and without any stimulation, as described in section 5.1.3.1. An equivalent number of two-hour collections during continuous secretin infusion (section 5.1.3.2) was carried out on each dog. The period between two successive collections 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 analysed at random for lecithin and lysolecithin concentration (5.1.4) and the pH was measured (section 5.1.5).

In the second part of the study each dog underwent a cholecystectomy and biopsies were taken from the fundus and antrum (section 5.1.1.4). After a recovery period of 2 to 3 weeks, gastric collections with or without secretin stimulation were performed

(section 5.1.3). Ten to 19 six-hour collections without stimulation were performed on each dog at intervals of 1 to 3 weeks. Eight to 11 two-hour collections during secretin stimulation were performed on each animal, with a minimal interval of 1 week between two consecutive collections. The specimens were stored at -20°C and were later analysed at random as described before. The experimental animals were sacrificed 3 to 6 months after cholecystectomy and the stomach was removed and examined histologically, as described in section 5.1.7.

5.1.11 Original techniques developed by the author

5.1.11.1 Measurement of D/G reflux

In this study D/G reflux was measured under fasting basal conditions over a continuous 6-hour period (section 5.1.3.1). During the experiments it became evident from the colour of gastric juice that reflux was occurring intermittently during the same test: very often the gastric juice was noted to be macroscopically clear initially, bile stained later and then again clear. This observation is in agreement with the findings of Sorgi et al (255). The motility pattern of the antrum, pylorus and the duodenum (an important factor in D/G reflux) is not stable but may be influenced by psychomatic (29) and local factors, such as secretory activity of the upper gastrointestinal tract. Its motility and secretory activity occurs in alternating cycles of activity and quiescence (30). By measuring reflux over a 6-hour period, the results attained were far more likely to be represen-

tative, than measurements taken over shorter periods. In addition to the 6-hour long period, reflux was estimated on a number of occasions in each dog. Six to 8 control tests and 13 to 19 post-cholecystectomy tests were done in each dog (section 5.1.10). This further enhances the accuracy of the picture of reflux. As far as the author is aware, this is the first time that reflux has been measured so many times in each experimental animal and over such long periods of time.

5.1.11.2 Duodenal markers used for measuring D/G reflux

In this study the amount of reflux was represented by the sum of the concentration of lecithin and lysolecithin in the stomach (section 5.1.2). In previous studies, reflux was measured by estimating only the concentration of lysolecithin in the stomach. However, this may lead to false conclusions in regard to the amount of reflux: lysolecithin is produced in the duodenum and upper jejunum from lecithin (section 3.5.1). The ratio of lecithin/lysolecithin in the duodenum varies from time to time and depends on the experimental conditions (eg. secretin infusion or vagotomy). An impression of increased reflux may be given when the concentration of lysolecithin in the stomach is high despite low concentrations of lecithin. Similarly, low concentration of lysolecithin in the stomach may suggest small amounts of reflux despite high concentration of lecithin. In addition, lysolecithin may be produced from lecithin *in vitro*, after the

collection of the specimen. By using the sum of both substances as an index of the amount reflux, the above problems were overcome. To the best of the author's knowledge, this is the first time that both phospholipids have been used for the estimation of D/G reflux.

5.1.11.3 The experimental study

As far as the author is aware, no previous experimental controlled study has been reported that investigated the relationship between cholecystectomy and bile reflux.

5.1.12 Statistical methods employed

Results were analyzed individually for each dog, and collectively (as a single group) for all 5 dogs. For statistical analysis of the amount of D/G reflux, the Mann-Whitney non-parametric test of uncontrolled data was used (256) because of the abnormal distribution of frequencies. The data concerning pH, ratios of lecithin to lysolecithin, and the volumes of gastric collections was suitable for t-test analysis, therefore this test was applied. For the statistical analysis of the histological findings the X^2 test was used.

5.1.13 Critical evaluation of the techniques used

5.1.13.1 Method used to measure D/G reflux

The technique used has certain disadvantages: (a) the presence of a gastrostomy cannula may interfere with gastric motility and intragastric pressure. This problem was minimized by the fact that a cannula was present during the control test as well. (b) This method does not give any information about the rate of gastric emptying, a factor which is a significant determinant of the damaging effect of the refluxed material on the gastric mucosa (33,75,76). (c) It does not take into account the rate of gastric secretion, a factor which may affect the concentration of duodenal contents in the stomach (233). However, it is the concentration of cytotoxic contents in the stomach that finally determines the mucosal damage, irrespectively of the various determinants of reflux (234). (d) This method requires time consuming chromatographic techniques (about 6 hours per test. Six samples could be assessed simultaneously).

The method, however, has many advantages: (a) it gives information about the concentration of cytotoxic agents in the stomach. Pathophysiologically this is more important than knowing the volume of reflux. (b) It is possible to get information about reflux over long periods of time. The importance of this has been discussed in section 5.1.11.1. The recently developed techniques which used radioactive biliary markers, such as

^{14}C-chenodeoxycholic acid and $^{99}TC^{m}$-EHIDA to study bile reflux, were considered unsuitable in this study because of certain problems (section 4.2.3). The more serious of the problems are that, firstly, there is always some retention of the tracer in the gall bladder, therefore the estimated amount of bile reflux before cholecystectomy cannot be compared with that after cholecystectomy. Secondly, excretion by the liver is completed in about 1 hour, therefore these markers cannot be used to study bile reflux over long periods of time (242).

5.1.13.2 Efficiency of phospholipid determination

The technique used for determination of phospholipids in the gastric juice involved many stages (section 5.1.4) and as a result there was some loss of lecithin and lysolecithin during the procedure. The efficiency of recovery of lecithin and lysolecithin from gastric contents was assessed by adding known quantities of pure lecithin and lysolecithin to gastric juice samples, in both the low and high ranges, and then recovering them.

The efficiency of recovery of lecithin and lysolecithin in high range concentrations was estimated as follows: Five 1,6 ml samples of gastric juice with a pre-determined concentration of lecithin of 28,305 mg% (452 µgr per 1,6 ml) and lysolecithin at a concentration of 3,733 mg% (60 µgr per 1,6 ml) were used. In each sample 150 µgr (15 µlt) of pure lecithin (Sigma) and 160 µgr

(6,4 µlt) of pure lysolecithin (Sigma) were added. The samples were analyzed and the recovery of lecithin was found to be 85,34 $\pm$ 1,046 % ($\bar{x} \pm$ SEM). The recovery of lysolecithin was 90,64 $\pm$ 1,292 % ($\bar{x} \pm$ SEM). Raw data is given in Appendix A.

The efficiency of recovering lecithin and lyslecithin from the specimens of low concentrations was estimated as follows: Five 1,6 ml samples of gastric juice with a known concentration of lecithin of 2,109 mg% (33 µgr per 1,6 ml) and 0,356 mg% (5,7 µgr per 1,6 ml) of lysolecithin were used. 10 µgr (1 µlt) of pure lecithin and 2,5 µgr (0,1 µlt) of pure lysolecithin were added to each sample. The samples were analyzed and the mean lecithin recovery was 90,94 $\pm$ 0,920 % ($\bar{x} \pm$ SEM). The mean recovery of lysolecithin was 89,52 $\pm$ 1,136 % ($\bar{x} \pm$ SEM). Raw data is given in Appendix A. Analysis of the specimens was done at random, therefore any changes due to new batches of chemicals, or improved techniques of the author were minimized.

5.1.13.3 Method used for histological assessment

Final histological assessment was by means of the 'Swiss roll' technique and the amount and distribution of any histological abnormality was estimated by Lawson's method (section 5.1.7). With this technique it was possible to examine the whole stomach and quantitatively record any abnormality. This is important because the distribution of gastritis is often patchy (211). Simple biopsies may miss areas of significant pathology.

Obviously the 'Swiss roll' technique could not be used during the histological evaluation of the control stage, when only relatively small amounts of biopsy material were available.

5.1.13.4 Design of the experimental study

Before starting to collect gastric juice under fasting basal conditions, the tightly fitted cap of the gastrostomy cannula had to be removed and the cannula cleared of any food remnants. These manoeuvres may promote reflux. To minimize this problem, a minimum period of 10 minutes was allowed before the collection was started (section 5.1.3.1). Another problem during gastric collection was the *in vitro* formation of lysolecithin from lecithin. This problem was solved by adding EDTA to the collection bags, thus innibiting this reaction (168).

5.2 RESULTS

5.2.1 Introduction

This study investigates the effect of cholecystectomy and secretin on bile reflux. Data is presented as follows:

a) Details of the experimental animals used.

b) Number and analysis of the tests done.

c) Data of bile reflux in control animals.

d) Data on the effect of cholecystectomy on bile reflux.

e) Data on the effect of secretin on bile reflux.

f) Data on volumes of gastric contents collected during the tests.

g) Data on pH of gastric contents collections.

h) Postmortem findings.

i) Histological findings.

5.2.2 Presentation of data

Data of the amount of reflux is presented in the form of a series of graphs. Results from each dog are shown on a separate graph and are statistically analysed individually and collectively as a single group. All raw data obtained in the study is presented in Appendices B, C and D. Histological data is presented in bar diagrams.

5.2.3 Experimental animals

Six mongrel dogs, each one acting as its own control, were used in this part of the study. There was one early death (Dog DD_1) therefore experiments were done on a total of 5 dogs (DD_2, DD_3, DD_4, DD_5, DD_6). All these animals remained healthy during the experiments. In none of them was there significant weight loss. The only complication encountered was minor sepsis around the exit of the gastrostomy cannula within the abdominal wall. Details of the animals used in the study are shown in Table 1.

TABLE 1 : DETAILS OF DOGS USED IN THE STUDY

Dog	Sex	Weight (Kg) *	**	***	Total duration of experiments (months)	Duration of experiments after cholecystectomy (months)
DD_2	F	17	18,7	22	6	4
DD_3	M	14	13,4	14,4	6	3
DD_4	F	24	28,1	29,5	9	6
DD_5	F	12	12,3	11	8	6
DD_6	F	9,6	8,6	11	8	6

* at the time of gastrostomy cannula insertion

** at the time of cholecystectomy

*** at the end of the experiments

5.2.4 Number of experiments carried out

A total of 193 tests were performed on 5 dogs. There were 35 measurements of reflux under fasting basal conditions before cholecystectomy, 35 with secretin stimulation before cholecystectomy, 80 under fasting basal conditions after cholecystectomy and 45 with secretin stimulation after cholecystectomy. A detailed breakdown of the tests is shown in Table 2.

TABLE 2 : NUMBERS OF TESTS CARRIED OUT

Dog	No. of tests under fasting conditions before cholecystectomy	No. of tests with secretin stimulation before cholecystectomy	No. of tests under fasting conditions after cholecystectomy	No. of tests with secretin stimulation after cholecystectomy	Total
DD_2	7	7	13	8	35
DD_3	7	7	10	8	32
DD_4	8	8	19	11	46
DD_5	7	7	19	9	42
DD_6	6	6	19	7	38
Total	35	35	80	43	193

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