

AN EXPERIMENTAL ENQUIRY INTO THE FORMATION OF GALL-STONES. BY VAUGHAN HARLEY, M.D., AND WAKELIN BARRATT, M.D. (Two Figures in Text.)

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IN the present paper an endeavour is made to throw some light on the formation of gall-stones by observing the effect of introducing cholesterol calculi or fragments of calculi into the gall-bladder of dogs, both in health and in various morbid states. Within the last decade many observers have studied experimentally the factors of cholesterol stone formation on the one hand and the conditions of production and consequences of cholecystitis on the other.

French observers seem to have obtained results more successful than those of German observers, for Gilbert¹, with Dominici and Fournier, succeeded in producing experimentally in dogs, rabbits, and guinea-pigs, biliary calculi after the injection of colon bacilli into the gall-bladder.

Mignot² found that non-virulent organisms introduced into the gall-bladder together with a foreign body—especially if porous, like cotton-wool—lead with the greatest certainty to the formation of gall-stones. The kind of bacteria injected seems to be of quite secondary importance. Those used were *b. typhosus*, *b. coli*, staphylococci and *b. subtilis*. Mignot found also that foreign bodies introduced into the gall-bladder can stay there for an indefinite time, provided they are aseptic, without causing inflammation or precipitating solids from the bile.

More recently, the German observers Ehret and Stolz³ have called attention to the difficulty of producing cholecystitis by artificial injection; these observers state that no result can be obtained by introducing

¹ *Arch. gén. de méd.* 1898, i. 2, 257–263. Also Gilbert et Fournier, *Bull. de la Soc. de Biologie*, 30 Oct. 1897.

² *Arch. gén. de méd.* 1898, i. 2, 263–282.

³ *Berl. klin. Wochenschr.* 1902, 39 Jahrg. 13, 15.

b. coli into the gall-bladder so long as the stream of bile is undisturbed. This is due to the movements of the gall-bladder and the action of normal bile. Injury to the motility of the gall-bladder, as by the presence of a foreign body, will alone lead to the appearance of organisms in the gall-bladder. Ehret and Stolz, and also Riedel¹ believe that gall-stones when present in a gall-bladder affected with cholecystitis are usually not the product of the inflammatory process. They further find that when aseptic glass balls or cotton-wool are introduced into the gall-bladder no immediate effect is produced, but after many weeks a suppurative cholangitis with numerous organisms in the bile is produced. The most common organisms are the b. coli group, but streptococci and staphylococci, b. mesentericus and sarcinae are also met with. Gilbert² also found that in seventy cases of cholelithiasis the colon bacillus was present in the bile in about one-third.

Naunyn also thinks that the presence of a foreign body in the gall-bladder is not essential to the formation of a gall-stone. He arrives at this latter conclusion partly from the results of some observations of Labes, who introduced various bodies, e.g. decomposing animal matter, acid and alkaline substances and gall-stones, into the gall-bladders of dogs without finding that any deposit of biliary matter formed on them. The gall-stones even disappeared after a time. Marcantonio found however that pieces of amber introduced into the gall-bladder of a dog became incrustated with a deposit of biliary matter³.

This brief *résumé* of the literature of the subject illustrates how diverse have been the results obtained by various observers; it therefore seemed that the question of gall-stone formation and cholecystitis left ample field for further investigation. Owing to the fact that a research like the present involves the necessity of keeping the animals under observation for a very long time, it has been in progress for some years, and was therefore commenced before the publication of some of the more recent experiments mentioned above.

Method. In the present series of experiments only dogs were used, and in order to make the paper as brief as possible the general operative procedure will merely be described, minor details being omitted. Healthy dogs, which had been accustomed to live in the laboratory, were anaesthetised by means of ether, and after the abdomen

¹ *Berl. klin. Wochenschr.* 1901, 38 Jahrg. 1.

² *Loc. cit.*

³ The above is quoted from E. M. Brockbank, *On Gall-Stones*, London, 1896, p. 67.

had been carefully shaved, well washed and treated with antiseptics, an incision two or three inches long was made in the middle line from the xyphoid downwards. The gall-bladder was then drawn forward and by gently squeezing, as much bile as possible was forced through the bile-duct, the gall-bladder being more or less empty; the fundus was then attached to the upper part of the abdominal incision, the lower part of the wound being closed, without contamination of the peritoneal cavity. A small incision was then made in the gall-bladder itself, and through the opening, fragments of gall-stone—the size of a hazel-nut or smaller, and with more or less rough edges—were inserted. The incision into the gall-bladder was then closed by means of fine silk, and the abdominal surface afterwards closed. In those cases where a bacteria culture or pus was inserted one was able by this means to avoid any contamination of the peritoneum, as was shown by the autopsy in no case revealing peritonitis, and in very few cases much adhesion. The edges of the wound having been united, a dressing of boracic acid was applied, and rapid healing took place. The animals were at first after the operation given milk together with white of egg, the diet being gradually improved; and during the rest of the time they were kept under observation they were fed with dog-biscuits and occasional meat.

The fragments of gall-stones were in all cases more or less similar. The composition of two of the fragments is shown in the following table:—

Water %	19.90	15.81
Solids %	80.15	84.23
Cholesterin %	77.24	77.30
CaO %	0.20	0.88
Ash %	2.75	2.71

From these two analyses we see that the calculi were principally of the cholesterin type, although there was a certain amount of bilirubin calcium present.

THE EFFECT OF HEALTHY DOGS' BILE ON A GALL-STONE WHEN INSERTED INTO THE GALL-BLADDER.

In this series we have 5 experiments, in 4 of which the gall-stone was merely inserted into the gall-bladder, and the animals kept alive from 6 months to 1 year.

EXP. 1. Fox-terrier, weight 7.500 kilos. Two gall-stones were inserted into the gall-bladder, each stone about the size of a hazel-nut. The gall-

bladder was not attached to the abdominal wall, but merely sutured and allowed to lie free in the abdominal cavity. After the dog had been kept in the laboratory for a year it was killed by chloroform. There were practically no adhesions, except that the gall-bladder was slightly adherent to the omentum; no signs of peritonitis were present. The mucous membrane of the gall-bladder was normal. The bile was also natural in aspect, being clear and quite free from suspended matter. The gall-stones introduced had entirely disappeared; the cystic duct was small in size and presented a normal appearance. Microscopical sections of the wall of the gall-bladder were typically healthy in aspect.

EXP. 2. Wire-terrier bitch, weight 7·200 kilos. Two fragments of gall-stones, measuring a little over a centimetre in the longest diameter, and just under a centimetre in the smallest diameter, were inserted into the gall-bladder with antiseptic precautions. The animal lived in apparently perfect health for some time, but towards the end refused to eat biscuits and had to be fed with meat. After 265 days the dog choked itself with a large lump of meat and was found to weigh only 4·800 kilos. The section showed no morbid changes in the abdomen. The gall-bladder appeared perfectly healthy and the microscopical examination of its wall showed nothing abnormal, the epithelium being unaltered. The bile was dark emerald-green and contained a good deal of mucus, but no *débris*; no gall-stones or remains of gall-stones were to be found within the gall-bladder, cystic duct or common bile-duct. No micro-organisms were found on the walls of the gall-bladder or in the bile. The liver substance was only slightly adherent to the gall-bladder.

EXP. 3. Fox-terrier. In this case the animal was about 7 kilos. Two fragments of gall-stones were introduced, as in the preceding experiment, into the gall-bladder, and six months later the animal was killed. The gall-bladder but for slight linear scarring on the outer surface was in every respect normal and contained bile which was free from suspended matter. The gall-stones introduced had entirely disappeared; the cystic duct and ductus communis choledochus were also unaltered in size and aspect.

EXP. 4. Fox-terrier. Two fragments, of rather larger size than the previous ones, were inserted into the gall-bladder. The animal was killed at the end of six months, during which time he had remained in perfect health. Careful examination of the gall-bladder, ducts, and surrounding parts failed to reveal any structural change beyond a slight scarring of the former. The bile was dark in colour and free from suspended matter. The cholesterin stones introduced into the gall-bladder had entirely disappeared.

EXP. 5. Small black and tan terrier, weight 4·500 kilos. With the ordinary antiseptic precautions the fundus of the gall-bladder was stitched

to the abdominal wall, and through an incision $\frac{1}{4}$ of an inch long into the gall-bladder three stones were inserted, the largest being a little over 1 centimetre long $\times 0.5 \times 0.5$ centimetres, the others slightly smaller. The gall-bladder was sutured and the fundus of the gall-bladder stitched to the abdominal wall, a knotted silk thread being drawn down through the wound to indicate the position of the gall-bladder. Eight days later a culture of bacillus coli was injected into the gall-bladder, taking the direction of the thick thread. The dog was killed 101 days later and was found to weigh 5 kilos. The tip of the gall-bladder was found to be adherent to the abdominal wall, the gall-bladder itself being somewhat elongated. Slight omental adhesions were present, but no peritonitis. The bile was thin and contained no gall-stones or *débris*, and was found to contain no micro-organisms. Microscopical examination of the gall-bladder wall showed it to be in every respect normal. The bile was thin and clear, and free from *débris* and suspended matter, and cultures from the bile all remained sterile. In this case, in all probability, the colon bacilli which were inoculated into the gall-bladder were washed out by the bile.

In these five experiments we found that in all cases the gall-stones disappeared in a period varying from 1 year to 6 months; and in those cases in which micro-organisms were searched for none were found, either in the substance of the gall-bladder or in the bile.

THE EFFECT OF THE CONTENTS OF DOGS' GALL-BLADDER UPON FRAGMENTS OF CALCULI WHEN CHOLECYSTITIS WAS PRESENT.

Exp. 6. Wire-haired fox-terrier, weight 7.650 kilos. The gall-bladder was stitched to the margin of the wound; the peritoneal cavity having been closed the gall-bladder was opened and a piece of gall-stone, 1.5 centimetres $\times 0.5$ centimetres $\times 0.5$ centimetres, inserted. At the same time, some pus was inoculated into the gall-bladder from a case of suppurative cholecystitis in a human subject. The pus had previously been found to contain the colon bacillus. The gall-bladder was then stitched, the wound carefully washed and sewn up in the ordinary way. The dog was killed 85 days later, and weighed 7.750 kilos. The gall-bladder was found adherent at its fundus, somewhat elongated, and the cystic duct was dilated as far as its juncture with the first hepatic duct. The gall-bladder contained light-coloured bile with streaks of mucus. The fragment of stone was near the entrance of the cystic duct, and was apparently of the original size, the edges still being sharp and the radiations of the calculus still distinct. The wall of the gall-bladder appeared to be thickened: microscopically, it showed changes in the mucous membrane, the epithelium of which was swollen and vacuolated, and

covered with an amorphous deposit. There was no clear increase in number, or change of character, in the sub-epithelial cells of the mucous coat or in those of the sub-mucous coat, which showed no marked structural change; no micro-organisms were recognisable in the deposit covering the surface or elsewhere. The gall-stone fragment was analysed. As compared with the fraction of the original gall-stone, the only apparent alteration it exhibited was a slight increase in the amount of water it contained, which had increased from 15·81% to 22·55%, while the solids had diminished from 84% to 77·45%. The total ash was 2·99%, the CaO appeared slightly increased, being 0·99% instead of 0·88%.

Exp. 7. Wire-haired Irish terrier, weight 6 kilos. The gall-bladder was stitched to the upper part of the wound and isolated from the peritoneal cavity, as in the previous case. Three pieces of the same gall-stone, together with dry pus from the same case, were inserted into the gall-bladder; two of the fragments of gall-stone were about as large as a mulberry, the third a little smaller. The animal was killed 56 days later, then weighing 7·5 kilos. The gall-bladder was firmly adherent to the abdominal wall, but no inflammatory process was found around, only some slight omental adhesions. The gall-bladder contained the two larger calculi, apparently unaltered in condition, together with one small black mass and three small lumps, the size of pins' heads. The bile was not very thick, although it had a good deal of mucus. Microscopically, the bile was found to contain a good deal of pigmented material together with bacilli forming fine threads. No cholesterin crystals were present. The epithelium of the mucous membrane of the gall-bladder was seen to be swollen and vacuolated; among the epithelial cells a few wandering ones were found. This change was less marked at the bottom of the recesses of the mucous membrane than at the summit of the projections which this coat presented. In a few places the surface of the epithelium had disappeared, leaving a raw surface. Lying upon the surface of the epithelium was an amorphous or finely reticular deposit, which did not stain well with a fibrin stain. In the connective tissue lying beneath the surface of the epithelium injected capillaries were seen where the epithelium was most altered. The number of the nuclei in the connective tissue of the mucous and submucous coats was considerable, but it is difficult to be sure that an actual increase of the cells had taken place. No micro-organisms were found on the surface or in the substance of the gall-bladder wall.

Exp. 8. Mongrel dog, weight 7 kilos. This animal was operated on exactly as the two preceding, fragments of calculi the same size being introduced into the gall-bladder, together with some pus containing virulent bacilli coli, which had been inoculated into a rabbit. The dog was killed 52 days later, and weighed 8·15 kilos, having been in very good health. The gall-bladder was firmly adherent to the abdominal wall; there were

slight mesenteric attachments, but no peritonitis. One fragment of gall-stone remained apparently unaltered, with sharp edges, while the other fragments had entirely disappeared. The gall-bladder appeared thickened, and the bile apparently normal. On microscopical examination, the surface epithelium of the gall-bladder was found to be swollen and vacuolated, with here and there wandering cells. On the summit of the elevations of the mucous membrane these changes were most marked, and occasionally in these situations a detachment of epithelium was found; but there was less alteration of the surface of the epithelium than in the previous case. A slight granular deposit staining diffusely, without any recognisable structure, covered the surface epithelium; there was some injection of the capillaries lying below the mucous membrane, but no cell infiltration around these vessels. No clear increase of the cells contained in the mucous or submucous coats could be made out. No micro-organisms could be demonstrated lying upon or within the wall of the gall-bladder.

EXP. 9. Fox-terrier, weight 6.75 kilos. The operation was exactly similar to the preceding, two fragments being inserted, one 3 centimetres, the other $1\frac{1}{2}$ centimetres long, both having diameters of 1 centimetre. At the same time some pus containing micro-organisms was inserted into the gall-bladder. The animal was killed 51 days later and weighed 6.9 kilos. Some adhesions were found round the gall-bladder, but no peritonitis. The wall of the gall-bladder appeared thickened; the two calculi were present, the smaller appearing exactly as it was originally, the larger fragment of gall-stone being surrounded by and embedded in pale yellow cheese-like substance. The epithelium of the mucous membrane of the gall-bladder was markedly vacuolated and swollen, and in some places disintegrated or shed. It was covered with an amorphous material, staining diffusely and containing bacilli, arranged singly in pairs or groups, and sometimes in short chains, resembling in size and shape the colon bacillus, exhibiting a variable staining capacity by Loeffler's method and retaining the stain by Gram's method. Where the surface of the epithelium was abraded these bacilli might be seen to penetrate a short distance into the sub-epithelial tissue, but no evidence of infiltration of multinuclear or mononuclear cells was to be found here or elsewhere. The elevations of the mucous membrane were elongated. There was some oedema of the mucous and submucous tissue, the capillaries of which were injected. Fibroid tissue was found to have developed all round the gall-bladder; the muscular coat appeared to be thickened. The caseous material from the gall-bladder consisted of granular matter together with fine needles, often arranged in rosettes, somewhat brown in colour. No epithelium or other formed material was present, and neither cholesterin nor fat globules were recognisable. On heating, the caseous material charred, and on incineration, a white residue was left which gave

off bubbles when moistened with 33% acetic acid. During charring an odour of burnt leather was given off. On staining by Loeffler's method, numerous bacilli about $2.9\ \mu$ in diameter were seen, resembling colon bacilli, and retaining the stain by Gram's method. The analysis of the caseous matter showed:—Water 5.19%, Solids 94.81%, Ash 9.54%, Lime 7.63%, Soluble matter in ether and alcohol (fat) 10.6%. There was no sulphur. Cultures from the caseous material gave the following results: On agar, a rapidly growing white film, resembling that of colon bacillus; on glucose gelatine, no liquefaction occurred, but an abundant formation of gas. Milk was curdled at the end of 48 hours; reaction at the end of 8 days was strongly acid. In broth a turbidity was produced at the end of 24 hours and a deposit of a whitish colour was seen at the bottom of the test-tube. The bacilli had the size and shape already described, were non-motile and were decolorised by Gram's method with great slowness. An offensive odour was given off in agar, gelatine, and broth culture, and to a less extent, in milk. Indol reaction in broth culture was not obtained. The growth was therefore one of bacillus coli.

Exp. 10. Brown terrier, weight 6.9 kilos. The operation was the same as the previous one, one large fragment, $2 \times 1 \times 1$ centimetres, being inserted. The animal was killed 40 days later, as it became ill, and during the last month rapidly lost weight, going off its food; when it was fed on meat it improved somewhat, but later again became thin, weighing only 4.75 kilos. The abdominal cavity showed some adhesions, but only round the gall-bladder; the gall-bladder itself was shrunken and contained very little bile, and the stone retained its sharp edges, only one being slightly rounded off; the size appeared to be unaltered. The gall-bladder itself appeared to be thickened, and microscopically there was marked vacuolation and swelling of the epithelium, which was in places diffusely stained and much disintegrated. The elevations of the mucous membrane were elongated; the sub-epithelium appeared œdematous and contained cells with multipartite nuclei in sparse numbers, and the capillaries were somewhat congested. The muscular coat was thin. There was much fibroid tissue surrounding the gall-bladder externally. The mucous membrane was covered with a deposit of an amorphous character, containing bacilli $2.2\ \mu$ long, varying much in staining reaction by Loeffler's method, decolorising slowly by Gram's method, and arranged singly or in short chains; these organisms usually stained uniformly, but some exhibited bipolar staining.

Exp. 11. Black and tan terrier, weight 6.4 kilos. Two fragments of gall-stone, 1.2 and 1.5 centimetres long respectively by 1 centimetre in the other direction, were inserted into the gall-bladder. In this case no pus was injected, but 27 days later the dog showed some twitching movements, and was found dead next morning. At the autopsy the gall-bladder

contained the two stones apparently unaltered, and was filled with dark-green bile. The wall of the gall-bladder was thickened and did not stain well. The epithelium was not markedly vacuolated and had become detached in some places; the nuclei of the epithelium assumed a flattened and shrivelled aspect. The sub-epithelial nuclei were not increased in number, and the submucous coat was œdematous in character. The muscular coat appeared thickened. On the inner surface of the gall-bladder lay much flocculent material, granular in character, and consisting of bacilli which did not stain well.

GENERAL RESULTS.

The results obtained in these experiments may be briefly summarised in tabular form.

TABLE I.—*Experiments in which cholesterin stones introduced into the gall-bladder subsequently became dissolved.*

No. of Animal	Operation	Result
Dog 1	One unbroken cholesterin-stone introduced into gall-bladder.	Necropsy 1 year later. Gall-stone had disappeared. Gall-bladder and cystic duct of natural aspect. Bile healthy in appearance and free from suspended matter.
„ 2	Two cholesterin-stone fragments introduced into gall-bladder.	Necropsy 265 days later. Both gall-stone fragments had disappeared. Gall-bladder, cystic duct and bile all healthy in aspect.
„ 3	Same as 1.	Necropsy 6 months later. Gall-stone had disappeared. Gall-bladder, cystic duct and bile all healthy in aspect.
„ 4	Same as 1.	Necropsy 6 months later. Gall-stone had disappeared. Gall-bladder, cystic duct and bile all healthy in aspect.
„ 5	Three cholesterin-stone fragments introduced into gall-bladder. (Eight days later a culture of <i>b. coli</i> was injected into the gall-bladder.)	Necropsy 101 days later. All three gall-stone fragments had disappeared. Gall-bladder and cystic duct healthy in aspect. Bile thin, free from suspended matter, containing no micro-organisms.

In Table I. all the animals remained in good health except dog 2, which lost weight. In all the gall-stones disappeared, the gall-bladder and its contents being in all respects normal.

In Table II. we see that in Exps. 6, 7, 8 and 9, the dogs continued apparently in perfect health; nevertheless, the autopsy revealed cholecystitis and no dissolution of gall-stones occurred. In Exps. 10 and 11,

the animals lost weight; as before, cholecystitis was present and the gall-stone fragments introduced were unaltered.

TABLE II.—*Experiments in which cholesterin stones introduced into the gall-bladder subsequently remained undissolved.*

No. of Animal	Operation	Result
Dog 6	One cholesterin-stone fragment introduced into gall-bladder, together with pus containing <i>b. coli</i> .	Necropsy 85 days later. Gall-stone fragment unaltered. Cholecystitis present. Bile light-coloured. Mucoid material on inner surface of gall-bladder free from micro-organisms.
„ 7	Three cholesterin-stone fragments introduced into gall-bladder, together with dry pus.	Necropsy 56 days later. Two gall-stone fragments present; also <i>débris</i> of the third fragment which had broken up. Cholecystitis present. Bile contained bacilli forming filaments.
„ 8	Two cholesterin-stone fragments introduced into gall-bladder, together with pus containing <i>b. coli</i> .	Necropsy 52 days later. One gall-stone fragment unaltered; the other had disappeared. Cholecystitis present. No micro-organisms could be detected in gall-bladder.
„ 9	Two cholesterin-stone fragments introduced into gall-bladder, together with pus containing <i>b. coli</i> .	Necropsy 51 days later. Both gall-stone fragments unaltered. Cholecystitis. Gall-bladder contained cheesy material in which <i>b. coli</i> was found.
„ 10	One cholesterin-stone fragment introduced into gall-bladder.	Necropsy 40 days later. Gall-stone fragment unaltered. Cholecystitis present. Bacilli present in contents of gall-bladder.
„ 11	Two cholesterin-stone fragments introduced into gall-bladder.	Necropsy 27 days later. Gall-stone fragment unaltered. Bacilli in flocculent deposit on inner surface of gall-bladder.

It would therefore appear that when the gall-bladder is healthy, gall-stones tend to disappear; on the other hand, when cholecystitis was present in our experiments, the gall-stones remained unchanged.

In the experiments (Table II.), the following changes were met with in the mucous membrane of the gall-bladder. The columnar epithelial cells were in every case covered with an amorphous or finely reticular deposit, which could not be well stained with a fibrin stain, nor with any of the other stains employed (methylene-blue, hæmatoxylin, basic fuchsin, methyl-violet, acid fuchsin erythrosin). Sometimes this deposit contained micro-organisms (in four cases out of six). The epithelial cells themselves were swollen, and presented in the middle and upper parts of their cytoplasm a clear vacuolated aspect, such as is shown in Fig. 1; Fig. 2 representing the normal uniform finely granular

appearance of the healthy epithelial cells similarly stained with methylene-blue for the sake of comparison. The outer denser peri-

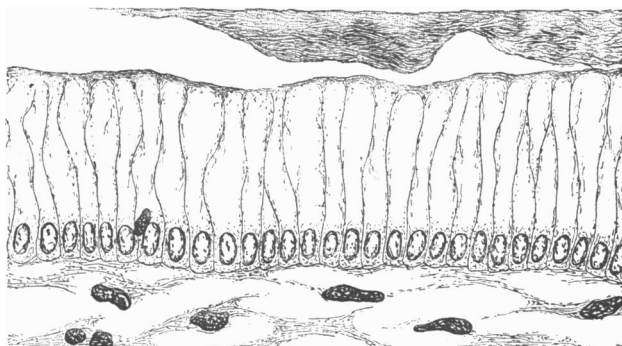


Fig. 1. Epithelial layer of mucous membrane of gall-bladder of Dog 7. The epithelial cells are swollen and the cytoplasm is clear in the upper part of the cell. On the surface is a deposit of amorphous material. In the sub-epithelial tissue no perivascular cell-infiltration is to be recognised. $\times 500$.

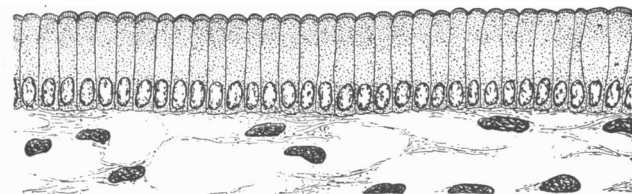


Fig. 2. Healthy epithelium of gall-bladder of dog for comparison with above. $\times 500$.

phery of the cell appeared less affected. Lying among the epithelial cells wandering cells could also be seen in sparse numbers. The connective tissue of the mucous coat sometimes appeared wide-meshed as if oedematous, but this character was not always present. The capillaries of the mucous membrane were sometimes congested, but no perivascular cell-infiltration was present here or elsewhere in the wall of the gall-bladder. The nuclei in the connective tissue of the mucous and submucous coats are normally numerous and collections of lymphoid tissue are met with, but no increase of nuclei could be clearly demonstrated in the sections. The muscular coat appeared unaltered. In some of the animals there was some development of fibrous tissue external to the gall-bladder, in others this was very small in amount.