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THESIS FOR THE DEGREE OF M.D.

"THE BACTERIOLOGY OF CHOLECYSTITIS, APPENDICITIS AND PEPTIC ULCER."

by

BRYAN WILLIAMS, M.B., Ch.B.

INTRODUCTION.

The importance in modern medicine of the three conditions of cholecystitis, appendicitis and peptic ulcer, together with their complications and sequelae, is soon appreciated from a survey of the cases that are to be found in a hospital ward, and even more by the statistical evidence that is available. Consultation of the records of the Edinburgh Royal Infirmary for the last decade of last century show that but few cases of gall-bladder disease were treated, and that those few cases in which a diagnosis of gall stones was made, were found almost entirely on the medical side of the hospital. The few cases of appendicitis that were operated on were those with abscess or peritonitis. The diagnosis of duodenal ulcer was never made, and gastric ulcer was a rarity. The present day frequency of these conditions is soon realised from an inspection of any operating list. More precise evidence also is not wanting. A very authoritative work on the subject (Graham, Cole, Copher and Moore, 1929) has estimated that no less than 40 per cent. of the adult population is suffering from gall-bladder disease. Stewart (1922) has shown that in a large number of autopsies, careful examination showed the presence of an active or healed gastric or duodenal ulcer in no less than 12 per cent. of cases. The common occurrence of appendicitis is a

matter of universal knowledge, and it might truthfully be said at the present day that the presence of a surgical scar in the right lower abdomen is even more the hallmark of civilisation than is the presence of vaccination marks. For it must be considered that these conditions are largely diseases of civilisation, and the evidence for this will be given later.

The investigation of these conditions, from any one of which we may consider ourselves lucky if we escape in a lifetime, seems to offer a fascinating field for study, and the present day phase of surgical treatment offers only too plentiful material. The following work was undertaken with the object of investigating the importance in these conditions of infection, and of determining whether it plays the predominating role ascribed to it by certain workers.

It must be obvious from a survey of the vast amount of work that has been done in this field that there is no simple and single explanation for any of these conditions, and though the presence of inflammatory changes is the striking feature that thrusts itself on our attention and demands our interference clinically, it does not seem justifiable from the evidence at present at our disposal to conclude that infection is the primary or the sole factor. Anatomical and biochemical factors also play a considerable part. That infection is a highly important factor in the manifestations which we find will be generally admitted. It provides most of the serious complications and the greater part of the

mortality. It is certainly the predominant factor in appendicitis, and to a less extent, in cholecystitis. Any increase, therefore, in our knowledge of the type, origin and route of infection, cannot but help in our knowledge of the treatment of these diseases, and what is even more important, in their prophylaxis.

OUTLINE OF THE WORK.

This work was originally undertaken in an investigation of certain aspects of cholecystitis - namely, the part played in its production by streptococci, and the question of their power of elective localisation. As the work progressed, however, it seemed desirable to enlarge its scope, to consider the part played by other organisms, to determine the pathway of infection to the gall-bladder, and also to add an investigation of appendicitis and peptic ulcer as being conditions with a possible aetiological relationship in which suitable material was available for examination.

The work detailed in this thesis, therefore, has consisted in the examination of fresh material removed at operation, its investigation for the type of organisms present and their origin, and an attempt to ascertain their pathological effects in animals. Similar methods have been used throughout, though of course the various conditions will have to be considered separately owing to individual differences in the material. Each part of the work will be

prefaced by some account of the investigations of other workers in the same field, and an attempt will be made to give the main present day theories of the aetiology of these conditions. Finally the whole work will be summarised, the relationship of the three conditions discussed, and an attempt made to assess the part played by both infection and by other factors, as a result of the findings of the present work and of the work of other observers.

The main results of the cholecystitis investigation have already been published in collaboration with Dr. D.G.S. McLachlan (Williams and McLachlan, 1930). The other findings have so far not been published.

PART I.

CHOLECYSTITIS.

INTRODUCTION.

The aetiology of cholecystitis still presents one of the big unsolved problems of Medicine and Surgery. It is recognised that many factors, pathological and biochemical, are involved in the genesis of cholecystitis and gall stones, but the relative importance of each is at present uncertain. It seems to have been agreed, however, since the work of Aschoff and Bacmeister (1909), that gall stones may form in the absence of an inflammatory process, and this has even been admitted in recent years by Naunyn, who was the pioneer of the theory of their inflammatory origin. There are doubtless also many disturbances of gall-bladder function which may give rise to the symptoms of more serious disease, and in which there is no evidence of inflammation, or at the most, only slight change. Such cases have been recognised more frequently since the introduction of cholecystography, and the term "cholecystopathy" has been suggested to cover them.

In the great majority of cases coming to operation, however, the presence of existing or past infection, whether such infection be primary or secondary, is undoubted. It is this infection, which,

superimposed on mechanical blockage of the biliary passages, gives gall-bladder disease its seriousness and mortality. It is this infection, also, which makes the gall-bladder such an important focus of sepsis, as emphasised by D.P.D. Wilkie (1929). It is essential, therefore, if we wish ever to control the incidence of this condition, to endeavour to determine the nature, origin, and route of infection, and to discover whether such infection is a primary or a secondary factor in the production of the disease.

In the present work, special attention has been paid to certain aspects of the problem which have been prominent in recent years, but an attempt has been made to review the whole question of the part played by infection in gall-bladder disease. Attention has been directed towards cholecystitis rather than cholelithiasis. It is impossible, however, to dissociate the two conditions in any experimental work. Undoubtedly either condition may occur without the other, and either may predispose to the other. In the great majority of cases dealt with clinically, we find that the two conditions coexist. In recent years, however, there has been a tendency to operate on more early cases, especially since the introduction of cholecystography. As a result, it is becoming commoner on the one hand to find the presence of slight cholecystitis without stones or of cholesterosis, or on the other hand to find non-inflammatory cholesterin or pigment stones without associated inflammatory changes in the gall-bladder wall. This opportunity of studying

the early stages rather than the late end results, or what Moynihan has called "the pathology of the living", should bring us considerably nearer the solution of this difficult problem.

HISTORICAL SURVEY OF BACTERIOLOGICAL AND EXPERIMENTAL WORK.

Galippe, in 1886, seems to have been the first to suggest the microbic origin of gall stones. He then advanced the theory that organisms formed the nucleus of stones. Naunyn, in 1892, was the first to advance the theory that inflammatory changes in the gall-bladder wall might be responsible for the formation of gall stones. He considered that an ascending infection from the intestine with B. coli occurred, and that a "lithogenous catarrh" of the mucous membrane was produced, with the result that cells were cast off, and acted as the nuclei for stones. He also considered that the gall-bladder excreted cholesterol - a view which he maintained till recent years.

Owing to the prevalence of typhoid fever at the end of last century, much attention was then paid to the complication of typhoid cholecystitis. The occurrence of this had been recognised by John Hunter in 1763, but the aetiological relationship was not proved till the demonstration of the typhoid bacillus in suppurative cholecystitis by Gilbert and Girode in

1890. In 1897, Gilbert and Fournier divided biliary lithiasis into colon and typhoid lithiasis, and this remained the main method of classification till the importance of the streptococcus was recognised in later years.

In the literature of this period, we note first of all that typhoid infections of the gall-bladder occupied a position that is out of all proportion to their present day importance, and secondly that studies were directed towards the occurrence of organisms in the bile rather than in the tissue of the gall-bladder wall itself. Little progress was made, therefore, till Rosenow showed the importance of examining actual tissues for organisms.

Pratt (1901) gave a most valuable account of the work done on typhoid cholecystitis and on experimental lithiasis up to that date. It gradually became realised that cholecystitis was a rare complication of typhoid fever, and also that typhoid bacilli might be frequently present in the bile of patients actually suffering from the disease or convalescent from it without inflammation being set up in the wall of the gall-bladder or gall stones being formed. Thus Osler (1925) recorded the occurrence of only nineteen cases of cholecystitis as a complication of 1,500 cases of typhoid fever, and Webb-Johnson (1919) found it to occur only fifteen times in 2,500 cases in the War. Pratt found typhoid organisms in the bile without any evidence of cholecystitis. It was realised also that typhoid bacilli might persist in the gall-bladder long

after the disease had occurred, and an interval between the primary disease and the complication as long as 41 years was recorded. (Pomeroy and Shen, 1925). Cases were also recorded in which typhoid cholecystitis occurred in which there had been no evidence of typhoid fever.

Although, therefore, typhoid cholecystitis has only a limited clinical interest for us at the present day, the bacteriological and experimental work that was done on it in former years is of considerable value in view of the present day tendency to ascribe to other biliary infections an intestinal origin.

Among other valuable work is that of Gilbert and Lipmann (1902). By making aerobic and anaerobic cultures of the gall-bladder contents of twelve cases, they found B. coli in six cases, the enterococcus in six cases, and B. welchii, which they seem to have been the first to describe in cholecystitis, in two cases. They also found some anaerobic organisms that do not seem to have been since described - B. ramosus, B. funduliformis, B. radiiformis, B. nebulosis and B. fragilis. Several of these organisms have been described in recent years in appendicitis, and more attention will be paid to them later.

A new era has been opened up since the work of Rosenow (1914, 1916). In his main article on the subject, he details the results of the examination of 47 cases. He gives in detail his methods of culturing the tissues, contents and gall stones, and also of his animal experiments - all of which follow the usual

lines of his work. In the 47 cases of cholecystitis, he demonstrated in the wall of the gall-bladder, in 66 per cent. of the cases in which it was examined, the presence of non-haemolytic streptococci, frequently in pure culture, but sometimes associated with other organisms, most frequently the colon bacillus. In a few cases he cultivated streptococci from the adjacent lymph glands. In eight cases in which the fluid contents of the gall-bladder were sterile, streptococci were obtained from the wall of the gall-bladder.

Intravenous inoculation of cultures of sixteen of the organisms isolated, including streptococci, B. coli and staphylococci, produced haemorrhagic or inflammatory lesions in the gall-bladder in 79 per cent. of 53 rabbits or dogs. Similar lesions occurred in the stomach, duodenum, intestines, joints and endocardium, but in a smaller percentage of animals. These results were obtained with freshly isolated strains and as the result of inoculating considerable quantities of culture.

Brown (1919) investigated 70 cases in a similar manner. He isolated streptococci from 30 per cent. of gall-bladders with evidence of slight chronic inflammatory changes and in 75 per cent. of those showing marked changes. B. coli occurred alone or in combination with streptococci in 18 per cent. of the former and in 15 per cent. of the latter. The intravenous injection of cultures in rabbits produced various lesions, including oedema of the gall-bladder wall and haemorrhages in the mucous membrane of the

stomach.

Other work on similar lines has been that of Judd, Mentzer and Parkhill (1927). Using Rosenow's methods, they examined material from 200 cases. In 200 cases in which they examined the gall-bladder wall, they found streptococci in pure culture sixteen times and in combination with B. coli ten times. B. coli was found alone nine times. B. welchii was found twice. The bile was examined in 193 cases. Streptococci were found alone fifteen times, and B. coli alone five times. They found that all specimens of bile which gave a growth were light coloured or lacking in pigment. Thick or "dirty" bile was nearly always sterile. The organisms found in the bile were usually similar to those found in the gall-bladder. In their animal experiments, 20 strains of organisms were injected into 43 rabbits, and of these, 36 received streptococci. Of these latter, lesions were produced in 75 per cent. Alterations in the colour of the bile are included under the term "lesions". Eight out of the 43 rabbits showed myocarditis or endocardial vegetations. They also found that B. typhosus had an "elective affinity" for the gall-bladder.

Other authors who have paid attention to the presence of streptococci are Reimann (1930), who found a streptococcus viridans in nine out of 45 cases, and who failed to produce cholecystitis in rabbits by intravenous injection of the organisms. Alvarez and his colleagues (1923) found streptococci in the wall

in nine out of 41 cases, and B. coli in five cases, and streptococci in the bile in two cases and B. coli in three. Mestitz and Rittner (1928) found B. coli in sixteen out of 80 cases, and streptococci in twelve. Moynihan (1925) reports the finding of streptococci in eight out of 100 cases, and B. coli in 46 cases.

Some important studies on the bile include those of Drennan (1922), who examined the fluid contents of 100 cases, and found organisms present in nineteen. B. coli was found twelve times, staphylococcus aureus four times, haemolytic streptococci twice, and a sarcina once. This author found that by making a series of cultures with varying dilutions of bile that a concentration of 70 per cent. inhibited the growth of most organisms.

Johnson (1925) also investigated the bile obtained from 100 cases by cholecystectomy, and found infection present in 32 per cent. B. coli was found eighteen times, and a streptococcus once, the other organisms present consisting of staphylococci and typhoid bacilli. He found the highest percentage of positive cultures to occur when mucus was predominant in the contents and the bile-salts were poorly concentrated.

Other studies have been those of Illingworth (1927), who found that in 100 cases streptococci were present in the gall-bladder wall 34 times and in the bile sixteen times, and B. coli in the wall seventeen times and in the bile twenty times. A.L. Wilkie (1928)[1] reported the examination of 50 cases. Streptococci

were found in the cystic gland in 43 cases, in the sub-mucosa of the gall-bladder wall in 21 cases, and in the bile in only two cases. B. coli was found in the cystic gland in one case, and in the bile in three cases. He considered that bile had an inhibitory effect on the streptococci present. He also found that on intravenous injection in rabbits of the streptococci isolated, a chronic progressive cholecystitis was invariably produced. Injection into the lumen of the gall-bladder produced no lession, whereas injection into the wall of the gall-bladder produced marked thickening. Previous ligation of the cystic duct did not prevent the occurrence of cholecystitis after intravenous injection of the streptococci, and he considered therefore that the organisms reached the gall-bladder by means of the systemic circulation.

Branch (1929) in 210 cases found streptococci in the wall eleven times, and in the bile fifteen times. B. coli was present in the wall 49 times and in the bile 50 times.

Nickel and Judd (1930) have reported the examination of 300 cases at the Mayo Clinic. Rosenow's technique was used. Fifty per cent. of the specimens gave no growth. In the others, streptococci were found in 44 per cent. and B. coli in 30 per cent. They classify their cases according to the duration of their symptoms, and found that the biggest percentage of positive cultures occurred in those cases in which the symptoms had existed for four months or less. They also investigated the effect of a dye used for

purposes of cholecystography - tetiothalein sodium N.N.R. - in influencing the results of cultures. They found that the interval between the giving of the dye and the making of cultures was least when the culture was sterile, being 8.2 days, and was greatest when a pure culture of streptococci was obtained, being then 12.8 days. They injected 72 strains of organisms into rabbits. Forty-one strains of streptococci were injected into 78 rabbits, and gall-bladder lesions were found in 45 per cent. of these. In only 9 per cent. of 54 rabbits injected with other strains were gall-bladder lesions produced. They do not consider that B. coli is merely a secondary invader. They discuss the reasons why a smaller percentage of positive cultures were obtained than in Rosenow's work. They consider that the examination of less grossly diseased specimens may partly be responsible, and also that the dye used for cholecystography may have had some bacteriostatic effect.

Gordon-Taylor and Whitby (1930) investigated the bacteriology of 50 cases. They made special search for B. welchii, which they found in nine cases, as well as in other cases on post-mortem examination. Streptococci were present in fourteen cases and B. coli in fifteen cases. They found that the streptococci were all non-haemolytic and of the faecalis variety, and consider that the bowel is the source of infection in cholecystitis. They have compiled a table from the work of several authors, and

they find that in 1,600 cases in which the contents were examined, infection was present in 31 per cent., and in 678 cases in which the wall was examined, infection was present in 67 per cent.

Amoss and Poston (1930) have recently described the occurrence of Brucella melitensis in a case of cholecystitis. This organism was isolated prior to operation by duodenal drainage, and subsequently after operation from the contents of the gall-bladder. This organism had previously been isolated from the gall-bladder contents by Bull and Gram (1911).

Among other uncommon organisms isolated has been B. enteriditis of Gaertner (Dean, 1911). The cholera vibrio has been found by Greig (1912), who found it in the bile in 81 out of 271 fatal cases of cholera. He considers that the gall-bladder may prove a source of re-infection in cases of cholera.

The entomoeba histolytica has been found in the bile by Coates and Hey Groves (1927).

Much work was formerly done on the experimental production of gall stones, such as that of Cushing (1899). This author produced stones in the gall-bladders of rabbits by the injection of organisms into their lumen and traumatisation of the gall-bladder. He quotes the work of Mignon who found experimental calculi in the guinea-pig, and who considered that the attenuation of the virulence of organisms was the main factor in the production of stones.

The work of Mann (1921) on the experimental production of cholecystitis by chemical methods is of

considerable interest. By giving intravenous injections of Dakin's solution to dogs in a dose of 5 to 10 cc. per kilo., he found by observation at laparotomy that within half an hour the production of a cholecystitis commenced, and was complete within 24 hours. He considered that the active substance was chlorine, and that it reached the gall-bladder by means of the systemic circulation, as it was produced with great rapidity and was not prevented by previous ligation of the cystic duct. The liver and spleen were also affected, but the action was mainly specific for the liver. The intensity of the action depended upon the excellence of the blood supply, and the appearances were those of intense inflammation. Dilatation of blood-vessels, sub-serous or inter-muscular haemorrhages, and even a gangrenous appearance were found. After three months, only a few white scars could be seen.

Several series of experiments have been done in recent years to determine the pathway of infection to the gall-bladder. Meyer, Neilson and Feusier (1921) give a most valuable account of their work on the mechanism of gall-bladder infections in laboratory animals. They consider the ascending route of infection via the bile passages to be exceptional, and they were unable to produce cholecystitis by the introduction of even enormous doses of typhoid bacilli into the duodenum close to the papilla of Vater. In summarising, they conclude that "for the experimental production of cholecystitis with intestinal organisms,

such as the typhoid-dysentery bacilli and cholera vibrios, the haemato-hepatogenous route is the only one which has been definitely proved." They consider that the streptococcus might be a secondary invader, reaching the gall-bladder by means of the lymphatics. They found that simple ligation of the cystic duct resulted in the production of gross lesions. "The majority of our attempts resulted in haemorrhagic infarction, or minor circulatory disturbances in the veins of the gall-bladder wall, which either became totally necrotic or showed haemorrhages and escape of blood into the lumen of the viscus." They found that in one per cent. of 500 rabbits a spontaneous cholecystitis occurred.

Meyer and Lowenberg (1926) injected rabbits intravenously with enterococci, streptococcus viridans and haemolytic streptococci. They found that of these three, only the enterococcus tended to localise in the gall-bladder, and consider that most of the streptococci isolated from cases of cholecystitis are of this class.

Wilkie (1928)[2] considered that infection reached the gall-bladder in the systemic blood stream via the cystic artery. He separated the gall-bladder from the liver in rabbits, and subsequently injected streptococci intravenously. A chronic cholecystitis was produced, and this occurred even after ligature of the cystic duct.

A valuable investigation on the route of

infection to the gall-bladder has been recently carried out by Patey and Whitby (1933). These authors consider the bowel to be the main source of the organisms found in cholecystitis, and by a valuable series of experiments which will be referred to in detail later, they consider that infection reaches the gall-bladder via the systemic circulation, though they do not think that the possibility of descending infection in the bile can at present be ruled out.

Among those who consider that infection is not the only factor in the production of gall stones are Aschoff and Bacmeister (1909), who were the first to advance the theory that in some cases gall stones might have an aseptic origin. They found that in those cases in which a single cholesterin stone, or "cholesterin solitaire" was present, evidence of inflammatory change in the gall-bladder wall might be completely lacking. They considered that once such a stone had formed, however, the way might be paved for the invasion of the gall-bladder by infected bile, with the production of numerous secondary "septic" stones. To a large extent, this theory, and the classification of stones into "aseptic" and "septic", is accepted at the present day.

Rovsing (1924) considers that infection is not the primary cause of gall stones. He considers that neither stasis nor infection are present in the majority of cases, and that when they are present they may exist for years without producing gall stones. In infective jaundice, both stasis and infections are

present, but gall stones do not occur in this condition. In addition, he finds that patients with visceroptosis seldom have gall stones.

METHODS AND TECHNIQUE OF GALL-BLADDER INVESTIGATION.

Over a period of two years, material from 117 cases has been investigated. This has been obtained from cases operated on in the Edinburgh Royal Infirmary, and in nursing homes in Edinburgh. Of this number, ten cases are excluded for the following reasons. In three cases the material had been accidentally subjected to gross contamination before it could be examined. In two cases a prolonged interval had elapsed between the time of removal and of examination of the material. It is interesting to note, however, that in one of these, in which the material was 24 hours old, a viridans streptococcus was obtained from the bile. In two further cases, in each of which a cholecystectomy had been carried out approximately a year previously, and in which a second operation was done for recurrent symptoms, specimens of bile were removed at the second operation, and were both found to be sterile. In three further cases, material was obtained during an operation for some other condition. One of these was a case of carcinoma of the caecum, in which a cholecystostomy was done after the bowel resection for the purpose of supplying fluid post-operatively. A streptococcus was grown

from the bile. Another was a case of carcinoma of the common bile duct, a specimen of bile from which was found to be sterile.

Of the remaining 106 cases, a few were admitted with acute symptoms, and were operated on as emergency cases. These are further referred to under the heading of Acute Cholecystitis. The remainder were operated on during a quiescent period for symptoms of varying duration.

The following technique was observed when specimens were taken at operation. Immediately after removal of the gall-bladder, the cystic gland, when palpable, was dissected off the cystic duct, and placed in a sterile test tube. The clamp on the cystic duct was then replaced by a ligature in order to prevent escape of bile, and the gall-bladder placed in a sterile dish or sterile swabs, and immediately removed to the laboratory. In most cases, the specimens have been cultured immediately after removal from the theatre. In some cases, an interval of a few hours has elapsed. Cases where the interval was very long, as already mentioned, have been excluded from consideration.

In the laboratory, the cystic gland was first cultured. With due precautions, it was transferred to a sterile covered mortar, minced up with sterile scissors, and emulsified rapidly in a small quantity of broth with a pestle. Tubes of warm glucose broth were then inoculated and incubated at 37°C. In some cases, blood-agar plates were also inoculated with

emulsion. Cultures were incubated for at least seven days, or until growth occurred.

Cultures were next made from the gall-bladder wall. Tissue was most commonly taken from the region of the gall-bladder attached to the liver, as it was considered that this would have been least subject to contamination during the ordinary procedure of removing the gall-bladder from the cystic duct forwards. Strips of the bluish-grey so-called "sub-mucous" coat were dissected away without opening the lumen of the gall-bladder, and emulsified and cultured in the same way as the cystic gland. When tissue from other regions of the gall-bladder was used, it was first necessary to reflect aside the peritoneum and the layer of sub-peritoneal fat which is usually present in grossly diseased gall-bladders.

The lumen of the gall-bladder was then opened, and cultures made from the bile, mucus, pus, "biliary mud" or other contents, or from the mucous membrane of the surface of stones in those cases in which the gall-bladder was tightly packed with stones and fluid contents were absent.

In cases in which the wall was only slightly thickened - and there were many of this nature in the series - it was not found possible to obtain an adequate amount of the sub-mucous layer for culture without opening the lumen of the gall-bladder; under these circumstances, fragments about 1 cm. square of the whole thickness of the wall were emulsified and

cultured. Some portions were washed with several changes of sterile saline in order to remove the bile, and other pieces were cultured after brief preliminary immersion in methylated spirit followed by washing with sterile saline, or momentary exposure to boiling water. An attempt was thus made to destroy surface organisms which might be present in the bile, without destroying those in the interior of the wall. It is of course possible that these methods may have failed to destroy organisms present in bile in crypts of the mucous membrane. Similar methods were also used in some cases in which it was possible to obtain "sub-mucous tissue". It is obviously of the highest importance to know whether the organisms found have been derived from the wall or from the bile - and further consideration will be given later to the readings of the results and the conclusions of other workers in this respect.

Cultures were made from gall stones after the surface had been sterilised by immersion in spirit and flaming. If large, the gall stones were cut with a sterile knife, the centre removed and emulsified in sterile broth, and the emulsion transferred to glucose broth with a sterile pipette. If small, the complete stone was emulsified.

The media employed were glucose broth, phosphate broth, glucose agar in deep tubes, and rabbit-blood-agar plates. Glucose brain broth was used in some cases. After inoculation, culture media

were examined daily, and were incubated for at least a week. Broth cultures were examined microscopically before being discarded. In a few cases, organisms resembling B. subtilis and unidentified cocci of the nature of sarcinae have been obtained. These have been regarded as contaminants, and have not been included in the tables.

Rosenow has stressed the importance of providing all grades of oxygen tension between complete aerobiosis and complete anaerobiosis by means of tall columns of fluid media. As noted by him, it was found in some cases that in the tubes of broth a growth of the organism could be seen steadily forcing its way up from the bottom of the tube as the oxygen was consumed. Fluid media have the disadvantage, however, that one organism may outgrow and suppress a more delicate organism also present. Thus it was often found that B. coli alone could be found in fluid media, whereas a mixture with other organisms might be found when the same material had been plated out.

The primary broth cultures were purified by plating out on blood agar plates. Single colonies of streptococci were picked off on to slopes of coagulated sheep's blood medium, and these were used as stock cultures.

CLASSIFICATION OF MATERIAL.

The classification of the material examined has presented some difficulty owing to the enormous

variation in the pathological changes present. Most methods of classification of gall-bladder disease seem to be needlessly complicated. Thus we find described catarrhal, suppurative, membranous, phlegmonous, gangrenous, fibrous, ulcerative, villous and papillomatous cholecystitis. Such descriptions are of little practical value. The same specimen may show more than one of these conditions. Clinically speaking, the important factors are the presence or absence of stones, and in particular, the presence or absence of obstruction in the biliary passages. As with appendicitis, the serious factor that gives gall-bladder disease its seriousness and mortality lies in obstruction, and due attention must be paid to this in any investigation. It seems to be of some value, to note in each case the presence or absence of stones and of any obstruction in the cystic or common bile ducts. The attention that has been paid to the condition of cholesterosis or "strawberry gall-bladder" in recent years and its recognition as a definite entity seem to entitle it to a separate place. The simple but convenient classification of Acute Cholecystitis, Chronic Cholecystitis with or without stones, and Cholesterosis has therefore been adopted.

ACUTE CHOLECYSTITIS.

The four cases grouped under the heading of Acute Cholecystitis derive their place rather from clinical reasons. All were admitted as emergency cases; three were operated on at once, and the other within a few days of admission. Two of the patients subsequently died. Gross evidence of inflammation was present in all - in two cases the gall-bladder being gangrenous - and in three cases, obstruction in the form of stones was removed. In only one of these was a cholecystectomy done. In the others, small pieces of the gall-bladder wall were removed for examination during the procedure of cholecystostomy.

TABLE 1.

ACUTE CHOLECYSTITIS.

(4 cases)

Case	Diagnosis	Wall	Contents
1	Acute cholecystitis with stones.	B. welchii	B. coli
2	Gangrenous cholecystitis with stones.	-	(B. coli (Enterococcus (B. welchii
3	Acute cholecystitis, gall-bladder being partly gangrenous.	B. coli	B. coli
4	Gangrenous cholecystitis with stones.	B. coli	B. coli

(- indicates no growth).

The bacteriological findings in each case are

shown in Table 1. It will be noted that B. coli occurred in every case, and B. welchii in two cases. In the second case, in which three organisms were found, direct films of the gall-bladder contents showed every field to be packed with organisms of the type isolated, and in addition a short Gram-positive bacillus which was not isolated on culture.

CHRONIC CHOLECYSTITIS.

The second group comprises the great majority of cases. In gross pathology the specimens showed enormous variation, all degrees of inflammation being represented. Thus several cases of empyema or mucocele of the gall-bladder are included, and also a number of cases of stone in the common bile duct, in which usually only the bile was examined. Many specimens showed evidence of recent inflammation in the form of haemorrhages or oedema. Stones were present in 72 out of the 93 cases classified under this heading. The results of the cultures in this group are shown in Table 2.

Cystic gland. The cystic gland was examined in 43 cases classified as chronic cholecystitis. It varied in size from that of a pinhead to a small bean. In some cases in which it could not be identified with certainty, the connective tissue from the neck of the gall-bladder was cultured, but as this was never found to give a growth unless contaminated with an infected bile, these cases have not been included in the tables.

On several occasions on which the gland was large enough to provide material to inoculate more than one tube, parallel series of cultures were carried out. In one series, the tissue was allowed to remain in the medium. In the other, it was removed at varying periods between three and seven hours after introduction into the broth, on the supposition that there might be some substance in the tissue which inhibited bacterial growth. No difference in the results of the two series, however, could be detected, and this practice was discontinued after it had been carried out in about 30 cases.

TABLE 2.

CHRONIC CHOLECYSTITIS, WITH OR WITHOUT STONES.

(93 cases)

	Gland (43 cases)	Wall (84 cases)	Contents (81 cases)	Stones (41 cases)
Streptococcus	7	10	15	2
Streptococcus plus *B. coli*	1	4	6	-
B. coli	6	15	10	5
Diphtheroid bacilli	4	4	1	-
B. welchii	1	3	1	-
Staphylococcus aureus	1	2	1	-
Staphylococcus albus	4	8	4	2
B. paratyphosus B.	-	-	1	-
No growth	19	41	43	(*B. pyocyaneus* -1) 31

It will be seen from Table 2. that streptococci

were obtained in eight cases (19 per cent.) in which the cystic gland was cultured. It is impossible to exclude contamination by bile of the cystic gland in some cases in which the same organism was obtained from both gland and bile, owing to the exposed position of the gland when the cystic duct is divided. Thus on four occasions on which a streptococcus was cultivated from the cystic gland, a similar organism was also cultivated from the bile. B. coli was obtained in seven cases (16 per cent.), and other organisms less frequently. The biological characters of the streptococci isolated are discussed later.

In one further case of chronic cholecystitis since examined, a cystic gland the size of a split pea was cultured, both aerobically and anaerobically. No growth was obtained, however.

Gall-bladder wall. The gall-bladder wall was examined in 84 cases, and the presence of streptococci, either alone or in combination with B. coli, was demonstrated on 14 occasions (17 per cent.), and B. coli was isolated in 19 cases (23 per cent.). On seven occasions a streptococcus similar to that found in the wall was obtained from the bile. In three further cases a similar organism was found in the gland, wall and bile, and in one other case in the gland and bile. Of the 24 cases in this group in which streptococci were found, in only five were they not found in the bile. In one of these the bile was not examined. In three others streptococci were found,

either alone or in combination with B. coli in the wall, while B. coli was found in the bile.

In deciding the significance of these results, several things must be borne in mind. It is important to know, as already noted, whether an organism found on culture of the wall is actually an intra-mural one or merely a surface organism present in the bile. It is obvious that if the bile contains organisms, a similar growth will be given by the whole thickness of the wall in virtue of the bile adherent to its surface. If, however, the tissues of the wall give a growth, and the bile is sterile, the organisms isolated are obviously intra-mural ones. The same may be said if a growth is obtained from the outer coats of the wall, even if the bile is infected, provided, of course, that contamination of the superficial tissues by bile can be definitely excluded. More difficulty arises, however, in drawing conclusions in those cases in which a growth has been obtained from the bile and also from the whole thickness of the wall after treatment by one of the methods already described. It has been concluded, however, that these methods have been sufficient to destroy organisms merely on the surface of the tissue. In the tabulation of the results, an organism has been considered to be an intra-mural one if it has been grown from the outer coats, or from the whole thickness of the wall when the bile has been sterile or after sterilisation by one of the methods described.

The results of the cultures have often been

surprising. On the whole it may be stated, as noted by Illingworth (1928), that the specimens most likely to give a growth are those with a thick and oedematous wall. Occasionally naked-eye appearances are extremely deceptive. Thus a gall-bladder that is angry red in colour and has a thick wall may give no growth, whereas a lax, apparently innocent looking specimen, or one with a "strawberry" mucous membrane may give a profuse growth. Very grossly diseased specimens, consisting of fibrous tissue, and packed with gall stones and lacking a mucous membrane - obviously the end results of a process many years old - were generally sterile.

Contents. The bile, mucus, pus, "mud" or other contents of the gall-bladder or the surface of stones have been examined in 81 cases of chronic cholecystitis, and the results are shown in Table 2. It will be seen that streptococci were isolated on 21 occasions (26 per cent.), on six of which they were associated with B. coli. B. coli was present in 16 specimens (20 per cent.) Two of the coliform strains were identical with B. lactis aerogenes.

As also noted by Illingworth, naked eye appearances are here also very deceptive. A purulent looking content may prove sterile, while thin bile may yield a growth. The important factor, however, is undoubtedly the concentration of the bile. Drennan (1922) has shown that a concentration of over 70 per cent. of bile inhibits the growth of all organisms,

and Johnson (1925) has also reported that thin bile containing mucus was more likely to give a growth. It has been found that thick tarry bile, so often looked upon with suspicion, is always sterile, and Graham, Cole, Gopher and Moore (1929) conclude that the presence of such bile is evidence of good concentrating function on the part of the gall-bladder wall. Judd, Mentzer and Parkhill (1927) also found that only lightly coloured bile gave a growth.

In 21 of the 93 cases classified under this heading, no stones were present. It is of interest to note that in only one of these 21 cases was a streptococcus isolated, and in that case it was obtained from the bile. B. coli was grown from the cystic gland twice, from the wall five times, and from the contents three times. In one case, B. paratyphosus B. was isolated from the discharge from a gall-bladder sinus. The patient had several months previously suffered from paratyphoid fever, and subsequently developed acute cholecystitis, for which drainage of the gall-bladder was carried out. The sinus continued to discharge for some time.

CHOLESTEROSIS.

Nine cases of this condition were investigated, and the results are shown in Table 3. The low percentage of specimens included under this heading may be explained by the fact that only those cases

showing a general "strawberry" appearance of the whole mucous membrane have been included.

TABLE 3.

CHOLESTEROSIS.

(9 cases)

	Cystic gland (4 cases)	Gall-bladder wall (8 cases)	Contents (8 cases)	Stones (3 cases)
treptococcus	-	1	1	-
. coli	-	1	-	-
taphylococcus ureus	-	1	-	-
taphylococcus lbus	-	1	-	1
o growth	4	4	7	2

Stones were present in six cases, two of them being single cholesterin stones.

The low incidence of organisms isolated in this condition, which is in accordance with the findings of Illingworth (1929) and Nickel and Judd (1930), is of interest when we consider that inflammatory changes of some degree are nearly always present. Thus Boyd (1923) found inflammatory changes in all of his 52 cases of cholesterosis, and Illingworth found them to be absent only once in 35 cases. Walton (1930) found inflammatory changes present in each of 23 cases in which a single cholesterin stone was present.

EXAMINATION OF DIRECT FILMS.

The examination of direct films of the gall-bladder contents has sometimes proved of interest. The films were stained by Kopeloff and Beerman's modification of Gram's stain. In a case of gangrenous cholecystitis which subsequently proved fatal, and from which B. coli, enterococci, and B. welchii were cultured, every field was found to be packed with Gram-negative coliform bacilli, oval Gram-positive diplococci, and large thick Gram-positive welchii-like bacilli. In addition, there was a short, straight, Gram-positive bacillus, occurring in pairs forming a wide angle, but attempts to isolate this were not successful. This may have been the B. ramosus of Gilbert and Lipmann (1902), which is further discussed in the appendicitis section. Gordon-Taylor and Whitby (1930), found in one of their cases in which they isolated B. welchii, that very numerous bacilli could be seen in every field.

In another case in which the gall-bladder contained semi-solid faecal smelling matter, numerous small round cocci in long chains were seen in every field, and streptococci were subsequently isolated. In another case, in which the contents had the appearance of pale-coloured faecal matter, diplo-strepto-cocci were seen in films but were not cultured.

Unfortunately an examination for spirochaetes by dark-ground illumination or by silver impregnation methods was not carried out as in the appendicitis

work, but it is suggested that such methods might be of interest in future work, in view of the frequency of spirochaetes in gangrenous conditions.

It is interesting to note that Soromenho (1930) has described the presence post-mortem in the contents of the gall-bladder in a case of Blackwater Fever of a spirochaete corresponding morphologically to Treponema Eugyratum.

EFFECT OF CHOLECYSTOGRAPHY.

In certain cases a previous cholecystographic examination by means of "STIPP", or sodium tetra-iodo-phenolphthalein or a similar substance, had been carried out prior to operation, and it was considered of interest to investigate the relationship of the radiological and cultural findings. Thirty-eight cases in which it had been given were thus investigated. In all but two of these, it had been administered by intravenous injection, usually in a dose of 3 grammes. It was found that out of fifteen cases in which a positive gall-bladder shadow was obtained, organisms were demonstrated in the wall on six occasions, and in the contents, in five out of eleven specimens examined; in 21 cases in which no shadow was obtained, organisms were found in the wall seventeen times, and in the contents, in six out of seventeen specimens examined. Owing to the many factors involved, however, and the small number of cases in which this point has been investigated, it does not seem justifiable to draw any

conclusions from the above results regarding the bacteriological action of "STIPP" when excreted by the liver and concentrated by the gall-bladder. It is obviously essential to know first whether the failure to obtain a shadow was due to failure of the dye to enter the gall-bladder owing to blockage of the cystic duct, or to deficient concentrating power of the gall-bladder wall owing to disease, and it is not always possible to obtain this information from an examination of the specimens removed at operation, as circumstances may then be different from those existing at the time of radiographic examination.

Nickel and Judd (1930) investigated the effect of a similar product, tetiothalein sodium N.N.R. (iodeikon) in 201 cases. They found that the interval between the time this substance was given and the time of operation was least in the cases in which cultures were sterile, whereas it was as long as twelve days in the cases in which streptococci were obtained. They consider that it may have some bacteriostatic action, and that its use in their cases may partly be responsible for the lower incidence of positive cultures than those reported by earlier workers.

CLASSIFICATION OF STREPTOCOCCI.

Few subjects are more debated and undecided in Bacteriology at the present day than the classification of streptococci. Many important attempts have been made to bring order out of the chaos, but there is so

far no generally accepted system. The present situation may best be understood by a brief review of past work. The first attempt was that of von Lingelsheim (1891) who originated the morphological classification of the streptococci into those with long and those with short chains - the former being considered to be the more virulent, and the latter including the less virulent normal inhabitants of mucous membranes. The next important step was that of Schottmüller (1903) who introduced the division of haemolytic and non-haemolytic varieties, and this is still the main division used at the present day. Gordon (1905) and Andrewes and Horder (1906) first emphasised the classification of streptococci by means of sugars, and introduced the use of such names as Streptococcus salivarius and faecalis. This method, usually in the modification of Holman (1916), who divided streptococci into sixteen varieties according to their action on mannite, raffinose and salicin, is still largely adopted at the present day. Since the War era, work has mostly centred round the identification of the enterococcus. This was extensively described by Dible (1921), who found it to be a frequent inhabitant of wounds. He considered its main property to be that of heat-resistance - i.e. the ability to withstand a heat of 60° C. for a period of fifteen minutes or longer. This property has been extensively investigated by Alston (1928) and Davidson (1928) in their researches on the gastro-

intestinal flora in Pernicious Anaemia and other conditions. The latter found that some strains even resisted a temperature of 80° C., a finding previously made by Logan (1914). Alston considered that bile resistance was a more suitable basis for division. Meyer and Schönfeld (1926) have recently claimed that the splitting of aesculin in a bile-salt medium is the most specific test of the enterococcus, being given by 98 per cent. of cases. It will thus be seen that of all the streptococci at the present day, the enterococcus is the one whose characters have become most definitely known.

Other important work is the recognition of strictly anaerobic streptococci by Schottmüller (1923) and Prévot (1925). The most comprehensive review of the streptococci is the monumental work recently undertaken by Thomson and Thomson (1927). After reviewing the whole history of previous researches on the streptococci, they have introduced a classification by means of the colony appearances on Warren Crowe's chocolate medium, and have utilised the aid of photography in the recording of the characteristics of the organisms.

It will be realised, therefore, that there is no generally accepted method of classifying the streptococci or of nomenclature. In the present work, however, an attempt has been made to investigate fully the streptococci that have been isolated, in order to determine as far as possible their origin in the body.

They have been analysed therefore by the following methods:-

(1). Macroscopic and microscopic appearances in broth and on 10 per cent. rabbit-blood-agar plates.

(2). Colour changes on coagulated blood media.

(3). Heat resistance of a broth culture at 60° C. for fifteen minutes.

(4). Ability to grow on a bile-salt medium.

(5). Fermentation tests for glucose, mannite, salicin and inulin on serum-agar slopes.

(6). Action on aesculin in a bile-salt medium.

(7). Appearances on Warren Crowe's medium.

(8). Serological reactions.

All the streptococci isolated in the cholecystitis work have been of the non-haemolytic variety. A few produced a tiny zone of haemolysis on blood-agar, but none of them showed true "beta" haemolysis.

Of the eight strains isolated from the cystic gland, four corresponded definitely to enterococci in their characteristics of heat-resistance, tolerance of a medium containing bile, and ability to split aesculin. The other four resembled salivary streptococci. Of fifteen strains obtained from the gall-bladder wall, seven were enterococcal in type, and of 23 strains cultivated from the bile, nine belonged to this group. Of the remaining strains, some resembled salivary types, while the rest were of the nature of gamma streptococci. A systematic study

of all strains has not been carried out on Warren Crowe's medium, but a certain number have been tested and have been found to present a variety of colony appearances.

A serological examination of the strains isolated showed that there was considerable antigenic heterogeneity. Agglutinating antisera were prepared by immunising rabbits with three strains of Streptococcus viridans previously isolated by A.L. Wilkie from the cystic gland. Two of these strains were serologically identical, and were quite distinct from the third strain. Agglutination and agglutinin-absorption tests were carried out according to the technique described by McLachlan and Mackie (1928). These tests indicated that one of the strains was related antigenically to two of the cystic gland strains, but was not identical with them, as it did not completely remove the agglutinin from the sera. A second strain likewise showed a partial antigenic similarity to the third cystic gland strain. The remainder of the viridans strains and all the enterococci which were tested did not react with these sera. Agglutinating sera were prepared for two strains of enterococcus, which were themselves serologically distinct as judged by agglutin-absorption tests. None of these enterococci or other streptococci which were studied reacted in a significant manner with these sera.

(Most of the serological tests were carried out by Dr. D.G.S. McLachlan).

OTHER ORGANISMS.

Of the other organisms isolated, B. coli has occurred most frequently and occupied the most important place. Its identification has been carried out by plating out on McConkey's medium. In the case of non-lactose-fermenting organisms, further identification has been carried out by means of the routine tests, including the fermentation of glucose, lactose, mannite, dulcite, inulin, growth in gelatine, and the indol and Voges and Proskauer reactions. With few exceptions the coliform organisms isolated showed the biological characters of "typical B. coli". In one case of chronic cholecystitis a non-lactose-fermenting variety was isolated from the bile and gall-bladder wall. In two cases, B. lactis aerogenes was found. In one of these, it was obtained from the bile and gall-bladder wall. In the other case it was found in the bile in conjunction with enterococci, B. welchii, and typical B. coli. In the tables, it has included under the heading of "B. coli".

B. paratyphosus B. was identified by the usual biochemical tests. In addition it was found to be agglutinated by an anti-serum up to its end titre, and also absorbed agglutinins from it for B. paratyphosus B.

The occurrence of B. welchii has been of some interest. Considerable attention has been paid in recent years to the part played by this organism in acute abdominal conditions, and also to the use of B. welchii anti-serum in acute intestinal obstruction

and in inflammatory lesions associated with the alimentary canal, especially in the presence of gangrene.

It seems to have been first described in cholecystitis by Gilbert and Lipmann (1902), and since then numerous cases have been described. It was found fairly frequently by Rosenow in his series, and in recent years, several isolated cases have been described, including those of Baugher (1914), Cottam (1917), Halle and Marquezy (1922), Brutt (1923), Kirchmayr (1925), and Pierce Gould and Whitby (1927). Recently Gordon-Taylor and Whitby (1930) have paid special attention to the occurrence of this organism in a series of 50 cases which were examined bacteriologically. By using anaerobic methods of culture they found this organism in nine out of the 50 cases, and attribute the lower incidence of the organism in the findings of other observers to the fact that anaerobic methods of culture have been insufficiently employed.

Identification of this organism was made by the typical appearance of the "stormy clot" reaction in milk.

In the present series it was found in six cases: in the gland once, in the gall-bladder wall four times, and in the contents twice. It is interesting to note that two of these six cases come under the heading of acute cholecystitis, and in one of these the gall-bladder was gangrenous.

ANIMAL EXPERIMENTS.

A series of animal experiments has been carried out. The first object of these has been to determine the capacity of the organisms isolated to localise electively in the gall-bladder on intravenous injection, and the second to attempt to ascertain the pathway of infection to the gall-bladder. Rabbits have been used throughout, and altogether 61 animals have been used in the experiments on cholecystitis. In addition a careful search has been made in the rest of the total of 103 animals used in the other part of the work for gall-bladder lesions.

With the object of ascertaining the normal appearances of the various organs of the rabbit, a large number of post-mortem examinations has been made. In the great majority of these cases the animals were perfectly normal stock rabbits which had been killed under an anaesthetic by heart puncture for the purpose of obtaining sterile blood for culture media. Special attention was paid to the stomach, duodenum, gall-bladder and appendix. The following conclusions have been drawn.

The gall-bladder is a very delicate organ. Anatomical variations in size and shape are common. In one animal it was found to be completely absent. In one of the animals reported in the Table in which a laparotomy was done a completely divided gall-bladder with a double cystic duct was found. The gall-bladder is attached to the liver along a narrow area, and is

freely moveable sideways. The main blood supply is derived from the cystic vessels. In appearance it is usually dark blue, and a few delicate transverse striations may be seen. The wall itself is of the thinness of tissue paper, and it collapses readily on being opened. The bile is usually thin, and of variable colour, being most commonly dark green. It has been always found, however, to be free from granules, and it seems important to emphasise this point. The possibility of a spontaneous cholecystitis has been borne in mind, but in no case has this been found. Meyer and his colleagues (1921) found a spontaneous cholecystitis to occur in one per cent. of 500 rabbits.

The stomach and duodenum have been searched for haemorrhages. In three cases, small haemorrhages have been found in the stomach in animals killed by heart puncture under an anaesthetic. In no case has a haemorrhage or other gross lesion been found in the appendix.

The experiments may be divided into two groups. In the first group intravenous injections alone were given. In the second group a laparotomy was carried out with or without some other procedure such as intravenous injection of organisms. The details of each experiment are shown in Table 16.

INTRAVENOUS INJECTIONS.

The organisms used were streptococci, staphylococci, B. coli, B. paratyphosus B, and

B. dysenteriae Shiga. All the strains of streptococci and staphylococci used were isolated from cases of human disease. The other organisms used included both stock cultures and strains isolated after animal experiment.

The strains tested were injected as soon as possible after isolation. Twenty-four hour broth cultures were employed, and the injections given into the marginal ear vein. Two methods of administering the organisms were practised. In the first, small doses of 300 to 500 million organisms were given at weekly intervals as advocated by A.L. Wilkie (1928). Up to nine injections were given. In the second method, a single large dose of 5,000 to 10,000 million organisms was given, and the animal killed in a few days if the injection did not prove fatal. This is the method practised by Rosenow. The enormous doses originally employed by Rosenow, such as the deposit from 45 cc. of a broth culture, were not used. Rosenow has stated (1928) that in more recent work on elective localisation he has only employed one-tenth to one-fifth of the quantities formerly injected.

When the injection did not prove fatal the animals were killed by chloroform at periods varying from a week or two up to several months. All the viscera were carefully examined at once, together with several joints, and usually also the brain. Cultures were made from the heart blood, bile, gall-bladder wall and joints, and from any organ showing a lesion .

Streptococci. Streptococci have been injected intravenously in 21 rabbits. Of these, five were injected with cystic gland strains, three with strains from the gall-bladder wall, ten with strains from the bile, one with a strain from a gall stone, and one with a strain recovered from the gall-bladder wall of a rabbit injected with a cystic gland strain. Thirteen animals received a single injection, and seven animals received multiple injections, the biggest number being nine.

A gross lesion developed in one rabbit (No. 11). This animal received a single injection of 7 cc. of a broth culture of a poorly growing streptococcus isolated from the bile and gall-bladder wall of a case of chronic cholecystitis. The animal remained apparently well and was killed after a week. The gall-bladder was found to be thick and oedematous, and covered with fibrin (Fig. 1.) Microscopic examination of sections of the gall-bladder wall showed considerable oedema of the tissue and infiltration by large numbers of polymorphonuclear leucocytes. The epithelium had desquammated, and streptococci were found in the wall after prolonged search. A streptococcus with the same biological characters as the strain injected was recovered from the wall. Intravenous injection of this organism into another rabbit (No. 12) failed to produce a gross cholecystitis, but a few black granules were present in the bile, and there were haemorrhages and ulceration in the cardiac end of the stomach.

In one other case (No. 5) a slightly oedematous gall-bladder was found after the injection two days previously of 7 cc. of a broth culture of a streptococcus isolated from a cystic gland. The organism was not recovered from the gall-bladder wall or bile.

In a further case (No. 7) the gall-bladder was found to be slightly congested and the bile to be blood-stained. There were also haemorrhages and congestion in many of the other viscera, and vegetations on the mitral and aortic valves (Fig. 12.) In another case (No. 9) there were a few tiny black granules present in the bile.

Apart from these lesions, the gall-bladder was not found to be involved in any other case. Gastric haemorrhages were found in eleven cases. Endocardial lesions were present in two animals. Iritis and conjunctivitis were present in one case, and a suprarenal haemorrhage in one case.

Staphylococci. A rabbit (No. 21) injected with a strain of Staphylococcus aureus isolated from a gall-bladder wall showed no lesion of the gall-bladder. Sub-endocardial haemorrhages were present, together with ulceration of the cardiac end of the stomach and congestion of other viscera.

B. coli. Eight rabbits were injected with different strains of B. coli. Two of these strains were isolated from the bile of cases of human disease,

and one from the gall-bladder wall. The rest were recovered from the heart-blood, bile or gall-bladder wall of other rabbits with an experimental cholecystitis.

A gross lesion of the gall-bladder was produced in three out of the eight rabbits. The first (No. 22) was killed two days after injection of a whole agar slope culture of B. coli isolated from the bile of a patient with chronic cholecystitis. The gall-bladder was found to be white and thickened (Fig. 2), and the bile to be turbid and full of black and yellow granules. There was also an extensive haemorrhage into the first part of the duodenum around the opening of the common bile-duct (Fig. 11). There were also haemorrhages into the mucous membrane of the Fallopian tubes and uterus, and in the pericardium, and congestion of the lungs and spleen.

B. coli was recovered from the heart-blood, bile, and gall-bladder wall. Injection of this organism into a further rabbit (No. 23) did not result in the production of a gross cholecystitis, but there were yellow granules in the bile, and B. coli was recovered from the gall-bladder wall and bile.

Another rabbit (No. 26) was given a single injection of B. coli recovered from the gall-bladder wall of a rabbit injected with streptococci isolated from a root abscess of the author. The gall-bladder was thick and white and showed sub-peritoneal haemorrhages. B. coli was recovered from the bile, and on re-injection into a further animal (No. 28) a gross cholecystitis was again produced, the gall-bladder being tense, white, thick, and with numerous haemorrhages on the surface. The lumen was packed with faeculent-looking matter, from which B. coli was again recovered. Further injection of this organism into another rabbit (No. 29) did not result in the production of cholecystitis.

Only one of the animals died as a result of the injection, but on the whole the effects were far more marked than after the injection of streptococci. Congestion of the viscera and especially of the lungs were more frequent.

B. paratyphosus B. Four rabbits were injected with cultures of this organism. Two were injected with a strain isolated from a gall-bladder sinus, one with a strain isolated from sewage, and one with an old stock culture.

Of the two animals injected with the gall-bladder strain, one died after three days (No. 30). The gall-bladder was thick, and full of muco-purulent contents and black granules (Fig. 3). B. paratyphosus B, together with B. lactis aerogenes and B. coli, were recovered from the bile. The spleen and Fallopian tubes were congested, and the lungs consolidated. The second animal also died after two days (No. 31). There was slight congestion of the gall-bladder, and the organism was recovered from the bile. There were haemorrhages and ulceration of the cardiac end of the

stomach, and haemorrhages in the duodenum around the opening of the common bile-duct. The spleen and other viscera were congested, and the lungs consolidated.

The rabbit injected with the strain isolated from sewage died three weeks after the first injection (No. 32). The gall-bladder was found to be thick and full of soft brownish concretions (Fig. 4), and B. paratyphosus B was recovered from the bile. There were also found to be present enteritis, colitis and pneumonia.

The fourth rabbit received a series of injections of a stock culture for the purpose of producing an anti-serum (No. 33). The first injections were of dead cultures, and the last four of live cultures. The animal was killed two and a half months after the first injection, and was found to have a thick gall-bladder containing black granules.

B. dysenteriae Shiga. One rabbit was injected with a stock culture of this organism for the purpose of producing an anti-serum (No. 34). The last four injections were of live organisms, and the animal was killed three and a half months after the first injection. The gall-bladder was found to be thick and white, and full of thick purulent material and numerous black granules (Fig. 5).

It is interesting to note that both the animals which received preliminary injections of dead organisms followed by live organisms were found to have a gross lesion of the gall-bladder.

DIRECT ATTACK ON THE GALL-BLADDER.

In order to determine the pathway of infection to the gall-bladder a series of experiments involving a laparotomy was carried out. These consisted of direct injection of organisms into the lumen of the gall-bladder, of injection of organisms into the wall of the gall-bladder, of intra-duodenal and intra-portal injections, and of traumatisation of the gall-bladder and ligation of the cystic duct followed by intravenous injection of organisms. Controls were carried out in the latter two series of experiments. The details of the experiments in 27 rabbits are given in Table 16.

Injection of organisms into the lumen of the gall-bladder. A laparotomy was carried out under ether anaesthesia, and the gall-bladder inspected. The gall-bladder was then partly aspirated with a fine needle in order to prevent over distension, and a small dose of the organisms injected in a broth culture or as a suspension in saline. A piece of omentum was placed over the site of injection, but it was found difficult to avoid leakage from the site of injection even though a very fine needle was used.

Two rabbits were injected with small doses of streptococci isolated from bile, and were killed five weeks after injection. In both there was some escape of bile. In the first (No. 35) the gall-bladder was found to be surrounded by adhesions and buried in the liver. The wall was considerably thickened, and the contents consisted of a small amount of turbid fluid and a soft greenish concretion. The same streptococcus was isolated from the bile. In the second animal (No. 36) the gall-bladder was also found to be submerged by the liver. Its wall was thick and white, and it contained caseous material from which staphylococci were isolated (Fig. 6).

B. coli was injected in two rabbits. The first was killed nine weeks after injection (No. 37). The gall-bladder was found to be buried in the liver and surrounded by numerous adhesions. The wall was thick, and the contents consisted of white inspissated material. In the second animal (No. 38) in which there was considerable escape of bile, death occurred nineteen hours after injection from general peritonitis. The gall-bladder was collapsed and inflamed, and contained a brownish friable concretion. The formation of this in such a short time is of considerable interest.

A stock culture of B. typhosus B. was injected into the lumen of the gall-bladder of one rabbit (No. 39). One month later the animal was killed, and the gall-bladder was found to be surrounded by adhesions, and with a white, thickened wall (Fig. 7). B. typhosus was recovered from the contents.

Similarly a stock culture of B. paratyphosus B was injected into the lumen of the gall-bladder of another rabbit (No. 40). Three weeks later the gall-bladder was found to be buried in the liver. The wall was much thickened, and there were several yellow caseous nodules in the wall. B. paratyphosus B was recovered from the contents.

Two animals were injected with B. welchii. The first (No. 41) died in twenty-four hours, and was found to have gas gangrene of the abdominal wall and general peritonitis. The gall-bladder was collapsed and inflamed, and full of reddish semi-solid material. The second animal (No. 42) died three weeks after injection. It was very emaciated. The gall-bladder wall and cystic duct were very markedly thickened (Fig. 8). The contents consisted of thick creamy material and a few brown particles, and B. welchii

was recovered from the lumen.

Injections of organisms into the wall of the gall-bladder. An attempt was made to produce a cholecystitis by injections of organisms into the wall of the gall-bladder. In order to perform this, the gall-bladder was exposed by a laparotomy. It was then steadied either with the finger or by picking it up with fine forceps, and injection made into the tissue of the wall by means of a fine needle with a short bevel. It was found to be very difficult to avoid escape of the emulsion either into the peritoneum or into the lumen of the gall-bladder.

Three rabbits received intra-mural injections of streptococci. The first (No. 43) received an injection at several places of a streptococcus derived from a gall-bladder wall. There was some escape of the emulsion, and the animal was found dead within twenty-four hours with general peritonitis. The second animal (No. 44) was found two and a half months after injection to have slight thickening of the gall-bladder wall, but no organisms were recovered. The third animal (No. 45) which was given injections of a streptococcus isolated post-mortem from a case of ulcerative endocarditis, was killed seven weeks later. The gall-bladder was found to be buried in the liver substance, but there was no very gross change in the wall itself.

As controls, two animals were given intra-mural injections of saline. In both cases the gall-bladder was found to be surrounded by adhesions. In the first (No. 46) the gall-bladder was thick and white at the site of inoculation. In the second (No. 47) there was slight thickening of the wall.

Ligation of the cystic duct. This procedure was carried out in seven animals. The cystic duct was exposed, and an attempt made to dissect off the cystic vessels. This was found to be very difficult owing to the tiny structure of these vessels in the rabbit, and it was sometimes impossible to avoid laceration of them. The cystic duct was then ligated with cotton, or, as on one occasion, with catgut.

Two of the animals were subsequently given intravenous injections of streptococci. The first of these (No. 53) was given one injection of a streptococcus isolated from bile. Five weeks later the gall-bladder was found to be thick, white and enlarged, and full of muco-purulent material from which streptococci were isolated. The second animal (No. 54) was found to have a double gall-bladder and cystic duct. One cystic duct alone was ligated, and subsequently three injections of a streptococcus grown from a cystic gland given. One and a half months later the animal was killed, and was found to have an enormous swelling in the right lobe of the liver, containing about 5 cc. of whitish-yellow semi-solid contents which gave a growth of staphylococci and B. coli on culture.

The other five animals were used as controls,

and ligation of the cystic duct alone carried out. In three of these a gross lesion of the gall-bladder was produced, and one of the specimens is illustrated (Fig. 9). In the other two, the gall-bladder was found to be buried in the liver. In one, the wall was thickened, but the contents were clear. In the other, there was no gross thickening of the wall, but the contents were thick and dark.

Traumatisation of the gall-bladder. Attempts were made to determine the localisation and growth of organisms in the gall-bladder by the use of trauma. In carrying out this procedure a laparotomy was performed under ether anaesthesia, and the gall-bladder was gently rubbed and squeezed with the finger. One animal (No. 55) was subsequently given three injections of a streptococcus isolated from a gall-bladder wall. There was found to be no gross change in the gall-bladder wall, but there were a few black and yellow granules present in the bile. In the other two rabbits the effect of repeated trauma without injection was tried. The first (No. 56) was traumatised on three occasions, and four months after the commencement the gall-bladder was found to be white, thick and full of inspissated material, and surrounded by numerous adhesions. The second (No. 57) was traumatised twice. Three months after the commencement the gall-bladder was found to be thick, white, and full of greenish particles.

Intraduodenal injection of streptococci. In an attempt to produce an ascending infection of the biliary passages, three animals received injections of organisms at a laparotomy into the duodenum in the region of the opening of the common bile-duct. The first (No. 58) received a dose of 6,000 millions of a streptococcus isolated from the lumen of an appendix, suspended in 1 cc. of saline. Five days later, it was killed. The duodenum was found to be thin and inflamed, and perforated on examination. The gall-bladder was normal, and no organisms were recovered from the bile. Two other animals (Nos. 59 and 60) received intra-duodenal injections of B. paratyphosus B. Both animals died before the conclusion of the anaesthetic, however, but it seems difficult to conclude that this was anything more than a coincidence.

Intra-portal injection of B. paratyphosus B. In order to determine the effect of injection of organisms into the portal circulation, one rabbit (No. 61) was given an injection of a stock B. paratyphosus B into the portal vein. A laparotomy was carried out, and one-fifth of an agar slope culture suspended in 1 cc. of saline was injected into the portal vein with a fine needle after it had been exposed and identified. The animal was found dead the next morning with a post-operative hernia and general peritonitis. The gall-bladder was slightly red, but the bile was clear. There were haemorrhages present in the duodenum.

THE PROBLEM OF ELECTIVE LOCALISATION.

Few theories in medical science have been so much debated as that of "elective localisation". Much attention has been paid to the long and fascinating series of experiments carried out over a considerable number of years by Rosenow. The gradual development of his ideas may be followed from one of his later articles (1928). While working originally on the transmutation of pneumococci into streptococci and vice versa, it occurred to him that many diseases might be due to a single member of the pneumococcus-streptococcus group having different localising powers. He found that the site of localisation varied with the virulence of the organism, and gradually the theory of "elective localisation" took shape. He found that two factors were of importance - first, the necessity of using media providing varying grades of oxygen tension, and secondly, the necessity of injecting freshly isolated strains into animals in order to obtain specific localisation. As the work developed, he came to stress the importance of organisms in suspected foci of infection, and found that intravenous injection of these also resulted in elective localisation. Altogether he claims to have substantiated his theories in the case of the following diseases - appendicitis, gastric and duodenal ulcer, cholecystitis, pancreatitis, parotitis, arthritis, myositis, iritis, erythema nodosum, herpes zoster, neuritis, myelitis, encephalitis, epidemic hiccup and

wry-neck, but records failures in pernicious anaemia, leukaemia, catarrhal and epidemic jaundice, and in some other conditions, and considers the results in diabetes only suggestive! He records a further series of recent investigations which confirmed his original work.

His results have been the subject of much attention, and on the whole they have not been generally confirmed. In some cases, indeed, as in the problem of epidemic encephalitis, much conclusive evidence to the contrary has been brought forward. His own figures have been keenly investigated, and Holman (1928) has made a very critical analysis of them. He has shown that there is very little difference in the percentage of lesions produced in various organs with specific and with non-specific strains, and that indeed in many cases the percentage is higher with the non-specific than with the specific strains. Gay (1918), however, has also analysed Rosenow's figures with the opposite result. He finds that among 833 animals injected with 220 strains, gall-bladder lesions were produced in 80 per cent. of cases with specific strains, and only in 11 per cent. with non-specific strains.

Zinsser (1927) after concluding that the weight of evidence is against Rosenow's claims, expresses himself as follows - "It is an interesting thought, yet a dangerous one to spread broadcast, since it has influenced clinical thinking to an extent not warranted by experimental fact".

In the work described above, no evidence in support of the theory of elective localisation has been obtained, though it must be admitted that the series of experiments and controls carried out are relatively small in number compared to those reported by Rosenow. Thus out of 21 rabbits injected intravenously with streptococci or staphylococci only two showed a gross lesion of the gall-bladder. With the coli-typhoid group, however, there has been found to be a greater pathogenic action on the gall-bladder, but this seems to be entirely non-specific, and to occur as readily with stock organisms or organisms isolated from other lesions as with organisms isolated from gall-bladder lesions. Thus out of thirteen animals injected, a gall-bladder lesion was found in nine, but in only four of these was it produced by a gall-bladder strain, either newly isolated, or after animal passage. Out of 29 animals injected with organisms isolated from the appendix, a gall-bladder lesion was found in six, and out of eight animals injected with teeth or tonsil strains, a gall-bladder lesion was found in one. Thus a higher percentage of gall-bladder lesions was produced with organisms from the appendix than from the gall-bladder, though it must be admitted that in the appendix experiments bigger doses were given and the organisms were more virulent.

If there is any tendency at all of an organism to show "elective localisation" it seems to depend on

its biological characteristics rather than on the site of its localisation in the human body. Meyer and Lowenberg (1928) showed that enterococci tended to localise in the gall-bladder experimentally, and it would seem that B. typhosus, whatever its origin, has an "elective affinity" for the gall-bladder.

DISCUSSION.

The bacteriological and experimental work detailed above was originally undertaken with the object of investigating the rôle of streptococci in inflammatory disease of the gall-bladder, and also their power of "elective localisation", or, in other words, their selective affinity for the gall-bladder tissue of animals. An attempt was also made to determine their origin and route of infection to the gall-bladder. It was found, however, that there was little difference in the incidence of streptococci and of B. coli. Thus out of 106 cases examined, streptococci were found in 26 cases, and B. coli in 28 cases. Streptococci were found in the cystic gland or gall-bladder wall in 16 per cent., and B. coli in 20 per cent. Streptococci were found in the contents in 20 per cent. and B. coli in 19 per cent. It was also noted that a large percentage of the streptococci isolated were undoubtedly of the "enterococcus" type, which is found naturally in the bowel. The others belonged to types which are known to occur both in the mouth and in the bowel. In

several cases undoubted enterococci were isolated from the cystic gland and from the gall-bladder wall. In each case also in which streptococci occurred in more than one site the strains isolated were found to have similar characters. It was also found that in some cases mixtures of streptococci and B. coli were obtained from various situations.

All these facts seem to indicate that the original site of the streptococci isolated was the same as that of B. coli (i.e., the bowel), and that therefore the route of infection to the gall-bladder was presumably the same. Gordon-Taylor and Whitby (1930) are strongly of the opinion that the bowel is the main source of infection , and found that in thirty-one out of their 50 cases the organisms isolated were of an undoubted bowel origin. There would seem to be no evidence that the streptococcus is the primary invader and B. coli the secondary invader. The part played by both in the invasion of the gall-bladder seems to be similar. Nickel and Judd (1930) also consider that B. coli is not merely a secondary invader.

It does not seem justifiable to conclude, however, that any of the organisms isolated is the primary factor in the production of inflammatory changes in the gall-bladder. It is conceivable that they may all play merely the rôle of secondary invaders when once some pathological change, toxic or biochemical, has already commenced in the gall-bladder.

Thus it is interesting to note that the highest incidence of organisms occurs in the cases of acute cholecystitis, and in such cases the infecting organism is almost always of a bowel origin. Thus in one of the cases of gangrenous cholecystitis, who died soon after operation, there were found to be present B. coli, enterococci, and B. welchii, as well as a further Gram positive anaerobic organism which was not isolated. On the other hand, the low incidence of organisms in cholesterosis has already been pointed out, although it is found that inflammatory changes are usually present in this condition. It has already been noted that in only one out of 21 cases of chronic cholecystitis without stones was a streptococcus found, which would seem to suggest that the presence of stones may to some extent be responsible for the invasion of the gall-bladder by organisms. Nickel and Judd have suggested that the lower incidence of organisms found in recent series investigated may be due to the fact that earlier cases of gall-bladder disease are operated on at the present day. Thus we tend now to carry out operation for "cholecystitis" rather than for "gall stones".

PATHWAYS OF INFECTION OF THE GALL-BLADDER.

Whatever the part played by organisms in the production of cholecystitis may be, however, it is obviously of the highest importance to determine the route by which they reach the gall-bladder, and at the

present day more attention is being paid to this aspect of the problem of cholecystitis than to the nature of the infection itself.

On theoretical grounds, organisms may reach the gall-bladder in one or more of the following routes:-

(1) By direct spread from a neighbouring viscus or the peritoneum.

(2) By ascending infection from the duodenum via the common bile and cystic ducts.

(3) By descending infection from the liver in the bile.

(4) By direct lymphatic spread from the liver.

(5) By blood spread from the systemic circulation via the cystic artery.

All these routes have at some time received support. Attention at the present day is usually confined to the latter three.

Route (1) must be a rare means of infection, and organisms probably only travel this way when the gall-bladder is pathologically adherent to an inflamed viscus. Rosenow considers that in one of his cases in which the gall-bladder was adherent to a duodenal ulcer the infection spread in this way. Rutherford (1930) has recorded a case of cholecystitis in which he considers that infection reached the gall-bladder from an inflamed appendix that was in apposition to it, as the inflammatory changes were more marked in the serous and muscular coats of the gall-bladder.

Route (2) was formerly considered to be the usual path of infection, especially in the days when

typhoid cholecystitis was common. As late as 1910, McCarty, at the Mayo Clinic, as the result of the examination of 365 gall-bladders, was impressed with the importance of this route, and with the frequent association of duodenal ulcer. He makes no mention of haematogenous infection.

Such infection may travel either in the lumen of the common bile and cystic ducts, or along the mucous membrane by direct spread of inflammation, or via the lymphatics. It is now generally realised, however, that a hollow viscus in communication with the exterior of the body, such as the bladder or uterus, will easily rid itself of at any rate an avirulent infection. It has long been known since the work of Cushing (1899) that the duodenum is relatively amicrobic. Meyer and Lowenberg (1928) have shown that the duodenal juice is actually bactericidal. Meyer, Neilson and Feusier (1921) were unable to produce an ascending infection of the bile passages by the injection of even enormous doses of typhoid bacilli into the duodenum opposite the Ampulla of Vater. Patey and Whitby (1933) failed to produce an infection of the bile after the ligature of the duodenum and the intra-duodenal injection of organisms. In the present work, in the only animal out of three which survived after the intra-duodenal injection of streptococci, no infection of the bile was found.

The difficulty of producing experimentally an ascending infection of the bile passages is thus seen. Such infection may occur more easily in the human body,

however, when there is any disturbance or lowering of resistance in the upper part of the gastro-intestinal tract. It must be noted that the freedom from organisms of this part of the alimentary tract is only relative. Thus in cases of achlorhydria, Davidson (1928) has shown that there may be an enormous quantitative increase in the bacterial flora of the gastro-duodenal region, especially in its streptococcal content. If smears are taken from the interior of the stomach at operation, even in cases of peptic ulcer, where there is often increased acidity, a profuse growth may be obtained, and in the first specimen removed by the duodenal tube in the Meltzer-Lyon test, a growth is frequently obtained, though in both these cases, of course, there is the possibility that a certain number of organisms may have been swallowed with the saliva. Miyaki (1900) found organisms normally present at the lower end of the common bile duct. The association of achlorhydria with cholecystitis is well known, and occurs in 40 per cent. of cases, according to Walton (1930).

On the whole, however, the ascending route of infection finds little experimental or other support at the present day.

Route (3) also formerly received much attention, and Patey and Whitby have recently re-investigated the possibility of infection travelling in this way. It has long been known that organisms derived from the area of portal drainage may appear in the bile, though it has been disputed whether the liver cells must be

damaged before such passage can occur. Futterer (1899) showed that organisms entering the portal vein began to be excreted within a few minutes.

It is urged against this route of infection that histological evidence of catarrh of the gall-bladder is rare, and that the incidence of organisms in the bile is lower than in the gall-bladder. Thus Gordon-Taylor and Whitby have collected a series of figures from various authors. Out of 1,600 cases in which the bile was examined, infection was present in 39 per cent., and in 678 cases in which the wall was examined, infection was present in 67 per cent. Even leaving aside the possibility of fallacy in these figures, such as the greater possibility of contamination of the wall - and the high percentage of staphylococci included by one author in the list suggest that this may be a factor - these figures do not seem necessarily to indicate that infection is present only secondarily in the bile. It is conceivably possible that infection may have reached the gall-bladder wall in the bile, which has then become sterile, while the infection has remained in the tissues of the wall. The constant flow of bile into the gall-bladder may be realised from the fact that the contents of the gall-bladder, which may hold 30 to 50 cc., are concentrated as much as ten times. Cushing (1899) found that typhoid bacilli introduced into the lumen of the gall-bladder of dogs disappeared from the bile within 24 hours, and Petersen (1899) found that the bile from fistulae often became sterile at the end of eight days,

and nearly always after three or four weeks. It has long been realised that bile is inhibitory to the growth of organisms, but this is a quantitative factor. Drennan (1922) has shown that all organisms are inhibited in their growth by a concentration of more than 70 per cent. of bile. As already mentioned, concentrated bile is usually sterile.

Patey and Whitby have found that after intra-portal injections of B. welchii in rabbits the bile does not tend to become infected for 48 hours, and then only in the minority of cases. Meyer, Neilson and Feusier, as the result of a large number of experiments, conclude that as regards the "intestinal organisms, such as the typhoid-dysentery bacilli, and cholera vibrios, the hemato-hepatogenous route is the only one that has been definitely proved."

It has already been shown that in all of the eight animals which were given injections of organisms into the lumen of the gall-bladder and which survived, a gross lesion was produced (Figs. 6, 7, and 8). It may, however, be urged against these findings that the organisms used were relatively virulent and were given in large doses, and that owing to the delicate structure of the rabbit's gall-bladder, any experiment involving direct handling of it may introduce the factor of trauma. In addition, it was difficult to prevent some escape of organisms from the puncture wound, and thus inflammatory change may have taken place from the serous surface rather than from the lumen.

A descending infection must, however, occur in those cases in which the biliary passages fill up with "biliary mud", and Moynihan (1922) has recorded one such case in which after seven previous operations, relief of symptoms was only obtained by removal of a spleen enlarged to thrice the normal size. He suggests that the spleen may be the source of infection in the portal area in such cases.

In cases of obstruction in the common bile duct the bile is usually infected. In such cases bile pigment is usually deficient, and the fluid contents of the duct may be of the nature of "white bile". In such cases the infection must also have descended from the liver, the liver cells being damaged by back pressure and toxic action. Netter (1886) found that the bile became infected within twenty-four hours of the ligation of the common bile duct.

This route, then, still demands our attention as a likely one, and as the probable one in cases of obstruction in the common bile duct.

Route (4), the lymphatic one, has received much attention since the original work of Graham (1918). This author found that in 87 per cent. of cases of cholecystitis the liver was enlarged. After examining sections of the liver in the neighbourhood of the gall-bladder he came to the conclusion that infection spread directly from the liver to the gall-bladder.

This theory has not received general acceptance, as it is usually considered that the changes in the liver are secondary to the gall-bladder changes.

Patey and Whitby have found no signs of spread between the liver and gall-bladder after injection of India ink particles and tumour emulsion. It is possible, however, that under exceptional circumstances this route of infection may be of considerable importance. Andrews and Hrdina (1931), by tying the cystic duct in dogs - great care being taken to avoid the cystic vessels - have found that infection of the gall-bladder very easily occurs, especially with B. welchii. They consider that this organism spreads directly from the liver, and that also it is the important factor in the highly fatal "biliary peritonitis". These observations are of considerable importance. The high incidence of infection in cases of acute cholecystitis, especially with B. welchii, has already been pointed out. The serious acute cases are those with a stone impacted in the cystic duct or neck of the gall-bladder, a condition which has been called "acute obstructive cholecystitis", analogous to the "acute obstructive appendicitis" of Wilkie. The indications for surgical intervention in acute cholecystitis are much disputed. Now, however, that the high incidence of anaerobic infections in the acute cases is realised, and that their probable pathway has been determined, it is possible that we may be able to define a clinical syndrome of "acute obstructive cholecystitis", and thus carry out operation in these cases, while deferring it in the others till a later date. The advisability of giving B. welchii antiserum in such cases is also indicated.

Route (5) - that of infection from the systemic

circulation via the cystic artery - has received much attention since the work of Rosenow. This view was first brought forward by the work of Koch (1909) and Chiarolanza (1909), and has received much support from A.L. Wilkie (1928). It has usually been considered that the origin of infection must lie in some distant focus, such as the teeth or tonsils, but Patey and Whitby have shown that the focus of infection may well lie within the portal area and yet reach the gall-bladder by the cystic artery.

The few experiments that have been carried out in the present work do not allow conclusions to be drawn from them. The control experiments have shown the importance of the part that may be played by trauma in such experiments as ligation of the cystic duct and intra-mural injections. The experiments of Patey and Whitby are of more value. These authors made a series of injections in rabbits, first into the systemic circulation via a marginal ear vein, and secondly into the portal circulation via a mesenteric vein. They used B. welchii on account of the ease of its recognition in cultures, and its lack of virulence towards rabbits. They found by making a series of cultures from various organs that the liver was not an efficient filter of organisms, and that infection was as easy by the portal route as by the systemic. They consider that the source of infection is undoubtedly an intestinal one, and that the most probable route of infection is via the portal and systemic circulations to the cystic artery.

On the whole, present day opinion is strongly in favour of this being the most likely route of infection. The histological evidence and the relative ease with which gall-bladder lesions may be produced experimentally via the blood stream compared to the difficulty with which they can be produced via the biliary passages all favour this view. The evidence is strong, however, that the infection arises in the bowel, and thus if it reaches the gall-bladder via the cystic artery it must have reached the systemic circulation after passing through the liver from the portal blood stream. The inefficiency of the liver as a filter of organisms in the portal blood stream has already been shown. The possibility of descending infection from the liver has already been demonstrated, but this is probably not the common route, and direct spread to the gall-bladder from the liver or neighbouring organs may also undoubtedly occur, but only in exceptional circumstances.

SUMMARY.

Infection has been found to be an important factor in the aetiology of cholecystitis and gall stones and their complications. The organisms most frequently found are B. coli and streptococci in about equal proportions, and the streptococci found are to a large extent similar to those normally present in the bowel. B. welchii also occurs, especially in cases of acute cholecystitis and when the factor of obstruction

is present in the gall-bladder. The organisms found, therefore, are predominantly of an intestinal type, and the bowel would thus seem to be the source of infection in most cases of cholecystitis. The pathway by which infection reaches the gall-bladder is still undecided, but in most cases it is probably either that of descending infection from the liver in the bile or that of blood borne spread from the systemic circulation via the cystic artery.

It still does not seem justifiable on the evidence at present available to consider that infection is the primary factor in the genesis of cholecystitis and gall stones. Inflammatory changes may occur, as in cholesterosis, and gall stones may be formed without it being possible for us to detect, at any rate by our usual methods, the presence of any organisms. When once inflammatory changes have taken place in the gall-bladder wall or stones have formed, a vicious circle may be established. Stasis and obstruction may occur, with the onset of definite infection and the formation of more stones, and the part played by infection in the later stages and in the complications, and the importance of the gall-bladder as a focus of infection are undoubted. While recognising, therefore, that infection is perhaps the most important individual factor in the aetiology of cholecystitis and gall stones, we are not yet in a position to say without further investigation of the earlier changes that it provides the primary factor in this condition.

PART II.

APPENDICITIS.

INTRODUCTION.

The bacteriological and experimental work on appendicitis was commenced simultaneously with the work on cholecystitis and on similar lines. At first the material examined consisted mainly of appendices removed as a routine during operation for some other condition, such as cholecystitis or peptic ulcer. The outer part of the appendix wall, together with any glands from the mesentery that were available, formed the main part of the material examined, and attention was chiefly directed towards determining the presence of streptococci. Later, however, it seemed desirable to make a more complete examination of the material, and to include specimens removed during an acute attack, in which undoubted evidence of inflammation could be found. Finally the investigation was confined to acute cases, and it was endeavoured to examine all cases by a comparable technique, with the object of determining the incidence of all the organisms present, especially of the anaerobes, and of their relative incidence in the various layers of the appendix.

HISTORICAL.

The literature on diseases of the vermiform appendix is vast, and is still being poured out in an endless stream. Only a relatively small proportion of it, however, deals with the aetiology of appendicitis, and there have been few exhaustive bacteriological investigations, at any rate in recent years, considering the importance of the condition in Medicine and Surgery.

The main historical landmarks are now well known. Mestivier, in 1759, made the first record of a case. Melier, in 1827, first distinguished appendicitis from other inflammatory diseases of the intestine, and even suggested the possibility of cure by operation. Fitz, in 1886, proved definitely that most inflammatory conditions in the right iliac fossa have their origin in appendicitis. Krönlein in 1884, was apparently the first to carry out removal of the appendix by operation.

The whole literature up to 1905 has been reviewed by Kelly and Hurdon in their large and beautifully illustrated volume of that date. It may be said that in the period of nearly thirty years that has elapsed since its publication there has been little important advance in our knowledge of the aetiology and pathology of the condition.

Weinberg and his colleagues (1928), in their valuable work which will be referred to later, divide the history of bacteriological investigations of appendicitis into three periods. In the first, which

was in the early days of both Bacteriology and of the study of appendicitis, attention was mainly paid to the presence of aerobic organisms. In the second, which commenced with the work of Veillon and Zuber in 1898, the presence of anaerobic organisms was mainly investigated. In the third and present day period, equal attention has been paid to both aerobic and anaerobic organisms. It is since the War that our knowledge of the anaerobic organisms has been placed on a sound basis, and at the present day no study of appendicitis, or for that matter of any inflammatory condition in which gangrene or tissue destruction occur*, can afford to neglect the factor of anaerobic infection.

Brief reference will be made to some of the more important bacteriological studies that have been carried out. The earlier work has been summarised by Kelly and Hurdon. The first investigation was apparently that of Larquelle, in 1889, who found B. coli in the exudate in general peritonitis following appendicitis. In the early days there was much dispute as to the relative importance of B. coli and streptococci in appendicitis and its complications, and indeed it may be said that this dispute has lasted till the present day. On the whole, however, the work of this period has now little interest for us, mainly on account of the lack of description of the streptococci found, and the confusion that existed in the terminology employed. Thus the name "Streptococcus pyogenes", which is now only applied to a haemolytic streptococcus,

was formerly applied to any streptococcus isolated from a pathological lesion.

Important work, however, was that of Tavel and Lanz, in 1893. These authors made a very thorough investigation of 24 cases, and found B. coli, streptococci, B. pyocyaneus, a diphtheritic bacillus, and a glanders-like organism as well as anaerobic organisms and several species which they did not succeed in isolating. Veillon and Zuber in 1898 examined 22 cases of foetid or gangrenous appendicitis, and their work has been strikingly upheld in recent years by Weinberg and his colleagues. They found anaerobic organisms in nineteen cases, including B. fragilis, B. ramosus, B. perfringens, B. fusiformis, and B. furcosus, and they considered these to be responsible for the gangrenous condition of the appendix and the toxaemia produced in the patient. Krogius (1899) found two species of anaerobes which he identified with their B. ramosus and B. perfringens.

Dudgeon and Sargent (1905) did valuable work on appendicitis and peritonitis. They considered that staphylococcus albus might produce some degree of immunity in the peritoneal cavity. They denied any importance to the anaerobes, however, in appendicitis or its complications.

Peronne (1905) reviewed the previous researches on appendicitis, "ni nombreuses, ni anciennes". He stressed the importance of the anaerobes, including both bacilli and streptococci. In 14 cases, he found diplo-streptococci six times, streptococcus pyogenes

four times, B. fragilis seven times, B. perfringens six times, and other organisms less frequently, including B. pyocyaneus, B. proteus, and B. fusiformis.

Heyde (1911) emphasised the importance of the anaerobes, which he found in 100 out of 102 cases. Jennings (1923) reviewed the literature on anaerobic infections, and describes thirteen cases of diffuse peritonitis in which B. welchii was present. He emphasised the value of B. welchii anti-serum in such cases.

Kraft (1921) made a special search for haemolytic streptococci. He found them to be present in two out of 48 normal appendices, and in four out of 77 pathological appendices. He considers that when they do occur, they are possibly the aetiological agent in the inflammation.

Dudgeon and Mitchiner (1924) examined 25 cases, paying special attention to the histological findings. They isolated B. coli in six cases, B. coli plus streptococci in fourteen cases, and B. welchii in five cases. Spirochaetes were also found in one case.

Warren (1925) reported the results of the investigation of 100 appendices out of many examined. Cultures were made from the serous, muscular and mucous coats. In 66 cases acute inflammation was present. He considered that appendicitis is not a specific disease due to one organism, but is due to many organisms, and decides that the evidence is against the view that it is due to a haematogenous infection.

Meyer (1928) found that the streptococci present in appendicitis were mainly of the nature of enterococci, and similar findings have been reported by many German workers quoted by Aschoff (1932).

Weinberg, Prevot, Davesne and Renard (1928), at the Pasteur Institute, have made what is perhaps the most thorough investigation of the flora of acute appendicitis so far published. They examined 160 cases, in 58 of which the appendix was gangrenous, and while they did not search for any primary causative organism, they endeavoured to make a complete investigation of all organisms present. Altogether they isolated fourteen different aerobic and fourteen different anaerobic organisms, including many species not so far described, and they found that an association of many organisms was frequent. They strongly advocate the use of serum in cases in which an anaerobic infection is predominant.

The importance of the worm Oxyuris vermicularis in producing appendicitis was first suggested by Still in 1899, and this was supported by Metchnikoff (1901), and subsequently by many other authors. The question was very thoroughly investigated by Eastwood (1923), who concluded that this parasite occurred in the same proportion in diseased and in normal appendices, and that there was no support for the theory that it played any important part in the production of appendicitis.

Among those who have produced appendicitis experimentally may be mentioned Poynton and Paine (1911, 1912). These authors found that a streptococcus

isolated from a joint in a case of acute rheumatism attacked the middle part of the appendix in rabbits on intravenous injection, both before and after animal passage. They also quote a case in which appendicitis followed an attack of tonsillitis, and in which they isolated a diplo-streptococcus from both the tonsils and appendix which produced appendicitis on injection in rabbits. Altogether out of 24 rabbits injected with a streptococcus obtained from a joint, appendicitis was produced in four rabbits, together with arthritis and mucous colitis.

The most important work in this direction is that of Rosenow (1915), though this does not seem to have been extensively repeated or confirmed. This author investigated the bacteriology of fourteen cases of acute appendicitis and six cases of chronic appendicitis, and also studied the bacteriology of suspected foci of infection, and the localising power in animals of organisms derived either from these or from the appendices themselves. B. coli was found in every case and streptococci in seventeen cases. It was found that streptococci predominated in the wall of the appendix and B. coli in the lumen. Other organisms, including B. welchii, B. pyocyaneus, diphtheroids, staphylococci, fusiform bacilli, spirilla, and anaerobic organisms were also found. On animal injection of the streptococci isolated, it was found that the tonsil strains produced appendicitis in nineteen out of 29 cases, and the appendix strains in

22 out of 30 cases. In 26 out of 31 rabbits injected with mixtures of streptococci and colon bacilli the appendix was attacked, though there was a greater tendency for the gall-bladder and intestine to be affected. Six out of eleven rabbits injected with pure cultures of colon bacilli showed lesions of the appendix. After animal passage, it was found that the elective affinity for the appendix tended to disappear, and that the stomach, duodenum and gall-bladder were attacked by the organisms to a greater extent.

It is important to note that the average dose of a broth culture given to a long series of which he gives details is no less than 33 cc. In conclusion he regards the infection in appendicitis as being haematogenous in origin, and the streptococcus as being the primary invader, and the colon bacillus as being usually the secondary invader.

Wilkie, in 1914, described the now classical syndrome of "acute appendicular obstruction". He stressed the importance of this condition clinically as opposed to that of acute catarrhal appendicitis. By producing a condition of closed loop obstruction in the isolated ileum of the cat, he found that a condition of gangrene rapidly supervened if the animal had been fed previously on a diet rich in protein, whereas a condition of empyema was found if the diet had been a vegetable one. He considered that appendicitis was associated with a civilised diet containing a high percentage of protein.

TECHNIQUE.

The following technique was adopted. In most cases the material was transferred to the laboratory immediately after removal. In some cases a few hours elapsed. The gland from the meso-appendix, when present, was placed in a sterile test-tube, usually by the surgeon. The appendix itself was placed in sterile swabs or in a sterile test-tube. In many cases in which peritonitis or local abscess were not present, a ligature was tied round the proximal end of the appendix, and the raw end carbolised in order to prevent contamination of the peritoneal surface by the contents of the lumen.

The gland was first cultured, and was ground up and inoculated into broth with all sterile precautions in the same way as the cystic gland.

Cultures from the peritoneal surface were then made. Smears were made on to blood agar and McConkey plates, and a small piece of the peritoneal coat or fat, or fibrin, when this was present, were inoculated into broth. Films were also made. Pieces of the outer coats of the wall down to the mucous membrane were then removed, ground up in a sterile mortar and cultured in the same way as the gall-bladder wall. The lumen of the appendix was then opened, and the appendix was split along its length with scissors. Cultures were then made from the surface of the mucous membrane at one or more places, gangrenous areas being avoided if these were only localised. The media used

were blood agar and McConkey plates, glucose broth and glucose agar in upright tubes, phosphate broth, and Robertson's bullock heart medium. The fluid media were heated before use in order to drive off oxygen, but were cooled before inoculation. In addition, other tubes of glucose broth and of bullock heart medium were heated at 60° C. for periods varying from fifteen minutes upwards, and also at 80° C. for half an hour, the former with the object of killing off non-heat-resistant organisms, and the latter with the object of killing off all non-sporing organisms. In some instances the plates were incubated in an anaerobic jar. It was found that this lessened the growth of such facultative anaerobes as B. coli, and thus favoured the growth of other organisms such as streptococci.

In cases in which it was expected that the number of organisms would be large, either from the nature and extent of the inflammatory changes present, or from the results of film examination, dilutions of the contents of the lumen were plated out. It was found impossible, however, even to make a rough estimate of the number of organisms present. On the whole it was found that when the changes were slight and only consisted of scattered haemorrhages in the mucous membrane, organisms were relatively few in number. In the presence of any obstruction their numbers were enormously increased. When gangrenous changes had passed a certain point, however, and the

appendix had become black or green in colour, the numbers again fell.

Fluid cultures were incubated for at least a week, and were examined microscopically before being discarded. The methods of identification and isolation of the various organisms will be described under the different headings.

RESULTS OF FILM AND DARK-GROUND EXAMINATIONS.

Films were made from both the peritoneal surface and from the mucous membrane, and were stained with Kopeloff and Beerman's modification of Gram's stain. This was found to give very good differentiation between Gram positive and Gram negative organisms, and to be especially useful for showing up the degenerated forms so common in the bowel. In addition, a dark-ground examination of the contents of the lumen was made in many cases, and films were stained for spirochaetes by Fontana's method.

The results of such film examination have presented features of considerable interest. Aschoff (1932) lays great stress on this method of examination, and has drawn important conclusions from his findings. It is possible to some extent to be able to tell the pathological condition of an appendix by means of the films taken from it. Many organisms have been seen, of course, that have not been isolated, and no attempt has been made, as was done so successfuly by Weinberg

(1929) and his collaborators, to isolate every organism seen in films.

In cases of slight catarrhal inflammation, the lumen of the appendix, which is usually empty of faecal matter, films showed few organisms or pus cells. In more advanced cases, Gram negative coliform bacilli usually predominated. Except in cases of gangrene or perforation of the appendix, organisms were seldom seen on the peritoneal surface, and pus cells were scanty. In cases of gangrene, however, both the peritoneal and mucous surfaces presented a striking and similar picture. Cells were almost completely absent. The organisms present were almost entirely Gram positive. A large variety of cocci and bacilli were packed together in masses, and often long filamentous organisms were seen. On several occasions long curved organisms were seen on dark-ground examination, but true spirochaetes were only found on one occasion in the contents of a faecal abscess. Every field was seen to be packed with long treponemata, which possessed numerous, fairly deep, regular coils. In appearance it was something similar to Treponema pallidum, but was slightly thicker and less glistening. Motility was not observed, but the specimen was not examined till 24 hours after removal. The spirochaetes were well shown up by Fontana's silver impregnation method. This organism would seem to correspond to Treponema eugyratum (Werner), a normal inhabitant of the alimentary canal. It could not be found in the stools or urine of the patient three days after

operation. The Wasserman reaction in this case was negative.

MATERIAL AND PATHOLOGICAL FINDINGS.

Altogether material from 84 cases has been examined. The greater part of this has been obtained from several theatres in the Edinburgh Royal Infirmary. A few cases were operated on in nursing homes, and some in the Royal Hospital for Sick Children.

Out of the 84 cases, seven are excluded from the present survey for the following reasons. In five of these cases, removal of the appendix was carried out when the clinical features suggested a diagnosis of appendicitis. In one of these, a subsequent diagnosis of Paratyphoid Fever was made on clinical grounds. In this case, streptococci were isolated from the peritoneum and outer coats, and streptococci, B. coli, and B. welchii from the lumen. On subsequent examination, the Widal reaction, blood culture and stools were persistently negative, but an outbreak of Paratyphoid Fever in the ward was considered to have originated in this case. In another case, pelvic adhesions following childbirth three and a half months before were found. In the third case, a ruptured pyosalpinx was found, and B. coli and streptococci were cultured from the peritoneum. In the fourth case, no pathological change was found in the appendix or elsewhere in the abdomen. In the fifth case, no lesion

was found in the appendix, but a peritonitis with free turbid fluid was present, which was evidently the local evidence of a septicaemia which persisted after the operation. Staphylococcus albus was obtained from the peritoneal fluid.

The sixth case was one of pneumococcal peritonitis in a male child of four years. A pneumococcus, Type IV, was isolated from the peritoneal exudate. The seventh was a case of streptococcal peritonitis following tonsillectomy in a boy of fourteen. There was a general peritonitis, and a haemolytic streptococcus was obtained from the peritoneal exudate. The appendix was acutely inflamed throughout all its coats, and B. coli and B. lactis aerogenes were obtained from its lumen.

Classification has again presented some difficulty, as with cholecystitis. In the absence of any known aetiological factors we are driven to classifying our cases on the basis of naked-eye appearance, and to some extent this is unsatisfactory, as one specimen may show a variety of changes. The important factor in the progress of the case is the presence of obstruction, and this has been emphasised by Wilkie (1914) in his classical description of the syndrome of "acute obstructive appendicitis". It is possible, however, for an appendix to be gangrenous without there being any obstruction present in the form of a stricture, concretion, kink or band, though such cases are uncommon.

In this survey, therefore, the cases have been

classified on a broad basis of naked-eye appearances, and they are analysed in Table 4.

ACUTE APPENDICITIS.

The cases of acute catarrhal appendicitis, six in number, are those in which the changes, in the form of haemorrhages, ulceration or oedema, have been confined to the mucous membrane. Involvement of the other coats in such cases is usually seen on microscopical examination, but there have been no gross changes on naked-eye examination in the muscular or serous coats.

The cases of acute interstitial appendicitis have been 21 in number, and have formed the biggest individual group. In these, the whole wall of the appendix has been diffusely inflamed, and the peritoneal coat has been involved, with dilatation of vessels, loss of lustre, and sometimes the presence of fibrin, without, however, there being involvement of the peritoneum elsewhere in the abdomen. Thread-worms were present in one case.

Six cases were partly or completely gangrenous. Concretions were not noted in any of these, though of course it is possible that they had escaped before examination. In two cases, the appendix was noted as being retro-caecal.

In five cases peritonitis was present. Three of these were of the acute obstructive variety, and perforations and concretions were present, the appendix

being wholly or partly gangrenous. One other was perforated and partly gangrenous, though no concretion was noted, and in the fifth, in which the peritoneal fluid alone was examined, a concretion and a very adherent appendix were found.

In three cases a local abscess was present. In the first, a gangrenous appendix was lying free in the abscess cavity and was removed. In the second, the abscess was merely drained. In the third, the appendix was found to be perforated and partly gangrenous, and a small abscess cavity shut off by omentum was present.

An empyema of the appendix was found in three cases. In one of these, the appendix was removed for symptoms of two days duration. In the second it was removed for recurrent attacks of abdominal pain, and in the third as a routine procedure during the operation of cholecystectomy.

A mucocele was present in one case in which two attacks of pain had occurred.

The classification adopted is to a large extent arbitrary, and of course it is impossible to avoid overlapping among the various sub-divisions. Thus doubtless in some of the cases classified as gangrenous appendicitis there must have been some degree of peritonitis present, and also the dividing line between catarrhal and interstitial appendicitis is very narrow. On the whole, however, such a classification is of help to us in associating together the pathological changes and the bacterial flora present.

Altogether forty-five cases are grouped under the heading of acute appendicitis. Almost all of these cases were operated on as emergency cases, and as in all of them a definite lesion was present, they offer a suitable series for investigating the bacteriology of acute appendicitis. A summary of the findings is given later in the text.

CHRONIC APPENDICITIS.

There has been much dispute as to whether a true chronic appendicitis can exist, i.e. a state of chronic inflammation of the appendix that is not the result of acute attacks. Histological evidence gives us little help, as with increasing age a higher incidence of fibrosis and obliteration of the appendix is found. On the other hand an appendix may show no evidence of previous inflammation even a short time after a definite attack.

Under this heading have been grouped the cases of sub-acute appendicitis, of "chronic" appendicitis, and of appendices removed as a routine during the performance of some other operation.

There were found to be seven cases of sub-acute appendicitis. Most of these were operated on for recent symptoms, and in all of them there was some evidence of recent inflammation in the form of redness, thickening of the wall or adhesions. Threadworms were present in one, and were associated with definite evidence of inflammation in the appendix.

Under the heading of "chronic" appendicitis are grouped ten cases in which the symptoms were of prolonged standing, and in which no other lesion was found that might have accounted for them. In three cases adhesions were present, in two cases constrictions or kinks, and in one case a bulbous tip.

The remaining fifteen cases comprise those in which the appendix was removed as a routine during the performance of a laparotomy for some other condition. There was a variable degree of fibrosis present in some of these specimens, but there were no gross pathological changes or evidence of active inflammation.

Altogether thirty-two cases have been included under this heading, and the bacteriological findings are discussed later.

DISCUSSION OF BACTERIOLOGICAL FINDINGS.

The bacteriological findings in the different categories are shown in Table 4. The number of cases in which glands, peritoneum, appendix wall and lumen have been examined in each category are also shown, and the bacteriological findings are given under each heading.

Acute catarrhal appendicitis. (Table 5). A gland from the mesentery of the appendix was examined in only one case, and no growth was obtained. The peritoneum was examined in four cases, but a growth was only obtained once, when B. coli was isolated. The former

TABLE 4.

ANALYSIS OF APPENDICITIS CASES.

cute catarrhal appendicitis...	6 cases
cute interstitial appendicitis	21 "
angrenous appendicitis..	6 "
cute appendicitis with peritonitis..	5 "
cute appendicitis with abscess	3 "
mpyema of the appendix..	3 "
ucocele of the appendix.	1 "
Total cases of acute appendicitis.....	45
ub-acute appendicitis...	7 cases
hronic appendicitis	10 "
outine appendectomy at operation for gastric ulcer..	1 "
" " " " " duodenal ulcer..	5 "
" " " " " cholecystitis...	9 "
Total cases of "chronic" appendicitis...	32
ases excluded. Pelvic adhesions..	1 case
Paratyphoid.	1 "
Streptococcal peritonitis ...	1 "
Septicaemia.	1 "
Ruptured pyosalpinx	1 "
Exploratory laparotomy.. ...	1 "
Pneumococcal peritonitis ...	1 "
Total of cases excluded.....	7
Total of all cases.....	84

dictum of Treves that "there is no appendicitis without peritonitis" would therefore seem to need revision at the present day. It is probable that earlier diagnosis and operation have provided us with material at an earlier stage of inflammation than was previously available. The numerical incidence of organisms on the peritoneal surface in the case in which they isolated was small, moreover, when judged by direct smears on plates.

The outer coats of the wall were examined in four cases. In one of these, no growth was obtained. In two cases, a pure growth of streptococci was obtained, and in one case a mixture of streptococci and an unidentified Gram positive anaerobic bacillus.

TABLE 5.

ACUTE CATARRHAL APPENDICITIS.

(6 cases).

	Gland. (1 case)	Peritoneum. (4 cases)	Outer coats. (4 cases)	Lumen. (4 cases)
No growth.	1	3	1	
B. coli.		1		1
Streptococci.			2	
B. coli plus streptococci.				1
Streptococci plus a Gram positive anaerobic bacillus.			1	
Streptococci plus *B. welchii*.				1
B. coli, *B. welchii*, *B. lactis aerogenes*, *B. pyocyaneus*.				1

The contents of the lumen were examined in four cases. *B. coli* was obtained once in pure culture, *B. coli* plus streptococci once, *B. welchii* plus streptococci once, and *B. coli*, *B. welchii*, *B. lactis aerogenes*, and *B. pyocyaneus*, once. The association in the latter case of *B. coli* and *B. pyocyaneus* does not seem to bear out the conclusion of Dudgeon and

Sargent that the latter organism exerts an inhibitory action on the former.

The cases under this heading in which examination of both the outer coats of the wall and the lumen were made are too few to enable one to draw conclusions about the numerical incidence of organisms in the various layers of the appendix. Rosenow found that streptococci were more numerous and B. coli less numerous in the outer coats of the wall than in the lumen.

Acute interstitial appendicitis. (Table 6). The cases under this heading form the biggest individual group in the series, and perhaps the most valuable from the bacteriological point of view. In such cases, inflammatory changes are definite and diffuse, and when gangrene has not commenced, other factors such as the presence of dead tissue and the cutting off of blood supply are not present to complicate the picture of the bacterial flora.

A gland was examined in five cases. No growth was obtained in two cases, B. coli in one, streptococci in one, and a mixture of B. coli, streptococci and B. lactis aerogenes in one case.

The peritoneal surface was examined in ten cases. No growth was obtained in four cases. A pure growth of B. coli was obtained in three cases, a mixture of B. coli and streptococci in one case, and of B. coli and B. lactis aerogenes in two cases.

In two of the cases in which B. coli was isolated, there is the possibility that contamination had occurred

TABLE 6.

ACUTE INTERSTITIAL APPENDICITIS.

(21 cases).

	Gland (5 cases)	Peritoneum. (10 cases)	Outer coats. (14 cases)	Lumen. (17 cases)
No growth.	2	4	4	0
B. coli.	1	3	4	4
Streptococci.	1			
Staphylococcus albus.			2	1
B. coli plus streptococci.		1	2	1
B. coli plus B. lactis aerogenes.		2		
B. coli plus a Gram positive anaerobic bacillus.				1
Streptococci plus B. welchii.			1	
B. coli plus streptococci plus B. lactis aerogenes.	1			
Streptococci plus B. welchii plus B. lactis aerogenes.			1	1
B. coli plus staphylococcus albus plus a sarcina.				1
B. coli plus streptococci plus B. welchii.				3
B. coli plus streptococci plus B. lactis aerogenes.				
B. coli plus B. lactis aerogenes plus a diphtheroid bacillus.				1
B. coli plus streptococci plus B. welchii plus a Gram positive anaerobic bacillus.				1
B. coli plus streptococci plus B. lactis aerogenes plus a Gram positive anaerobic bacillus.				1
B. coli plus streptococci plus B. welchii plus B. lactis aerogenes plus a Gram positive anaerobic bacillus.				1
B. coli plus streptococci plus B. welchii plus a diphtheroid bacillus plus Gram positive and negative anaerobic bacilli.				1

from the mucous membrane of the divided end of the appendix. The incidence of infection of the peritoneal surface of these cases is thus relatively low. It must be emphasised as already pointed out that one specimen may show several types of inflammatory change, and in some of the cases placed in this group patches of gangrene were already commencing. In such cases, of course, the flora of the peritoneal coat will be similar to that of the lumen. The impression has been formed that the peritoneal coat is very seldom infected when the external changes merely consist of redness, oedema, loss of lustre, and even the presence of fibrin or turbid reactionary fluid. Any green or black discolouration, however, suggests that the organisms present in the wall or lumen have reached the peritoneal surface, and that the incidence of anaerobic organisms will be found to be high.

The ordinary physical characteristics of the peritoneal fluid or peritoneal reaction are also of great practical value in the diagnosis of the organisms present. Sterile fluid is completely odourless, whereas when once infection has occurred the peritoneal exudate will have a definite smell, the nature of which will depend on the nature of the organism or organisms present. The smell attributable to B. coli infection is well known, although it has been alleged that pure B. coli pus is odourless, and that the odour is due to other organisms present. The presence of anaerobic organisms produce a characteristic smell that can be learned by experience and the handling of

anaerobic cultures, though it is usually in appendix and other faecal abscesses that this is found, and seldom in the peritoneal cavity. The practical value of such observations will lie in their helping to decide the question of drainage and the treatment of the abdominal wall.

The outer coats were examined in fourteen cases. No growth was obtained in four cases, B. coli alone was obtained in four, staphylococcus albus in two, B. coli plus streptococci in two, streptococci plus B. welchii in one, and streptococci, B. welchii and B. lactis aerogenes in one case.

The contents of the lumen were examined in seventeen cases, and positive cultures obtained in all. B. coli alone was obtained four times, staphylococcus albus once, B. coli plus streptococci once, B. coli, streptococci and B. welchii three times, streptococci, B. welchii and B. lactis aerogenes once, B. coli, staphylococcus albus and a sarcina once, B. coli and a Gram positive anaerobic bacillus once, B. coli, streptococci, B. lactis aerogenes and a Gram positive anaerobic bacillus once, and B. coli, B. lactis aerogenes and a diphtheroid bacillus once. In addition, combinations of five organisms were found twice, and of six organisms once, and these are detailed in the Table. A Gram negative anaerobic bacillus occurred in one of these cases.

Altogether B. coli occurred alone or in combination in nineteen cases, streptococci in ten cases, and B. welchii in seven cases. One organism

alone was isolated in six cases, two organisms in four cases, three organisms in five cases, four organisms in three cases, and five and six organisms each in one case.

Gangrenous appendicitis. (Table 7). A gland was examined in one case, and B. coli isolated. The peritoneum was examined in two cases, and B. coli alone obtained. This doubtless, however, does not adequately represent the flora present, as complete anaerobic cultures of the peritoneal coat were not always carried out. The results of film examination suggest that when gangrene is present there is no difference in the flora present on the peritoneal surface and in the lumen.

The outer part of the wall was examined in five cases. In three of these, B. coli alone was obtained, in one a mixture of B. coli and streptococci, and in one a mixture of B. coli, streptococci, B. welchii and a Gram positive anaerobic bacillus. In three cases in which the outer coats of the wall and the lumen were examined, the results of cultures were identical, except that in one case an additional organism in the form of a Gram positive anaerobe was obtained from the lumen.

The lumen was examined in four cases. B. coli alone was obtained in one case, B. coli plus streptococci in one case, B. coli, streptococci and a Gram positive anaerobe in one case, and B. coli, streptococci, B. welchii and a Gram positive anaerobe in one case.

Altogether B. coli was obtained in six different cases, streptococci in three, and B. welchii in one. One organism alone was found in three cases, and two, three and four organisms respectively in one case.

TABLE 7.

GANGRENOUS APPENDICITIS.

(6 cases).

	Gland. (1 case)	Peritoneum. (2 cases)	Outer coats. (5 cases)	Lumen. (4 cases)
No growth.	0	0	0	0
B. coli.	1	2	3	1
B. coli plus streptococci.			1	1
B. coli plus streptococci plus a Gram positive anaerobic bacillus.				1
B. coli plus streptococci plus B. welchii plus a Gram positive anaerobic bacillus.			1	1

Appendicitis with peritonitis. (Table 8). The peritoneum was examined in three cases. B. coli plus streptococci were obtained in one case, B. coli plus B. welchii in one case, and B. coli plus B. lactis aerogenes in one case. The outer coats were examined in two cases, and B. coli alone obtained in one case, and in combination with streptococci in one. The lumen was examined in three cases, and B. coli plus streptococci obtained in one case, B. coli, streptococci and

B. lactis aerogenes in one case, and B. coli, streptococci, B. welchii, B. lactis aerogenes, and Gram positive and Gram negative anaerobic bacilli obtained in one case. Of the two cases in which cultures were made from both the peritoneal and mucous surfaces, in one case identical organisms were found, and in the other two organisms were found on the peritoneal surface and six on the mucous surface.

Altogether B. coli has been found in five cases, streptococci in three, and B. welchii in two. One organism was found in one case, two in two cases, and three and six each in one case.

TABLE 8.

APPENDICITIS WITH PERITONITIS.

(5 cases).

	Peritoneum. (3 cases)	Outer coats. (2 cases)	Lumen. (3 cases)
No growth.	0	0	0
B. coli.		1	
B. coli plus streptococci.	1	1	1
B. coli plus B. welchii.	1		
B. coli plus B. lactis aerogenes.	1		
B. coli plus streptococci plus B. lactis aerogenes.			1
B. coli plus streptococci plus B. welchii plus B. lactis aerogenes plus Gram positive & negative anaerobic bacilli.			1

Appendicitis with abscess. (Table 9). The contents of an appendix abscess were examined in three

cases. In one, B. coli alone was found. In the second, B. coli, streptococci, spirochaetes and fusiform bacilli were found, and in the third, B. coli, streptococci, B. welchii and staphylococcus albus.

TABLE 9.

APPENDICITIS WITH ABSCESS

(3 cases)

B. coli.	1 case
B. coli plus streptococci plus spirochaetes plus fusiform bacilli.	1 "
B. coli plus streptococci plus B. welchii plus staphylococcus albus.	1 "

Empyema of the appendix. (Table 10). The outer coats were examined in two cases. In one, there was no growth, and in the other, B. coli was obtained. The pus in the lumen was examined in all three cases. In one, B. coli alone was obtained, in the second, B. coli plus streptococci, and in the third, B. coli, streptococci, B. welchii, B. proteus and a Gram positive anaerobic bacillus.

TABLE 10.

EMPYEMA OF THE APPENDIX

(3 cases)

	Outer coats. (2 cases)	Lumen. (3 cases)
No growth.	1	
B. coli.	1	1
B. coli plus streptococci.		1

(Contd. on next page).

TABLE 10. (contd.)

	Outer coats.	Lumen.
B. coli plus streptococci plus B. welchii plus B. proteus plus a Gram positive anaerobic bacillus.		1

Mucocele of the appendix. (Table 11). In this case, a mixture of B. coli and streptococci was obtained from the outer coats, and a diphtheroid bacillus from the lumen.

From the four cases in the last two groups, B. coli has been obtained five times, streptococci four times, and B. welchii once.

TABLE 11.

MUCOCELE OF THE APPENDIX.

(1 case)

	Outer coats.	Lumen.
B. coli plus streptococci.	1	
Diphtheroid bacillus.		1

Summary of findings in cases of acute appendicitis. Among the 45 cases examined under this heading, a growth of organisms has been obtained in every case but two. In one of these a gland only was examined, and in the other the outer coats of the wall alone. Altogether thirteen different types of organisms have been isolated, and these will be discussed later.

One organism alone has been isolated in fourteen

cases, two and three organisms respectively in nine cases, four organisms in seven cases, and five and six organisms in two cases respectively. Among the individual organisms isolated, B. coli heads the list, being found in 41 cases, alone or in combination. Thus in only two out of 43 cases in which a positive culture was obtained was it not found. Streptococci were found in 25 cases, B. welchii in 14 cases, B. lactis aerogenes in 6 cases, Gram positive anaerobes in 10 cases, Gram negative anaerobes in two cases, staphylococcus albus in two cases, diphtheroid bacilli in three cases, and a sarcina, B. proteus, B. pyocyaneus, spirochaetes and fusiform bacilli each in one case.

In analysing these results, several factors must be borne in mind. When numerous organisms are present in actively inflammed tissue, great difficulty is often experienced in separating and isolating them. The ubiquitous presence of B. coli, which, as has just been shown, occurs in almost every case, and its tendency to swamp all other organisms both in plate and fluid cultures, has provided the principle difficulty. Great difficulty has also been found in isolating and identifying the anaerobic organisms which are so frequent and so numerous when inflammation is severe or gangrene present. Thus on several occasions in bullock heart medium a mixture has been obtained of coliform organisms, streptococci, B. welchii and other organisms, which it has not been possible in the present work to separate. Great credit must be given

to Weinberg and his colleagues of the Pasteur Institute, who, with all the experience which they gained during the War of investigating gas gangrene have been at such pains to isolate every organism seen in films, and have also described several hitherto unknown organisms. They have well said that "l'isolement des microbes de l'appendicite est surtout une question de patience".

It may be well at this point to compare the present results with those of Weinberg and his colleagues. These latter examined 160 cases, of which 58 cases were gangrenous. Altogether fourteen different aerobic and fourteen anaerobic organisms were described. In the vast majority of cases more than one organism was present, and in two-thirds of the cases, either two or three organisms were isolated. As many as seven different organisms were isolated in a single case. They emphasise that they have aimed at identifying all the organisms present, especially in gangrenous cases, and have not searched for any causative organism. They consider that all the intestinal flora can be found in cases of appendicitis. The principle individual organisms found were B. coli in 87 per cent., enterococci in 30 per cent., B. welchii in 30 per cent., B. ramosus in 10 per cent., Gram negative anaerobes in 39 per cent., and anaerobic cocci in 18 per cent. The anaerobic forms they found to be especially associated with gangrenous appendicitis, in which their numbers are far greater than in other

forms of appendicitis.

In the present series, less attention has been paid to cases of gangrenous appendicitis and material from only a small number of these has been examined. The average number of organisms found in each case has been far smaller, and in the biggest individual group only one organism has been isolated. In addition the same total of organisms, and especially of anaerobic organisms, has not been found. The differences in results may partly be due to differences in the material, but in the main are no doubt due to the exhaustive technique adopted by the Pasteur Institute workers. On the whole the main results are similar in their general findings, in the incidence of the principle organisms such as B. coli, streptococci and B. welchii, and in the absence of pneumococci, which are considered by some authorities, especially German workers, to be common.

BACTERIOLOGICAL FINDINGS IN "CHRONIC" APPENDICITIS.

Sub-acute appendicitis. (Table 12). The gland has been examined in four cases. No growth was obtained in one case, B. coli was found in two cases, and B. coli and B. welchii in one case.

The peritoneum was examined in one case, and no growth obtained.

The outer coats were examined in five cases. No growth was obtained in one case, B. coli in two cases, B. lactis aerogenes in one case, and B. coli and

streptococci in one case.

The lumen was examined in four cases. B. coli was obtained once, B. coli plus streptococci once, B. coli, streptococci and B. welchii once, and B. coli, streptococci, staphylococcus albus and a Gram negative anaerobic bacillus once.

TABLE 12.

SUB-ACUTE APPENDICITIS.

(7 cases)

	Gland. (4 cases)	Peritoneum. (1 case)	Outer coats. (5 cases)	Lumen (4 cases)
No growth.	1	1	1	
B. coli.	2		2	1
B. coli plus streptococci.			1	1
B. lactis aerogenes.			1	
B. coli plus B. welchii.	1			
B. coli plus streptococci plus B. welchii.				1
B. coli plus streptococci plus staphylococcus albus plus a Gram negative aerobic bacillus.				1

"Chronic" or interval cases. (Table 13). A gland was examined six times. No growth was obtained in four cases, B. coli in one, and B. welchii in one.

The outer coats of the wall were examined in seven cases. There was no growth in three, and B. coli, streptococci, B. welchii and staphylococcus albus were each found in one case.

The lumen was examined in five cases. B. coli was found once, B. lactis aerogenes once, and mixtures

of B. coli and streptococci three times.

TABLE 13.

"CHRONIC" APPENDICITIS.

(10 cases)

	Gland. (6 cases)	Outer coats. (7 cases)	Lumen. (5 cases)
No growth.	4	3	
B. coli.	1	1	1
Streptococci.		1	
B. welchii.	1	1	
B. lactis aerogenes.			1
B. coli plus streptococci.			3
Staphylococcus albus.		1	

"Routine" appendectomies. (Table 14). A gland was examined in four cases. No growth was obtained in one case, B. coli was found in two cases, and B. subtilis in one case.

The outer coats were examined in twelve cases. No growth was obtained in ten cases, and B. coli in two cases.

The lumen was examined in one case, in which a mixture of B. coli and streptococci was obtained.

TABLE 14.

ROUTINE CASES.

(10 cases)

	Gland. (4 cases)	Outer coats. (12 cases)	Lumen. (1 case)
No growth.	1	10	
B. coli.	2	2	
B. coli plus streptococci.			1
B. subtilis.	1		

Out of the 32 cases classified under the heading of "chronic appendicitis" B. coli has been found in sixteen cases, streptococci in eight cases, and B. welchii in three cases.

NATURE AND IDENTIFICATION OF THE ORGANISMS ISOLATED.

Attention has been paid to the nature of the organisms isolated with the object of determining the site of their origin in the body and the method by which infection spreads in appendicitis.

B. coli has been the organism occurring most frequently, and has been identified in the usual way by its cultural characteristics and its fermentation of lactose. In a few cases it has been of an atypical variety, or a late lactose fermenter. B. lactis aerogenes has also been identified by its cultural characteristics. Owing to its frequent occurrence it has been thought advisable to give it a separate place rather than include it under the heading of B. coli as was done in the cholecystitis work.

The streptococci isolated have presented great variety in their characteristics. Of those that have been fully tested out the "enterococcus" has provided the biggest group. This organism most frequently occurs in the form of lance-shaped diplo-cocci or diplo-streptococci, produces a diffuse haze in broth, is heat resistant and sometimes bile-resistant, though it has often been found difficult to get it to grow in a bile-containing medium on first isolation. It also

splits aesculin, and is relatively avirulent to laboratory animals. It seldom produces colour on a coagulated blood medium on first isolation, though as noted by Rosenow, after repeated sub-culturing green pigmentation may be produced. Several strains also of viridans streptococci have been isolated, frequently of a non-mannite fermenting character.

Anaerobic streptococci were sometimes found in mixtures on anaerobic cultivation in cases in which they did not appear in the aerobic cultures. Attempts to isolate these were found to be very difficult owing to their strict anaerobic requirements and the constant presence of coliform organisms. In one case tiny streptococci were found that would appear to correspond to the Streptococcus micros of the Pasteur Institute workers.

Haemolytic streptococci were not found in any case, though as already mentioned one case of haematogenous peritonitis due to this organism was examined. The tiny zone of haemolysis noted in some of the cholecystitis strains also was not found in the appendicitis strains.

Owing to the difficulties of classification of the streptococci it has been considered desirable to include them all under the collective heading of "streptococci" in the tables. As already stated, a large percentage of them were undoubtedly "enterococci" by all tests. Others undoubtedly belonged to the "viridans" class, but there were many strains that could be placed in neither category according to our

present standards. Thus it was found that several non-viridans streptococci were not heat resistant, and an even bigger number were not bile resistant. The fermentation of mannite has been found to be of no help in the classification of the strains, and it seems desirable at the present day that the names "salivarius" and "faecalis" should be dropped, as they suggest an origin in the body for the streptococci which they describe which is not always justified. In addition, several strains of strictly anaerobic streptococci were found which it has not been found possible to clasify further.

B. welchii, which has been found with some frequency, has usually been found in association with B. coli and streptococci in the anaerobic cultures. Its identification has been made by the typical "stormy clot" reaction in milk.

The Gram positive anaerobes which have been found in ten cases have not been isolated and identified, but morphologically most of them would seem to correspond to the Bacillus ramosus which was first found in appendicitis by Veillon and Zuber in 1898, and which has recently been exhaustively studied by the Pasteur Institute workers. These latter have found that it does not appear on sub-culture for sometimes as long as eight days, which may explain the difficulty of its isolation in the present series.

The Gram negative anaerobes which have been found in two cases appear to correspond morphologically

to the Fusobacterium biacutum of the Pasteur Institute workers.

The spirochaete found on dark-ground examination in the contents of an appendix abscess seems to correspond morphologically to Treponema eugyratum (Werner), which, so far as I have been able to find, has not so far been described in appendicitis. Weinberg described a spirillum, and Dudgeon and Mitchiner (1924) also found a spirochaete in appendicitis, but neither seems to correspond to the one described above. True syphilitic appendicitis is rare. A case has been described by Evans and Rowlands (1930).

ANIMAL EXPERIMENTS.

Experiments have been carried out on 30 rabbits, and these are detailed in Table 16. The object of this part of the investigation was to determine the power of the organisms isolated, and especially the streptococci, to localise electively in the appendix. Rosenow has strongly supported the existence of this power of "elective localisation," and succeeded in producing lesions in the appendix in 86 out of 143 animals injected.

Eighteen animals were injected intravenously with streptococci. One of these received a single injection of a haemolytic streptococcus derived from a case of streptococcal peritonitis which has been excluded from the appendicitis series. Four days after

injection, it was killed. The gall-bladder was red and oedematous, haemorrhages were present in the stomach and caecum, and there was also pericarditis and general congestion of the viscera.

All the other seventeen animals were injected with non-haemolytic streptococci derived from appendices, ten different strains of these being used. Except in one case, single injections only were given, and bigger doses were used than in the cholecystitis experiments. The enormous doses given by Rosenow, however, were not used, the biggest being 35 cc. of a broth culture. Although the mortality was small, the virulence of the organisms was fairly high. Most of the animals were killed within a few days of the injections, and marked congestion of the viscera was common.

In one case only, a few tiny haemorrhages were found in the appendix. In two cases, small retention cysts were found in the mucous membrane of the appendix, but these are of very doubtful significance.

Haemorrhages were found in the stomach, duodenum or small intestine in seven cases. The bile was found to contain black particles in two cases. There were arthritis in one case, endocarditis in one, and pancreatic haemorrhages in one case.

B. coli isolated from the appendix wall, lumen, or from an abscess, was injected in four animals, a different strain being used on each occasion. In one case there was slight congestion in the distal part

of the appendix, together with definite cholecystitis, the gall-bladder being thick and whitem and full of purulent material. In another case there were haemorrhages in the stomach and duodenum. Otherwise no gross lesions were found.

A strain of B. lactis aerogenes isolated from the lumen of a case of acute catarrhal appendicitis was injected into two animals. No lesions of the appendix were noted in either. In one the gall-bladder was thick and white, and contained black granules and purulent matter from which B. lactis aerogenes was isolated. In the other there was an acute arthritis of the knee-joint, together with congestion of the lungs. In both animals the spleen was enlarged and congested.

The strain of B. pyocyaneus isolated from the lumen of a case of catarrhal appendicitis was injected into two animals. In neither was any lesion of the appendix noted. In one the gall-bladder was thick and full of white granules, and the other viscera were very congested. In the other the gall-bladder was oedematous and full of soft brownish concretions. There were also vegetations on the wall of the right ventricle, haemorrhages in the stomach, and congestion of the other viscera.

Two animals were injected with small amounts of the primary bullock-heart-medium culture containing B. coli, B. lactis aerogenes, streptococci, B. welchii and a Gram positive anaerobic bacillus isolated from the lumen of a case of interstitial appendicitis.

Both were killed after four days, and neither showed any lesion of the appendix. In one, there was a definite cholecystitis, and in the other, a haemorrhage in the pyloric end of the stomach.

One animal injected with a sarcina isolated from the lumen of a case of acute interstitial appendicitis showed no lesion after receiving several injections over a period of three months.

An attempt was made in one rabbit to produce appendicitis by direct injection of organisms into the appendix. The appendix was exposed by a laparotomy, and its wall injected in five places with a small amount of an emulsion of a streptococcus isolated from the wall of a case of acute interstitial appendicitis. Four days later the animal was killed. There were adhesions at the base of the appendix, and sub-peritoneal haemorrhages in the caecum, but the appendix itself showed no gross lesion, and streptococci were not recovered from its wall. As this difficulty of producing appendicitis by direct injection of organisms was in accordance with the experience of other workers, the experiments in this direction were not continued.

Two experiments were carried out in order to determine the part played in peritonitis by staphylococcus albus, to which considerable attention has been paid by Dudgeon and Sargent. In the first rabbit an intra-peritoneal injection was given of a streptococcus isolated from the peritoneal exudate in a case of perforated duodenal ulcer. The animal

remained well, and was killed fifteen days later. There was no general peritoneal inflammation, but the coils of intestine at the site of injection were white and thickened, and the mesentery was full of hard glands, from which B. coli, B. welchii and streptococci were isolated. The gall-bladder was thick, and filled with semi-solid greenish material.

In the second animal, a preliminary intra-peritoneal injection of staphylococcus albus isolated from the same case, followed ten days later by the same injection of streptococci as in the first case. Four days after the second injection the animal was killed. There was no gross lesion present, and the peritoneal cavity was sterile. This finding would seem to support the theory that staphylococcus albus plays some part in producing immunity in the peritoneal cavity.

DISCUSSION OF EXPERIMENTAL WORK.

It is thus seen that it is very difficult to produce experimental appendicitis in the rabbit by the intravenous injection of organisms, and the theory of "elective localisation" has not been upheld. Out of 30 rabbits injected intravenously with organisms, a slight or doubtful lesion was only produced in three cases. Gall-bladder lesions were found with greater frequency, occurring in seven cases, and haemorrhages in some part of the intestinal canal were found in ten cases. Care was taken to use freshly isolated strains,

and in some cases the primary culture was used. Moderate doses were given, but the large doses originally used by Rosenow, who in his appendicitis work gave an average dose of 33 cc. of a broth culture to a big series of animals, were not used. Rosenow has stated, however, that in more recent experimental work on elective localisation, considerably smaller doses have been used. In the present work, none of the organisms that have been isolated from cases of acute appendicitis have been found to have any selective affinity for the appendix of experimental animals.

AETIOLOGY AND PATHOGENESIS OF APPENDICITIS.

In spite of the frequency and gravity of appendicitis, we still know little about its aetiology and pathogenesis. There is probably no other condition in Medicine which has such a mortality and morbidity and which may be so easily cured, and yet about the aetiology which we know so little. The present work was undertaken with the object of ascertaining the bacteria present in the early stages of acute inflammation of the appendix, and of determining whether there was any organism present that may have acted as the primary factor in inducing the inflammation. No special organism or association of organisms have been found that do not ordinarily occur within the lumen of the bowel. In one of the

cases excluded from the appendicitis tables, a haemolytic streptococcus was found in the appendix in a case of streptococcal peritonitis following tonsillectomy, but otherwise, no definite association, clinical or bacteriological, has been found in the cases examined between appendicitis and throat infections. Stress has been laid on this association by many authors such as Rosenow, since attention was called to it by Kelynack in 1893 and by Adrian in 1901, and Hilgermann and Pohl (1929) claim to have found similar organisms in the throat and appendix in 300 cases. They found pneumococci in 50 per cent. of cases, and do not consider that tonsillar strains may be found in the appendix by chance, as the diphtheria bacillus and the organisms of Vincent's angina have never been found in the normal appendix.

The anatomical similarity of the tonsil and appendix, which has been called the "ileo-caecal tonsil", and the undoubted occurrence of appendicitis as a result of tonsillitis in some cases, all favour the possibility of haematogenous infection sometimes occurring. Rosenow and Dunlap (1916) have recorded an epidemic of appendicitis, which also favours this possibility.

Most authors agree, however, that the organisms that can be isolated from inflamed appendices are predominantly, if not entirely, of an intestinal type. Thiercelin, in 1899, first described the enterococcus, which he found in 21 cases of appendicitis, and this organism has since been found frequently by Meyer (1928)

and other workers. In the present work, it has been the main individual type of streptococcus isolated, whatever the pathological condition of the appendix may have been. Aschoff (1932) considers that in over 70 per cent. of cases the streptococci of the intestines are responsible for appendicitis.

The evidence, then strongly favours the view that in the majority of cases the infection in appendicitis is an enterogenous one. The nature of the exciting organism, however, has been much disputed. It was previously considered to be B. coli, owing to the universal presence of this organism in inflamed appendices. The tendency of this organism to outgrow other organisms in culture, however, which provides such a difficulty in investigating this disease, has probably given a wrong impression of its importance in former years. More recently the streptococcus has been considered to play the primary role. Rosenow found the incidence of streptococci to be greater in the outer coats of the appendix, which he considered favoured the theory of the infection being blood-borne.

Aschoff has recently reported work on this subject carried on for many years, and including the examination of 1,000 appendices. He investigated the problem by means of smears, sections and cultures. He concludes that in over 70 per cent. of cases the streptococci of the intestines are responsible for appendicitis, and, less frequently, pneumococci and B. coli. Other organisms which he considers assist in

the development of the attack are B. coli, when it is not the primary exciting agent, a Gram positive bacillus, (probably the B. ramosus of Weinberg which has been found several times in the present work) and a Gram negative influenza-like bacillus. He considers that the streptococci which cause appendicitis are those which occur normally in the intestine, and which are found in profusion in the distal part of the appendix, where they constitute a special appendix flora.

The part played by the anaerobes in the development of appendicitis has been known since the work of Veillon and Zuber in 1898, and on the whole it may be said that we have made little important advance in our knowledge of the bacteriology of appendicitis since their day. It is strange to note, however, that Dudgeon and Sargent, in 1905, in their otherwise valuable work on the bacteriology of peritonitis, deny any importance at all to the anaerobes in appendicitis. Since then, however, numerous authors have ascribed to them an important rôle. Heyde (1911) found them in 100 out of 102 cases, and considers them to play an important part, at least as secondary invaders. Rosenow also considered them to be an important factor in the later stages of the disease. The recent work of Weinberg and his colleagues has already been referred to, and provides the most full account of these organisms that has so far been reported, including the description of several new species. They ascribe, however, the primary rôle in the

pathogenesis of appendicitis to B. coli and to B. welchii, and are struck by the frequent association of these two organisms, which they found together in 51 out of 160 cases, and also by the association of the enterococcus and B. coli, which they found together in 41 cases.

In the present series of cases, the number of gangrenous specimens examined is too few to prove definitely the part played by the anaerobes in the later stages of appendicitis. It has been found, however, in the cases of interstitial appendicitis examined, that the numbers of individual anaerobes and also of the different strains present rise as the inflammatory and gangrenous changes become more marked. The evidence, therefore, supports strongly the view that the anaerobes act merely as secondary invaders when the inflammatory changes are well advanced.

If considerable oedema is present, especially in the meso-appendix, the blood supply of the appendix may be interfered with, and this will be especially easy when the appendix is long, or when the meso-appendix is short. This interference with the blood supply will of course favour the multiplication of the anaerobic organisms. If any obstruction in the form of a stricture, adhesion, kink or concretion is present, gangrenous changes may proceed with great rapidity, and the condition of the "acute obstructive appendicitis" of Wilkie be brought about. It was formerly considered that obstruction at the base of the appendix by the

valve of Gerlach might be responsible for the development of most cases of appendicitis, but that this cannot be the case is shown by the absence of faecal matter in most cases of catarrhal or interstitial inflammation, and the occurrence of inflammation in the foetal type of appendix, with a broad funnel shaped opening into the caecum, of which type one case has been examined in the present series. Other anatomical factors, such as the position of the appendix and the nature of the omentum, in limiting the infection to the appendix or allowing it to spread are well known, and are rather of clinical interest.

So far no mention has been made of the primary factor in the production of appendicitis. The geographical incidence of this condition and the ratio of its occurrence among civilised and non-civilised peoples have been much discussed, and the evidence strongly favours the view that it is a disease of civilisation and that its increase is associated with an increasing use of the diet of civilisation. Appendicitis has been referred to by D'Arcy Power as the great endemic disease of the civilised world. Williams (1910) made a thorough investigation of the problem, and came to the conclusion that appendicitis was on the increase and that it was associated with the eating of meat. By personal enquiry among practitioners in all parts of the world, he found that it was rare or uncommon in Denmark, Spain, Italy, Roumania, Egypt, Persia, China, Polynesia, Morocco,

Central Africa, Abbysinia, and the West Indies. It was found to be more common among Europeans living in some of these countries, however, such as Persia. It was frequent in Norway and Finland. A similar investigation has been carried out by Rendle Short (1920), who points out that the death rate is high in Holland and the United States. In addition, he has made an important statistical investigation of appendicitis. He concludes, as the result of the study of a considerable mass of data that there has been a real increase in the frequency of the condition in civilised peoples, and that this increase has been most marked in the period between 1895 and 1905, since when it has remained more or less stationary. The rise has been mainly in the towns, in the male sex, and among the better-off classes, and the inhabitants of institutions have been relatively immune. He concludes that neither meat-eating nor the addition of any particular food substance to the diet seems to account for all the facts, but suggests that the cause is the relatively smaller quantity of cellulose consumed in a modern civilised diet. More recently (1934) he has reviewed further evidence in favour of this theory, and has shown that it is rare among vegetarians and among those who were affected by the hunger blockade during the War.

Theobald (1930) as the result of personal investigation in Asia has found that appendicitis is rare in Southern China. He has found that in Japan,

however, it is as common as in Europe, and that also in Jerusalem and Cairo it is on the increase, and that this increase has synchronised with a rise in the consumption of tinned meat.

It would appear, therefore, from the above evidence and from much more, that appendicitis is undoubtedly a disease of civilisation and one for which a civilised diet is responsible. The exact factor responsible is still uncertain. It may either be an absolute or relative increase in the amount of meat eaten, or an absolute or relative decrease in the amount of cellulose consumed. Possibly a vitamin deficiency may also be responsible. McCarrison (1920) has shown experimentally that a deficiency of vitamins may lower the resistance of the alimentary canal to infection.

It is probable that as a result of the dietetic factor a slight catarrh of the mucous membrane of the appendix may be produced, and a transient undiagnosed attack may occur, a condition which Aschoff considers to be of common occurrence. Such an attack may be followed by some fibrosis or by a stricture of the wall, with resulting stagnation and possibly the formation of a concretion. If, then, a further attack occurs, a favourable opportunity may be afforded for the aerobic organisms normally present to multiply and increase in virulence. As the inflammation progresses, and the blood supply is interfered with, the conditions that have been found to be favourable to the development of the anaerobes in wound infection will be reproduced,

with the resulting invasion of these organisms and an increase in their virulence. Such a condition will be favoured by any anatomical abnormalities and especially by the presence of any obstruction.

SUMMARY.

The primary factor in the aetiology of appendicitis is probably a dietetic one, and is found in the nature of the food eaten under civilised conditions, which tends to contain an excess of protein and a deficiency of cellulose as compared with that consumed in more natural surroundings. The secondary factor in the great majority of cases is an enterogenous infection arising in the bowel, and the progress of this infection may be assisted by various anatomical factors and by the presence of obstruction in the lumen of the appendix. In the later stages when the circulation is interfered with and tissue destruction is present, a great increase in the numbers and virulence of the anaerobic organisms occurs, and it is these which provide the gravity of the infection in the more serious cases of appendicitis and in its complications. Their presence and pathogenic action renders advisable the use of a polyvalent anti-serum to a greater extent than is generally used at the present day.

PART III.

PEPTIC ULCER.

INTRODUCTION.

It has been considered desirable to include both gastric and duodenal ulcer together in the present survey. It is probable that their aetiology is similar, and the present tendency is to consider them together under the term "peptic ulcer". The difference in their diagnosis, complications, and treatment, is, of course, well recognised. Thus a simple gastric ulcer may undergo malignant change - though the percentage risk of this complication occurring has been hotly debated - whereas a duodenal ulcer almost never becomes malignant. The greater liability of duodenal ulcers to perforate is also well recognised. Hyperchlorhydria is more commonly associated with duodenal than with gastric ulcers, and it is in such cases that there is a great tendency for an anastomotic ulcer to occur after operative treatment. Moynihan (1923) has emphasised the definite anatomical distinction between the two kinds of ulcer, and has shown the undesirability of the term "juxta-pyloric". In spite, however, of many important differences between the two kinds of ulcer and of the practical bearing of these, similar theories have been advanced to account for them both, and these can best be

reviewed together.

HISTORICAL SURVEY OF EXPERIMENTAL AND BACTERIOLOGICAL WORK.

A vast amount of research work has been done on the subject of peptic ulcer, though on the whole, bacteriological investigation and experimental work form a relatively small part of this. It has been found easy in experimental animals to produce an acute ulcer or erosion of the stomach, and to a less extent, of the duodenum, though it has been found more difficult to produce a callous or chronic ulcer similar to those found in the human subject. Lists of the methods employed are given in the articles of Turck (1906) and Rosenow (1916). They include bruising, excision, and ligation of the mucous membrane; cauterisation, clamping, application of hot substances, of nitric acid and silver nitrate, and of hydrochloric acid combined with trauma; sub-mucous injection of silver nitrate, nitric acid, adrenaline and alcohol; injection of fat and lead chromate into the gastric artery; ligation of the oesophagus and pylorus, ligation of the portal vein, section of the vagus and of the sympathetic nerve supply of the stomach and of both; injection of alcohol into the vagus, intersection of the thalami and peduncles, removal of the adrenals; and the intravenous injection of snake venom, pilocarpine, atropine, chloroform, phenol, copper sulphate, bile, bile salts, beta-tetra-

hydronaphthylamine, adrenaline, diphtheria toxin; of B. pyocyaneus, and of dysentery, lactic acid and colon bacilli, and streptococci; and also several other methods. The great majority of the ulcers produced by these methods, however, are of an acute type and heal readily. The experiments which have most nearly reproduced the chronic ulcers occurring in man have been those carried out in the dog by Rosenow (1916) by the intravenous injection of streptococci and by Mann and Williamson (1923) by operative interference with the mechanism for neutralising gastric acidity. These two series of experiments are of great importance and will be referred to again later.

As a result of this experimental work and of observation on the human subject, four factors have been held responsible for the production of peptic ulcer. These may be called the vascular, the acid, the neurogenic and the infective factors, and they will each be considered separately.

(1) The vascular factor. Virchow, in 1853, was the first to suggest that a gastric ulcer might be similar to an infarct in other organs, and that it might be due to a septic embolism from some distant focus lodging in one of the end arteries of the gastric mucosa, with subsequent digestion of the mucosa of the infarcted area by gastric juice. This received considerable support in the case of duodenal ulcer from the observation of Mayo (1908), who found that on traction of the stomach at operation an anaemic spot appeared in the anterior wall of the duodenum, which is

by far the commonest site for ulcer, and also from the work of Wilkie (1911)[1], who showed that the first part of the duodenum was supplied to some extent by an end artery.

The possibility of ulcer being due to venous thrombosis and embolism was first suggested by the occurrence of a haematemesis after operations upon the lower abdomen. Experimental evidence in favour of this was found in the work of Wilkie (1911)[2] who showed that a thrombosis of the mesenteric veins could be easily produced in cats, and that such thrombi could separate and result in the production of a gastric ulcer. He considered that the omental vessels might form a connecting link between the appendix region and the region of the stomach, and thus explain the occurrence of peptic ulcer as a complication or sequel of appendicitis.

It was also formerly thought that the endarteritis present in the vessels in the neighbourhood of ulcers might have played some part in their production, but more recently it has been concluded that this is a sequel of the ulceration.

(2) <u>The acid factor</u>. The high incidence of hyperchlorhydria in peptic ulcers, the effect of alkali treatment in improving symptoms and assisting healing, the rarity of ulcers in cases with achlorhydria, such as Pernicious Anaemia or chronic gastritis, and the tendency to recurrent or anastomotic ulceration in cases with a high acidity, have long been considered to indicate the importance of a high level of acidity

in the production of ulcers.

The importance of the acid factor has been demonstrated in a striking manner by the experiments of Mann and Williamson (1923) at the Mayo Clinic. By a series of operations on dogs they showed that when the alkaline bile and pancreatic secretions were diverted from the part of the intestine accustomed to receiving them, there was a marked tendency for ulceration to occur in the intestine receiving the un-neutralised acid gastric secretion, such as the jejunum. They also found a streptococcus present in the base of one of their experimental ulcers on culture, and in several cases also in sections.

(3) The neurogenic factor. The importance of the action of the autonomic nervous system in the aetiology of peptic ulcer has long been realised. The part played by worry in the exacerbation of an ulcer is well known. Treatment of ulceration by section of the vagus nerves in order to cut off the para-sympathetic nerve supply has been practised by Mayo and others. As long ago as 1862, Schiff produced ulceration experimentally by intersection of the thalami and peduncles, and the importance of the nervous mechanism has been demonstrated very vividly by Cushing (1930). This writer found that in three cases after radical extirpation of a cerebellar tumour, fatal perforations of peptic ulcers occurred. The parts of the alimentary tract affected were the stomach, duodenum and oesophagus respectively, the perforations being multiple in the first two cases.

The fourth ventricle had been widely opened in two of the cases. He concluded that something had upset the nervous mechanism which protects the mucosa from digestion by gastric juice. He had also been struck by the frequency of symptoms suggestive of ulcer in patients with tumours in the region of the third ventricle.

(4) The infective factor. Fenwick, in 1900, suggested the importance of infection, and Turck, in 1906, considered B. coli to play an important part, and produced ulcers in dogs with this organism. Dawson (1912) stressed the association of ulcers with mouth infections.

Most attention has been paid to this aspect of the subject since the work of Rosenow (1916), however. This author isolated streptococci from a large percentage of gastric and duodenal ulcers, and considered that they tended to localise electively in the stomach and duodenum of experimental animals. He found streptococci to be present, either alone or in combination with other organisms, in 42 out of 52 typical chronic ulcers. On injection of 23 of these strains into 117 animals-many of these being dogs - he found that haemorrhages were produced in 61 per cent. and ulcers in 60 per cent. of cases in the stomach or duodenum. Feeding the animals with large amounts of cultures of the organisms, together with meat and particles of dried splintered bone, produced no lesions in the stomach or duodenum. After animal passage, the percentage of haemorrhages and ulcers

produced dropped to 23 and 33 per cent. respectively. Streptococci isolated from the tonsils and the roots of infected teeth of patients with ulcers also produced ulcers on injection.

He found that the location of the ulcers corresponded strikingly to the location of ulcers in man, and considered that the state of function of the animal's stomach was of importance in determining the production of ulcers. He also found that infection inhibited locally the secretory functions of the gastric cells, and considered that therefore digestion of the damaged cells was due to the gastric juice formed in other portions of the stomach. He quotes work on two epidemics of duodenal ulcer in children reported by Gerdine and Helmholz (1915) and Helmholz (1909), which were due to a streptococcus. He also quotes work to show that spontaneous ulcer of the stomach in dogs, calves, cattle and sheep is commonly due to a circumscribed streptococcal infection. He concludes that the usual ulcer of the stomach and duodenum is primarily due to a localised haematogenous infection of the mucous membrane by streptococci.

Similar work has been reported by Haden and Bohan (1925). Other organisms have also been considered to have an important aetiological relationship. Hoffmann (1925) isolated a bacillus which he considered fulfilled Koch's postulates. He found it in two cases in stomach washings and once in the tonsils. In character it was a tiny Gram-negative organism which grew on ordinary media, and produced

lesions in the duodenum of experimental animals with great frequency, and could be isolated again from such lesions.

Askanazy (1920) has considered a fungus infection to play an important part.

TECHNIQUE AND MATERIAL OF PEPTIC ULCER INVESTIGATION.

The material examined in this work has consisted of gastric ulcers removed by local excision or by partial gastrectomy, of the edges of perforated duodenal ulcers, and of gastric, duodenal, jejunal and mesenteric glands removed at operation in cases of peptic ulcer. Rosenow in his work carried out at examination of both ulcers and glands.

The following technique has been adopted. After removal in the theatre, the specimens were placed in sterile swabs or glass containers and transferred to the laboratory, where immediate culture was carried out. The glands were cultured in the same way as the cystic gland. In the case of the ulcers, the peritoneal surface was seared with a spatula, and the peritoneal coat reflected with sterile instruments. Small pieces of the fibrotic tissue of the ulcer were then removed. These were taken rather from the periphery of the ulcer than from the centre, and care was taken not to perforate the mucous surface of the ulcer. The tissue was then emulsified in a sterile mortar, and cultured in broth in the same way as the gall-bladder wall.

In the case of the duodenal ulcers, the material

has simply consisted of a small piece of the edge of a perforated ulcer prior to its closure by suture. Cultures were then made from the surface of the tissue, or from another specimen of peritoneal exudate if this were available, and the tissue was immersed in spirit, washed in sterile saline, ground up in a sterile mortar and cultured in broth.

BACTERIOLOGICAL FINDINGS.

The findings in this part of the investigation are shown in Table 15. Material from 21 cases has been examined. Two other cases have been excluded in which glands were examined but which were proved to be not cases of peptic ulcer. A gastric ulcer alone was found in five cases, and was excised either locally or as part of gastric resection in all of these cases. The ulcer was therefore available for examination in all of these cases, and in three of them a streptococcus was found, no growth being obtained in the others. In one of these latter, however, a streptococcus was found in a gastric gland. In two further cases, a gastric ulcer was found in combination with one or more duodenal ulcers. In one of these, the gastric ulcer was available for examination, but no growth was obtained. A staphylococcus aureus was obtained from a gastric gland in the same case.

In twelve cases, a duodenal ulcer alone was found. In three of these cases, operation was carried out for perforation. In two of these cases, no growth

TABLE 15.

PEPTIC ULCER CASES.

Case No.	Age	Sex	Diagnosis	Material	Findings	Remarks
1		M	Gastric ulcer	Gastric ulcer Gastric gland	Streptococcus -	
2	45	M	Gastric ulcer	Gastric ulcer Gastric gland	- Streptococcus	Perforation of duodenal ulcer & gastro-enterostomy 2 years previously.
3	37	M	Old duodenal ulcer	Gastric gland Duodenal " Jejunal "	B. coli B. coli B. coli	Gastro-enterostomy 6 years previously. Gastro-duodenostomy.
4	42	M	Duodenal ulcer	Duodenal gland	-	9 years symptoms.
5	48	M	Perforated duodenal ulcer	Edge of ulcer	-	Symptoms of several hours standing. Death.
6		F	Gastric ulcer	Gastric ulcer	-	
7	44	M	Duodenal ulcer & papillomatosis of stomach	Gastric gland	B. welchii	Very high acidity.
8		M	Gastric ulcer	Gastric ulcer	Streptococcus	
9		F	Duodenal ulcer with stenosis	Duodenal gland	-	
10	35	M	Duodenal ulcers	Duodenal gland	-	Anterior & posterior ulcers.
11	31	M	Jejuno-colic fistula	Jejunal gland	-	Gastro-enterostomy 11 years previously. Operation for jejuno-colic fistula of 2 years standing.
12		M	Duodenal ulcer	Duodenal gland	-	
13	48	F	Pyloric stenosis	Pyloric gland	B. welchii	30 years history. Symptoms of tetany.
14	26	F	Perforated duodenal ulcer	Edge of ulcer	-	
15	58	F	Gastric and duodenal ulcers	Duodenal gland	-	
16	33	M	Duodenal ulcers with stenosis	Pyloric gland	-	14 years history. Anterior & posterior ulcers. Very high acidity.
17	40	M	Gastric ulcer	Gastric ulcer	Streptococcus	Also chronic cholecystitis. Cystic gland, gall-bladder wall & bile all sterile.

(contd. on next page).

8	M	22	Duodenal ulcers	Duodenal gland	-	Anterior and posterior ulcers.
9	M	36	Gastro-jejunal ulcer	Mesenteric gland	Staphylococcus albus	Gastro-enterostomy 9 years previously.
10	M	32	Gastric ulcer Duodenal ulcers	Gastric ulcer Gastric gland	- Staphylococcus aureus	12 years symptoms. Anterior and posterior duodenal ulcers, and gastric ulcer.
11	M	48	Perforated duodenal ulcer	Peritoneal exudate	Staphylococcus albus Streptococcus	Perforation about 5 hours old. Uneventful recovery of patient.

(- indicates no growth.)

was obtained from the edge of the ulcer. In the third, the peritoneal exudate alone was examined, and a culture of staphylococcus albus and a streptococcus obtained.

Of the nine other cases of duodenal ulcer, in three the ulcer was healed and stenosis was present. In a further one a gastro-enterostomy had been carried out six years previously, but the ulcer had not healed. In none of them was the ulcer available for examination. In one case, B. coli was obtained from gastric, duodenal and jejunal glands. In another case of pyloric stenosis, B. welchii was obtained from a pyloric gland. In a third case, in which papillomatosis of the stomach was also present, B. welchii was obtained from a gastric gland. In the other six cases, cultures from neighbouring glands proved to be sterile.

Of the two remaining cases, in one there was a jejuno-colic fistula following a gastro-enterostomy eleven years previously. No growth was obtained from a jejunal gland. The other was a gastro-jejunal ulcer following a gastro-enterostomy nine years previously. Staphylococcus albus was obtained from a mesenteric gland.

DISCUSSION.

It will be seen therefore that the amount of material available in the bacteriological investigation of peptic ulcer has not equalled that in the other

investigations. The most valuable material has been that of the actual gastric ulcers, and out of the five examined, a streptococcus was found in three. On the whole, these findings are in accordance with those of most observers, namely that a streptococcus may be found in the base of a large percentage of ulcers if care is taken to avoid surface infection. As stated already in the historical summary, Rosenow isolated a streptococcus in 42 out of 54 ulcers.

The streptococci isolated have been non-haemolytic and mainly of the viridans variety. In one case of gastric ulcer two kinds of streptococci were isolated, one belonging to the viridans class, and the other one being of a non-green-producing heat-resistant variety.

ANIMAL EXPERIMENTS.

Two rabbits were injected with separate strains of streptococci isolated from gastric ulcers. The first, (No. 102) which received several small doses, showed no lesion. The second, (No. 103) which received a large injection of a primary broth culture, apparently developed melaena the day after injection, but on post-mortem examination twelve days later showed no lesion of the stomach or duodenum. The gall-bladder was healthy, but the bile contained numerous small black granules. The patient from whom this strain had been isolated had also had a chronic cholecystitis, but no growth was obtained from the gall-bladder on culture.

It has already been noted that haemorrhages in

the stomach or duodenum occur relatively frequently after intravenous injection of organisms. The significance of these is doubtful. Three gastric haemorrhages of small extent were found in a normal animal killed under an anaesthetic. They have been noted frequently among the animals receiving intravenous injections. Thus they were found in fifteen of the 34 animals receiving gall-bladder strains, and in nine out of 23 receiving appendix strains. Out of 37 animals injected with streptococci they were found in nine cases, and out of nineteen animals receiving coliform organisms they were found in six cases. The incidence is thus seen to be less in the animals injected with streptococci than with other organisms.

DISCUSSION.

The evidence that infection is the primary factor in the production of peptic ulcer is much stronger than it is in the case of cholecystitis or appendicitis. The high percentage of cases in which organisms, predominantly streptococci, can be isolated from the base of ulcers, and the ease with which haemorrhages, and to a less extent ulcers, can be produced by intravenous injection of organisms into animals, all favour the view that it is a highly important factor in the production of ulceration. The difficulty, of course, is to determine whether it is a primary or a secondary factor. The finding by Mann and Williamson of a streptococcus in the base of one of the peptic

ulcers which they produced in dogs by preventing the normal neutralisation of acid gastric juice suggests that infection is only a secondary factor. The perforation of a jejunal ulcer following an attack of acute appendicitis noted by Gordon-Taylor (1928), and the fatal perforation of a duodenal ulcer which one has known to follow an attack of otitis media and lateral sinus thrombosis may also be open to the same interpretation. It is more difficult to explain, however, the haematemesis that may follow appendicitis or any other septic infection, or the duodenal ulcer that may follow burns, if we conclude that infection is only a secondary factor. The epidemics of duodenal ulcer that have been recorded and the occurrence of similar spontaneous ulcers in domestic animals all favour the theory that infection is the primary factor. The clinical observation that a duodenal ulcer may follow a short time after an attack of acute appendicitis also seems to support this theory. Once an erosion or acute ulcer has commenced, several factors may then come into action. A vicious circle may be established, and a chronic ulcer may thus commence. The normal mechanism for protecting the mucous membrane from digestion may be disturbed, and Cushing has shown how this may be brought about by the action of the sympathetic nervous system. The chronic ulcers which we have the opportunity of examining bacteriologically are, of course, the end results of a process which is usually many years old, and one must draw conclusions from the findings in

such cases with some hesitation. Suitable control cases might be found by examining bacteriologically the base of carcinomatous ulcers, but this does not seem to have been done on any large scale.

Whatever the part played by the infection may be, however, its origin and the route by which it reaches the site of the ulcer is even more uncertain. Of recent years, the general trend of opinion, largely as a result of Rosenow's work, has been to believe that the infection is a haematogenous one arising in some distant focus such as the teeth or tonsils. Rosenow produced haemorrhages or ulcers with great frequency by intravenous injection of organisms, whereas feeding the animals with the same organisms together with irritating substances produced no lesion.

In the present state of our uncertainty about the classification of the non-haemolytic streptococci we cannot definitely assign a place of origin to any non-haemolytic strain which we may isolate. The characters of the enterococcus are fairly well known, and normally it is confined to the bowel. The characters of the other non-haemolytic streptococci, however, are not so well defined, and it is known that they may occur in any part of the alimentary tract. The streptococci found in peptic ulcers are usually of the viridans class, though enterococci may also occur, as in one case in the present series, in which a mixture of viridans streptococci and enterococci were found. Davidson (1928) found that in achlorhydric conditions the streptococci found in the gastric

contents were predominantly of the salivarius type, but that enterococci were present in small numbers, and he concludes that these latter were derived from the food. He has found that in normal persons, presumably without hyper-achlorhydria, micro-organisms are actually or practically absent in the gastric contents. This latter finding, however, is not in accordance with a few investigations which have been made in the present work, in which swabs taken from the mucous membrane of the stomach or the surface of ulcers have given a profuse growth, predominantly of streptococci. It must be admitted, however, that a possible source of fallacy may have been that as the specimens were taken at operation a large quantity of saliva swallowed at the commencement of anaesthesia may have been present, and that the secretion of gastric juice may have been inhibited - whereas the specimens in the work of Davidson were taken from perfectly healthy subjects.

Though hyperchlorhydria is found in the majority of cases of peptic ulcer, it is not universal, and in many of the cases in which it is present, it may be a secondary effect due to pyloric spasm as a result of the ulcer. Even in cases with a high acidity there must be periods of considerable length after neutralisation in which opportunity is given for multiplication of the innumerable organisms brought down with the food and saliva. When, therefore, the surface of the mucous membrane is exposed constantly to the presence of streptococci, direct infection seems

a more likely possibility than blood borne infection. The presence of pyorrhoea may provide a gross source of infection, although it has been shown that the greatest age incidence of pyorrhoea does not correspond to that of appendicitis, and the same is true, to a less extent, of peptic ulcer.

The importance of infection in peptic ulcer, however, whether it is a primary or secondary factor, is undoubted. As to a large extent the organisms found are similar to those found in the mouth, the gravity of the complications of peptic ulcer demand that till our knowledge on the subject is more definite we must make every effort to eradicate thoroughly all septic foci.

(A general summary of the whole work is given at the end of Table 16.)

TABLE 16. ANIMAL EXPERIMENTS.

CHOLECYSTITIS EXPERIMENTS.

Intravenous injections of streptococci.

No. of rabbit	No. of case Source	No. of injections & approximate dose. (no. of organisms or amount of broth culture)	Interval between first injection & post-mortem	Post-mortem findings.
1	2 (gland)	9 300 million up to 4 cc.	4½ months	Killed. Gall-bladder - no lesion. Haemorrhages in cardiac end of stomach. Streptococci recovered from stomach.
2	94 (bile)	4 1,000 to 2,000 millions	5½ months	Killed. Gall-bladder - no lesion. Haemorrhages in cardiac end of stomach. Small endocardial vegetation. Spleen very soft.
3	78 (wall)	5 3,000 to 6,000 millions	3½ months	Died. Gall-bladder - no lesion. Other viscera - no lesion.
4	145 (bile)	One 6 cc.	9 days	Killed. Coccidial cyst fixing gall-bladder to liver. Corneal ulceration & opacity & exudate in anterior chamber. Haemorrhage in pyloric end of stomach.

5	165 (gland)	One 7 cc.	3 days	Killed. Gall-bladder wall oedematous. No growth from wall or bile. Lungs congested. Haemorrhages in pyloric end of stomach. Urinary cystitis.
6	158 (wall)	8 all 1 cc.	3½ months	Killed. Gall-bladder - no lesion. No other lesions.
7	156 (bile)	2 each 1 cc.	12 days	Died. Gall-bladder - slight congestion. Bile blood-stained. Vegetations on aortic & mitral valves. (Figure 12.) Extensive ulceration & haemorrhages in cardiac end of stomach. General congestion of viscera, especially of Fallopian tubes, lungs, spleen and thyroid. Haemorrhages into ovaries.
8	164 (bile)	6 all 1 cc.	3 months	Killed. Gall-bladder - no lesion. Large cystic swelling of Fallopian tube. Haemorrhages in cardiac end of stomach. No other lesion.
9	142 (wall)	One 1 cc.	3 months	Killed. Gall-bladder - no lesion. A few tiny black granules in bile. Other viscera - no lesion.
10	175 (stone)	One 7 cc.	2 days	Killed. Gall-bladder - no lesion. Haemorrhages in pyloric end of stomach & small intestine. Congestion of spleen.

11	197 (bile)	One 7 cc.	7 days	Killed. Gall-bladder thick & oedematous, & covered with fibrin. (Fig. 1.) Sections showed considerable oedema of the tissue, & infiltration by numerous polymorphonuclear leucocytes, with desquamation of the epithelium. Streptococci seen in sections & recovered from the wall on culture. Haemorrhages & ulceration in cardiac end of stomach. Slight inflammation of urinary bladder.
12	197 (recovered from gall-bladder wall of previous animal)	One 5 cc.	7 days	Died. Gall-bladder - no lesion. A few black granules in bile. Haemorrhages and ulceration in cardiac end of stomach.
13	201 (bile)	One 5 cc.	7 weeks	Killed. Gall-bladder - no lesion. Haemorrhage in cardiac end of stomach.
14	119 (bile)	One 1 cc.	2 weeks	Killed. Gall-bladder - no lesion. Other viscera - no lesion.
15	183 (bile)	One 6 cc.	6 days	Killed. Gall-bladder - no lesion. Other viscera - no lesion
16	152 (gland)	One 7 cc.	8 days	Killed. Gall-bladder of an hour-glass structure but healthy. Haemorrhage in pyloric end of stomach. Cloudy swelling of the kidneys.

17	153 (gland)	One 7 cc.	6 days	Killed. Gall-bladder wall healthy - but a few white granules present in the bile. Other viscera healthy.
18	204 (bile)	One 8 cc.	8 days	Killed. Gall-bladder - no lesion. Other viscera healthy.
19	210 (bile)	One 8 cc.	7 days	Killed. Gall-bladder - no lesion. Haemorrhages into medulla of suprarenals.
20	3949 (gland)	4 .2 cc. up to .8 cc.	6 weeks	Killed. Doubtful thickening of gall-bladder.
	Intravenous injection of staphylococci.			
21	66 (staphylococcus aureus from gall-bladder wall)	4 one agar slope culture	6 months	Died. Gall-bladder - no lesion. Ulceration of cardiac end of stomach. Congestion of spleen, meninges & urinary bladder. Sub-endocardial haemorrhages in left ventricle.
	Intravenous injections of B.coli.			
22	183 (bile)	One whole of an agar slope culture	2 days	Killed. Gall-bladder wall white & thickened, & bile turbid & full of tiny yellow granules, together with several black granules. (Fig. 2.) B.coli isolated from the bile & gall-bladder wall.

(contd. on next page)

22 (contd.)				Extensive haemorrhage in first part of duodenum, around opening of common bile duct. (Fig. 11.) Haemorrhages into mucous membrane of Fallopian tubes and uterus, & in the pericardium. Congestion of lungs & spleen.
23	183 (heart blood of previous rabbit)	One 4 cc.	2 days	Killed. Gall-bladder wall healthy. Tiny yellow granules in bile. B.coli recovered from wall & bile. Sub-pericardial haemorrhages.
24	194 (bile)	One 15 cc.	2 days	Killed. Gall-bladder - no lesion. Haemorrhage in pyloric end of stomach. Extensive enteritis. General congestion of viscera. Haemorrhage into right ovary.
25	195 (wall)	One 5½ cc.	Died soon after injection	Dilatation of right heart. Haemorrhages & congestion of lungs.
26	14 (recovered from wall of gall-bladder of Rabbit 100)	One 18,000 millions	7 days	Killed. Gall-bladder wall thick & white, & showing sub-peritoneal haemorrhages. B.coli recovered from bile. Lungs & kidneys congested.
27	15 (same as previous animal)	One 2,000 millions	21 days	Killed. Gall-bladder - no lesion. Haemorrhage in pyloric end of stomach.

28	18 (recovered from bile of Rabbit 26)	One whole agar slope culture	7 days	Killed. Gall-bladder tense, white, thick & with numerous haemorrhages on surface. Lumen packed with faeculent-looking matter, from which B.coli was recovered. Cystic duct markedly dilated. Kidneys congested.
29	23 (recovered from bile of Rabbit 28)	One 1/5 of an agar slope culture	4 days	Killed. Gall-bladder - no lesion. Congestion of lungs.

Intravenous injections of B.paratyphosus B. and B.dysenteriae Shiga.

30	8253 (B.paratyphosus B. isolated from the discharge from a gall-bladder sinus)	One 2½ cc.	3 days	Died. Gall-bladder thick & full of muco-purulent contents, together with numerous tiny black granules. (Fig. 3.) B.coli, B.lactis aerogenes, and B. paratyphosus B. recovered from bile. Congestion of spleen and Fallopian tubes. Consolidation of lungs.
31	8253 (same as previous animal)	One 6½ cc.	2 days	Died. Slight congestion of gall-bladder. Haemorrhage and ulceration of cardiac end of stomach. Haemorrhages of duodenum around opening of common bile-duct. Marked congestion of spleen & other viscera, & consolidation of lungs. B.paratyphosus B recovered from bile.

32	728 (*B. paratyphosus B.* recovered from sewage)	2 2½ cc. each	3 weeks	Died. Gall-bladder small, white & thick, & full of soft brownish concretions. (Fig. 4.) *B. paratyphosus B.* recovered from bile & gall-bladder wall. Enteritis, colitis & pneumonia.
33	(stock *B. paratyphosus B.*)			Injected for the purpose of producing a stock antiserum. Given about eight injections, only the last four being of live organisms, and the maximum dose being a whole slope culture. Animal killed 2½ months after the first injection. Gall-bladder found to be thickened, and containing numerous small black granules. Cultures not made.
34	(stock *B. dysenteriae* Shiga.			Injected for the purpose of producing a stock antiserum. Given about ten injections - the last four being of live organisms - and the maximum dose being a whole slope culture. Killed 3½ months after the first injection. Gall-bladder found to be thick and white, full of thick purulent material, and containing numerous medium-sized black granules. (Fig. 5.) Cultures not made.

Most of the injections of the last two rabbits were given by Miss Winifred Clark, B.Sc.

Injection of streptococci into the lumen of the gall-bladder.

35 Gall-bladder exposed, contents partly aspirated, and a dose of about 1,800 millions of a streptococcus isolated from bile, and suspended in .1 cc. of saline, injected into lumen. Subsequently there was some escape of bile and partial collapse of the gall-bladder. A piece of omentum was fixed over the gall-bladder. Animal killed five weeks later. Gall-bladder found to be surrounded by adhesions and buried in the liver. Wall considerably thickened, and contents consisted of a small amount of turbid fluid and a soft greenish concretion. A streptococcus similar to that injected was recovered from the bile.

36 Same procedure as in last animal, except that 4,500 million organisms suspended in .25 cc. of saline were injected. Slight escape of bile. Killed five weeks later. Gall-bladder found to be submerged by liver, and thick and white, and full of caseous material, from which staphylococci were isolated. (Figure 6.)

Injection of B. coli into the lumen of the gall-bladder.

37 Similar procedure, gall-bladder being partly aspirated, and injected with 1/5 cc. of a broth culture of B. coli isolated from bile. Slight escape of bile. Animal killed nine weeks later. Gall-bladder found to be buried in the liver and surrounded by numerous adhesions. Wall very thick, and contents consisted of white inspissated material.

38 Similar procedure, .25 cc. of a broth culture of B. coli being injected. Marked escape of bile. Omentum fixed over gall-bladder region. Animal found dead nineteen hours later. Signs of peritonitis and general toxaemia present. Gall-bladder collapsed and inflamed, and contained a brownish friable concretion. B. coli recovered from bile, peritoneum and heart blood.

Injection of B. typhosus and B. paratyphosus into the lumen of the gall-bladder.

39 Similar procedure, about 500 millions of a stock B. typhosus in a volume of .2 cc. being injected into the lumen of the gall-bladder. Slight escape of bile.

Animal killed one month later. Gall-bladder found to be surrounded by adhesions, and with a thickened white wall. (Figure 7.) B. typhosus recovered from contents.

40 Similar procedure, gall-bladder being completely aspirated, and injected with about 2,000 million of a stock B. paratyphosus B. in 1 cc. Slight escape of emulsion. Animal killed three weeks later. Gall-bladder buried in liver. Wall much thickened, and with several yellow caseous nodules in wall. B. paratyphosus B. recovered from contents.

Injection of B. welchii into the lumen of the gall-bladder.

41 Similar procedure, about .1 cc. of a broth culture of B. welchii isolated from bile being injected into the lumen. Marked escape of bile. Animal found dead the following morning, with gas gangrene of abdominal wall, peritonitis and signs of general toxaemia. Gall-bladder collapsed, inflamed, and full of reddish semi-solid material. B. welchii recovered from abdominal wall and region of gall-bladder.

42 Similar procedure, .25 cc. of same culture being injected. Marked escape of bile. Animal died three weeks later. Found to be very emaciated. Gall-bladder wall and cystic duct very markedly thickened. (Figure 8.) Contents consisted of thick creamy material and a few brown particles, from which B. welchii was recovered.

Injection of streptococci into the wall of the gall-bladder.

43 Gall-bladder exposed. Intra-mural injection attempted at several places with a streptococcus (78) derived from the gall-bladder wall. A very fine needle was used, and the emulsion used contained about 3,000 million organisms per cc. Some emulsion escaped both into the peritoneum and into the lumen of the gall-bladder. Animal found dead twenty-two hours later, with peritonitis and oedema of the abdominal wall. Gall-bladder inflamed and ruptured. Streptococci recovered from abdominal wall and gall-bladder region.

44 Similar procedure, about 120 million of the same organism being injected intra-murally at one point. Animal killed two-and-a-half months later. Slight thickening of gall-bladder wall, no organisms being isolated from the wall or bile.

45 Similar procedure, a small dose of a broth culture of a streptococcus isolated post-mortem from a case of ulcerative endocarditis being injected. Animal killed seven weeks later. Gall-bladder found to be buried in the liver substance, but showing no gross change in its wall.

Injection of saline into the wall of the gall-bladder.

46 Intramural injection of sterile saline attempted. The gall-bladder was perforated, and there was some escape of bile. Animal killed seven weeks later. Fundus of gall-bladder surrounded by numerous adhesions. Gall-bladder white and thick at the site of inoculation.

47 Intramural injection of sterile saline at several places attempted. The gall-bladder was perforated, with some escape of bile. Animal killed one month later. Gall-bladder found to be surrounded by adhesions, and with slight thickening of its wall.

Ligation of the cystic duct.

48 Cystic duct ligated with cotton. Animal killed one month later. Numerous adhesions found in the region of the gall-bladder. Gall-bladder buried in liver tissue. Wall thickened. Contents clear.

49 Cystic duct ligated with catgut. Animal killed one-and-a-half months later. Found to have numerous adhesions, and enormous swelling in right lobe of liver, full of caseous material. (Figure 9.)

50 Cystic duct ligated with cotton. Animal died two-and-a-half months later. Numerous adhesions present. Gall-bladder buried in liver, and its wall found to be thick and white.

51 Cystic duct ligated with cotton. Accidental puncture of gall-bladder, with escape of bile. Killed one-and-a-half months later. Gall-bladder buried in liver and surrounded by adhesion. Contents purulent, and gave a growth of staphylococcus on culture.

52 Cystic duct ligated with cotton. Animal killed five weeks later. Gall-bladder submerged in liver, but showing no gross thickening of wall. Contents thick and dark.

Ligation of cystic duct followed by intra-venous injection of streptococci.

53 Cystic duct ligated with cotton. Animal subsequently given 2½ cc. of a broth culture of a streptococcus (156) isolated from bile. Killed five weeks later. Gall-bladder thick, white and enlarged, and containing muco-purulent material from which streptococci were grown.

54 Found at laparotomy to have completely divided gall-bladder and double cystic duct. Left duct only ligated with cotton. Immediately afterwards 2½ cc. of a broth culture of a streptococcus (153) isolated from a cystic gland were given intravenously, and subsequently two further injections. Killed one-and-a-half months later. Found to have enormous swelling in right lobe of liver, containing about 5 cc. of whitish yellow semi-solid contents which gave a growth of staphylococci and B. coli on culture. No evidence of original structure of either half of the gall-bladder.

Traumatisation of the gall-bladder followed by injection of streptococci.

55 Gall-bladder gently traumatised at a laparotomy with the finger. Animal afterwards given 3,000 million of a streptococcus (78) isolated from a gall-bladder wall. Two further injections given. Animal killed three weeks later. No gross change in gall-bladder wall. A few black and yellow granules present in bile.

Traumatisation alone of the gall-bladder.

56 Gall-bladder traumatised three times at weekly intervals. Animal killed four months after commencement. Gall-bladder found to be white, thick and full of inspissated material, and surrounded by numerous adhesions.

57 Gall-bladder traumatised twice with an interval of two weeks between. Animal killed after an interval of three months. Gall-bladder found to be thick, white, and full of greenish particles.

Intraduodenal injection of streptococci.

58 Laparotomy. Dose of 6,000 million of a streptococcus (232) isolated from the lumen of an appendix and suspended in 1 cc. of saline injected into the lumen of the first part of duodenum in region of opening of bile duct. Animal killed five days later. Duodenum found to be thin and inflamed, and it perforated on examination. Gall-bladder and other viscera normal. No organisms isolated from bile.

59 & 60 A similar intraduodenal injection of paratyphoid bacilli was carried out in two rabbits, a dose of half an agar slope culture in 1 cc. of saline being used. Both animals died, however, before the termination of the anaesthetic.

Intraportal injection of B. paratyphosus B.

61 Laparotomy. Portal vein identified, and 1/5 of an agar slope culture of a stock B. paratyphosus B. suspended in 1 cc. of saline injected into its lumen. Some leakage of blood occurred. Animal found dead next morning with post-operative hernia. Some degree of peritonitis present, together with haemorrhages in the duodenum and signs of general toxaemia. Gall-bladder slightly red, but bile clear. Cultures not made.

APPENDICITIS EXPERIMENTS.

Intravenous injections of streptococci isolated from cases of appendicitis.

No. of rabbit.	No. of case. Source.	No. of injections & dose.	Interval between commencement & post-mortem.	Post-mortem findings.
62	25 (gland)	6 2-4,000 million	4½ months	No gross lesions.
63	63 (wall)	One 10 cc.	2 days	Congestion and haemorrhages of duodenum and small intestine. A few pin-point haemorrhages of appendix. Streptococci not recovered from appendix. Slight haemorrhage into knee-joint.
64	63 (wall)	One 14 cc.	2 days	Killed. Haemorrhages in small intestine.
65	102 (lumen)	One 20 cc.	2 days	Killed. Cloudy swelling of kidneys. Sub-fascial haemorrhage in region of right knee-joint
66	113 (wall)	One 20 cc.	1 day	Killed. Haemorrhages in pyloric end of stomach and duodenum. Congestion & enlargement of spleen.
67	144 (peritoneum haemolytic streptococcus).	One 1 cc.	4 days	Killed. Haemorrhages in pyloric end of stomach & caecum. Gall-bladder red & oedematous. Pericarditis. Congestion of lungs, meninges, spleen and kidneys.

68	231 (lumen)	One 15 cc.	8 days	Killed. No gross lesion.
69	231 (lumen)	One 15 cc.	5 days	Killed. Retention cysts in appendix. Numerous black particles in bile.
70	232 (lumen)	One 6 cc.	8 days	Killed. Congestion of lungs & spleen. Cloudy swelling of kidneys.
71	232 (lumen)	One 4 cc.	8 days	Killed. Haemorrhage in pyloric end of stomach. Haemorrhage in pancreas. Black particles in bile.
72	229 (abscess)	One 5 cc.	2 days	Killed. Infarct of lung. Enlargement and congestion of spleen.
73	229 (abscess)	One 5 cc.	2 days	Killed. No gross lesion.
74	227 (lumen)	One 6 cc.	3 days	Killed. Congestion of lungs.
75	227 (lumen)	One 4 cc.	3 days	Killed. Retention cyst in appendix. Small pin-head fibrinous nodules on aortic valve.
76	232 (lumen)	One 35 cc.	4 days	Killed. Haemorrhage in beginning of duodenum. Cloudy swelling of kidneys.

77	232 (lumen)	One 35 cc.	4 days	Killed. Haemorrhage in pyloric end of stomach. Cloudy swelling of kidneys.
78	236 (lumen)	One 10 cc.	4 days	Killed. Congestion of kidneys & Fallopian tubes. Haemorrhages in cardiac end of stomach.
79	236 (lumen)	One 8 cc.	4 days	Killed. Congestion of kidneys.
		Intravenous injection of B.coli.		
80	6 (wall)	5 40 to 2,000 million and one injection of 9 cc. of a streptococcus.	3 months	Died. Haemorrhages of pyloric end of stomach and duodenum.
81	40 (lumen)	6 100 to 4,000 million	3½ months	Killed. No gross lesion.
82	50 (abscess)	5 500 to 4,000 million	3½ months	Killed. No gross lesion, apart from coccidiosis in region of gall-bladder.
83	236 (lumen)	One 3 cc.	4 days	Killed. Gall-bladder thick, white, and full of purulent material. Slight congestion of distal part of appendix.
		Intravenous injection of B.lactis aerogenes.		
84	225 (lumen)	One whole agar slope culture	2 days	Killed. Congestion & infarcts of lungs. Thick haemorrhagic effusion into right knee joint. Enlargement and congestion of spleen.

85	225 (lumen)	One 1/5 agar slope culture	4 days	Killed. Gall-bladder thick, & white, & full of purulent matter & one black granule. B.lactis aerogenes recovered from contents. Enlargement & congestion of spleen.
		Intravenous injection of B.pyocyaneus.		
86	225 (lumen)	One 1/5 agar slope culture	4 days	Killed. Gall-bladder thick & full of white granules. Pelves of kidneys inflamed & full of pus. Cloudy swelling of kidneys. Congestion of lungs. Organism recovered from kidneys and bile.
87	225 (lumen)	One One agar slope culture	2 days	Killed. Vegetation on wall of right ventricle. Gall-bladder oedematous & full of soft brownish concretions. Haemorrhages in stomach, congestion of lungs & spleen.
		Intravenous injection of a sarcinae isolated from the lumen of an appendix.		
88	40 (lumen)	7 rising to one agar slope culture	Animal killed after 3 months.found	No gross lesion.
		Intravenous injection of other organisms isolated from appendix.		
89	236 (lumen)	One 1/4 cc. of primary mixed culture in bullock-heart-medium. (B.coli, B.lactis aerogenes streptococci, B. welchii & a gram positive anaerobic bacillus).	4 days	Killed. Gall-bladder thick, white, & full of purulent material.

90	236 (lumen)	½ cc.	One same culture as 89	4 days	Killed. Haemorrhage in pyloric end of stomach.

Injection of streptococci into the wall of the appendix.

91 Laparotomy. Appendix exposed, and its wall injected in each of five places with about .2 cc. of a thin emulsion of a streptococcus (113) isolated from the wall of an appendix. Animal killed four days later. Found to have adhesions at base of appendix, and two sub-peritoneal haemorrhages in the caecum. Appendix itself showed no gross lesion. Streptococci not recovered on culture of appendix wall.

Intraperitoneal injections.

92 Intraperitoneal injection of a streptococcus (223) isolated from peritoneal exudate in a case of perforated duodenal ulcer given in a dose of 6,000 million in 2 cc. Animal remained well, and was killed 15 days later. Found to have no general peritoneal inflammation, but the coils of small intestine at the site of injection were white and thickened, and the mesentery was full of hard, white glands, from which streptococci, B.coli and B.welchii were isolated. Gall-bladder found to be thick, and filled with semi-solid greenish material.

93 Intraperitoneal injection of staphylococcus albus isolated from the same case injected in a dose of 4,000 millions in 2 cc. Ten days later, 2 cc. of a broth culture of the streptococcus used in the previous experiment, diluted with 2 cc. of saline, were injected intra-peritoneally. The animal was killed 4 days later. There was no gross lesion present, and the peritoneal cavity was sterile.

OTHER EXPERIMENTS.

Intravenous injections of streptococci derived from pyorrhoeal pockets, roots abscess or tonsils.

No. of rabbit	No. of case Source	No. of injections & dose	Interval between commencement & post-mortem	Post-mortem findings
94	15 (Pyorrhoeal pockets of patient with a duodenal ulcer)	5 600 - 2,000 million	3 months	Black granules in bile. No other gross lesion.
95	14 (Pyorrhoeal pockets of patient with gallstones)	5 500 - 4,000 million	1½ months	No gross lesion
96	9 (Pyorrhoeal pockets of patient with pyelitis)	3 1,000 million	2 months	Haemorrhage in pyloric end of stomach. Cysts in kidneys.
97	10 (Pyorrhoeal pockets of patient with chronic nephritis)	6 1,000 million to 5 cc.	2 months	Small fibrinous mass on tricuspid valve.
98	51 (Pyorrhoeal pockets of patient with perforated duodenal ulcer)	One 23 cc.	3 months	Haemorrhages in cardiac end of stomach
99	(Root abscess of author's tooth)	One 4 cc.	18 hours	Died. Congestion of lungs, cloudy swelling of kidneys, haemorrhages in cardiac end of stomach.

100	(Same as in previous case)	One $3\frac{1}{2}$ cc.	$3\frac{1}{2}$ weeks	Died. Gall-bladder thick & white, & containing one large black stone. (Fig. 10.) B. coli isolated from wall & bile. Haemorrhage in cardiac end of stomach.
101	111 (Extirpated tonsils of person otherwise healthy)	14 cc.	2 days	Killed. Haemorrhage in pyloric end of stomach. Two tiny haemorrhages in small intestine. Small sub-endocardial haemorrhages in right ventricle. Consolidation of bases of both lungs.
102	Intravenous injection of streptococci derived from gastric ulcers.			
	30	5 4,000 million up to 5 cc.	7 weeks	Killed. No gross lesion noted.
103	177	One 7 cc.	9 days	Killed. Numerous black granules in bile found, but streptococci not recovered. Haemorrhage into ovarian follicle noted.

GENERAL SUMMARY OF THE WHOLE WORK.

In the work detailed in this thesis a bacteriological investigation has been made of the three conditions of cholecystitis, appendicitis and peptic ulcer. This was originally undertaken with the object of confirming the work of Rosenow in this direction. On the whole, it may be said that his general findings with regard to the nature of the organisms isolated and their presence within tissues has been supported, though a somewhat different incidence has been found which may possibly be due to differences in the type of material examined. Special attention has been paid to the biological characters of the organisms isolated, especially the streptococci, with the object of determining the site of their origin in the body. It has been found that in cholecystitis the organisms present are predominantly of a character similar to those found normally in the bowel. In peptic ulcer less evidence has been available, but on the whole, mouth types of organisms seem to be more frequent.

No evidence has been found of any tendency towards "elective localisation" of the organisms isolated. Cholecystitis may be produced relatively easily in rabbits by intravenous injection of coliform organisms, but such action seems to be entirely non-specific, and to occur as readily with organisms isolated from other lesions as with those isolated from

cases of cholecystitis. Animal experiments suggest that infection reaches the gall-bladder either through the systemic circulation or by descending infection from the liver in the bile, though in exceptional circumstances it may travel by other routes. Lesions in the appendix have not been produced to any extent with organisms in the present work. Haemorrhages in the stomach and duodenum have been found with great frequency in experimental animals after the intravenous injection of organisms, but it does not seem justifiable to draw any conclusions from these findings as regards the aetiological relationship to peptic ulcer in man of the organisms injected.

No evidence has been found so far that in the human subject infection is the primary factor in the production of cholecystitis and gall stones. In the early stages of cholecystitis organisms are found with difficulty, but the incidence rises as complications, such as the formation of stones and the presence of obstruction, occur. In the syndrome of acute obstructive cholecystitis the presence of a secondary anaerobic infection is of great practical importance. It is possible that the organisms isolated in this condition may all play the part of secondary invaders when once some change, toxic or biochemical, has already commenced in the biliary tract.

In appendicitis, the primary factor is probably a dietetic one, and the evidence is strongly in favour of this condition being a disease of civilisation. A

decreased consumption of cellulose and an increased consumption of protein seem to be the important factors, and they probably act by the encouragement of stasis and the production of catarrh in the appendix, with the passage of organisms through the mucous membrane. In the vast majority of cases the infection in appendicitis is undoubtedly an entero-genous one, though rarely it may be a haematogenous one arising in some distant focus such as the tonsils. Anatomical factors, such as the position of the appendix and the nature of its blood supply, probably play an important part in the progress of the inflammation. As the inflammation progresses, the incidence and virulence of the organisms present in the appendix rises, and this is specially the case with the anaerobic flora. In obstructive and gangrenous conditions it is the anaerobes which are mainly responsible for the complications and mortality.

In the case of peptic ulcer, the evidence is perhaps a little stronger that infection, usually with streptococci, is the primary factor responsible. Once ulceration has occurred, a vicious circle becomes established, and a secondary infection may be added. Whether infection originates or maintains the condition, however, its importance is such as to render absolutely necessary in treatment the eradication of all septic foci in the abdomen and elsewhere.

In recent years since the "pathology of the living" has been more extensively studied, considerable

stress has been layed on the relationship of these three conditions, which have been referred to by Wilkie as the "abdominal triad". There is definite clinical evidence that in some cases they are related, and that the appendix provides the original focus. In the present work, material from too few cases in which all three lesions have been treated surgically has been examined to justify any conclusion on the nature of the infection present. Till our knowledge about the origin and pathway of infection in these conditions is more definite, however, and till we have determined what is the primary factor, dietetic or otherwise, that allows infection to occur, our therapeutics must continue to rest on the principle that the infection is implanted within the tissues of the organ diseased, and that unless natural resolution can be shown to occur, the continuation of the infection and its spread to other organs can only be prevented by surgical treatment.

The above work was carried out in the Edinburgh University Bacteriological Department during the tenure of a Vans Dunlop scholarship. Part of the expenses were paid by a grant from the Moray Fund. The surgeons of the Edinburgh Royal Infirmary and the Royal Hospital for Sick Children have been at great pains to provide me with the material examined. I am indebted to my brother (D.C.W.) for the photographs.

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ILLUSTRATIONS.

Figure 1. (Rabbit No. 11). Gall-bladder after intravenous injection of streptococci isolated from bile.

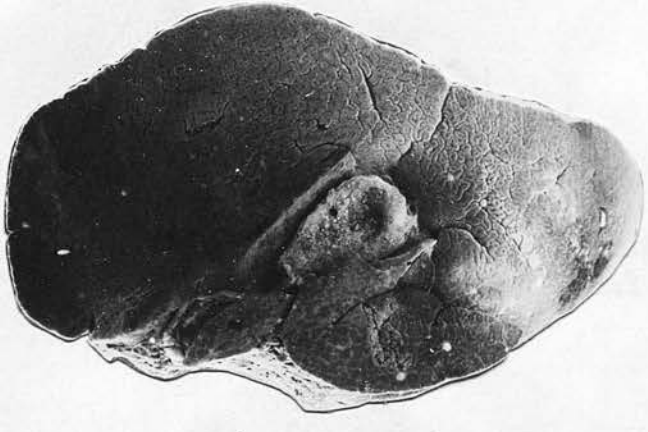

Figure 2. (Rabbit No. 22). Gall-bladder after intravenous injection of B. coli isolated from bile. A small stone is seen.

Figure 3. (Rabbit No. 30). Gall-bladder after intravenous injection of B. paratyphosus B. isolated from a gall-bladder sinus. Several tiny black granules are present.

Figure 4. (Rabbit No. 32). Gall-bladder after intravenous injections of B. paratyphosus B. isolated from sewage.

Figure 5. (Rabbit No. 34). Gall-bladder after intravenous injections of B. dysenteriae Shiga. Several small stones are present.

Figure 6. (Rabbit No. 36). Gall-bladder after injection into its lumen of a streptococcus isolated from bile.

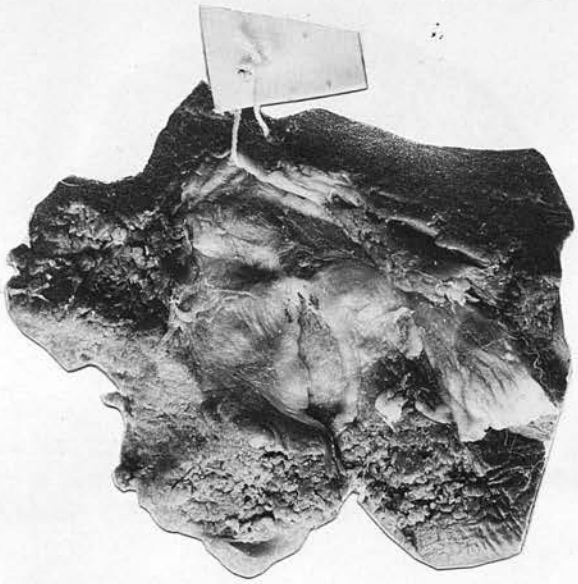

Figure 7. (Rabbit No. 39). Gall-bladder after injection into its lumen of a stock B. typhosus.

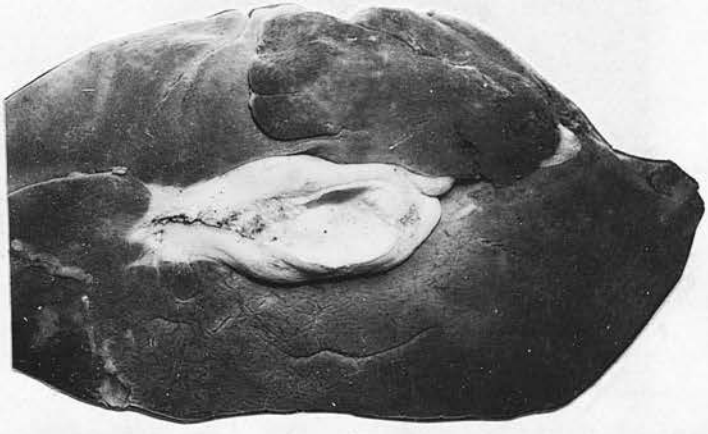

Figure 8. (Rabbit No. 42). Gall-bladder after injection into its lumen of B. welchii isolated from bile.

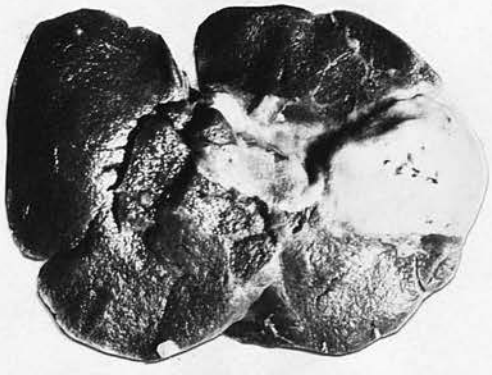

Figure 9. (Rabbit No. 49). Gall-bladder six weeks after ligation of the cystic duct.

Figure 10. (Rabbit No. 100). Gall-bladder after injection of streptococci isolated from author's root abscess. A large black concretion is present

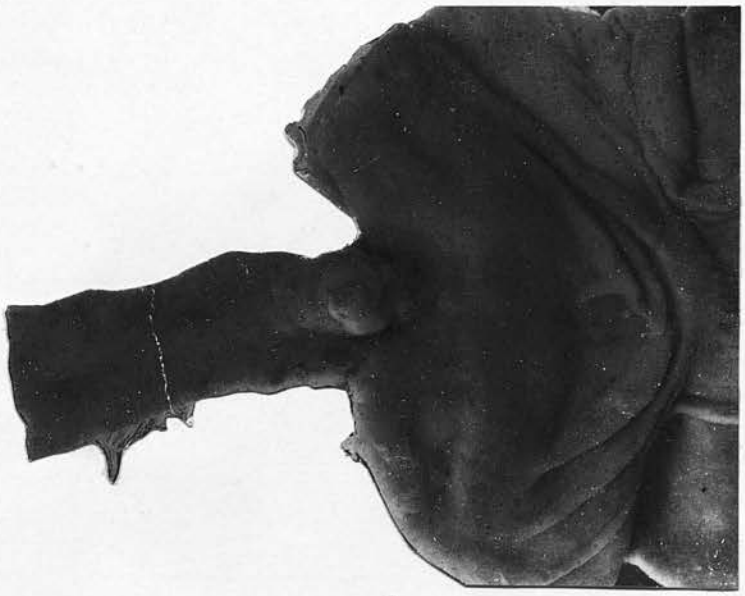

Figure 11. (Rabbit No. 22). Stomach and duodenum after intravenous injection of B. coli isolated from bile. Haemorrhages are seen in the first part of the duodenum.

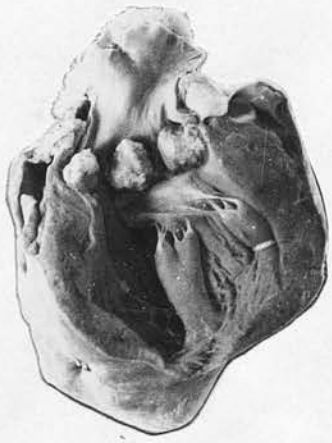

Figure 12. (Rabbit No. 7). Heart after intravenous injections of streptococci isolated from bile. Large vegetations are seen on the cusps of the aortic valve. Smaller vegetations are also present on the mitral valve.