M. Sc. EXPERIMENTAL SURGERY.

# AN EXPERIMENTAL STUDY OF CHOLELITHIASIS AND CHOLECYSTITIS IN LABORATORY ANIMALS

by

Eugene G. Caira
L.R.C.P. L.R.C.S. (Edin), L.R.F.P.&S. (Glas).

Research Assistant in Experimental Surgery, 1956-57
Demonstrator in Anatomy, McGill University, 1956-57

Thesis submitted to the Faculty of Graduate Studies & Research in partial fulfillment of the requirements for the Degree of Master of Science.

July, 1957. McGill University.

"GALLSTONES ARE A METAMORPHOSIS OF BILE"

(Klebs, 1889).

## PREFACE

This thesis has been the result of a year spent as a Research Assistant in the Experimental Surgical Laboratories at McGill University.

Using recently discovered reliable and near-physiological methods for producing biliary tract disease, an altered approach has been made to the problem. Biliary calculi have been examined by means of geological petrographic technique, in an attempt to deduce the exact mode of calculus formation. The vital role played by the liver and the comparatively unimportant one of the gallbladder has been demonstrated.

Suggestive evidence has been obtained demonstrating certain similarities of physical structure in human and experimentally produced calculi.

I have enjoyed this year of my life spent in research and regret it is not usually possible to do more of it, combined concurrently with everyday clinical work. Part of the enjoyment has been due to the debates and friendly association with the other members of the research group; Drs. A. L. Saunders, F. G. Inglis, W. E. Wilson, D. P. Goel, A. R. C. Dobell and E. R. Duchesne.

ACKNOWLEDGEMENTS

I wish to thank Dr. D. R. Webster for arousing my interest in this subject and for his continual encouragement throughout the year.

I am grateful to Dr. S. C. Skoryna of the Department of Experimental Surgery for his invaluable aid and supervision of my work at all stages of the experiments. His help with translations of the German texts is also acknowledged.

Dr. A. C. Ritchie of the Department of Pathology, Royal Victoria Hospital, very kindly advised on and examined the histological sections. Mr. H. Torunski prepared and stained the sections.

Mrs. E. J. Rumball and Mrs. C. K. Duerr carried out all the biochemical studies on blood, bile and calculi most competently. Their contribution was of considerable value.

Professor R. N. Reed and Dr. C. Butas arranged for the bacteriological work and interpreted the results obtained at the Bacteriology Department, Royal Victoria Hospital.

Dr. A. H. Sehon, Ph. D. kindly supplied information on matters pertaining to colloidal chemistry.

Mr. P. Roustan was responsible for the excellent micro-photographs and general photography of charts and calculi. I wish to express my appreciation of his work, and that of the

other members of the Staff for their care of the animals and the help rendered in the operating room.

Dr. J. S. Stevenson of the Department of Geology, McGill University advised on the interpretation of the petrographic sections and was responsible for their preparation.

Dr. O. F. Denstedt of the Department of Biochemistry, and Drs. G. C. McMillan and D. Waugh of the Department of Pathology freely supplied information in their respective fields and gave advice in solving the difficulties which arose during the experiments.

Dr. D. A. Murphy provided useful references from the Veterinarian literatury and obtained information from the Veterinary College, Toronto.

I wish to thank the Secretary of the Department, Miss B. M. Macario for doing all the clerical work and for typing this lengthy thesis.

To my wife I express my gratitude for her understanding and help during this financially difficulty period of adding to my knowledge and understanding of disease processes.

My gratitude is finally due to the older authors whose excellent work on the subject stimulated me throughout the year.

I also wish to thank the following Pharmaceutical Companies for their gratuitous supply of products as enumerated:

| | |
|---|---|
| U. S. A. Vitamins | (Vitamins A, D) |
| Merck Company | (Dr. Laurie)(Vitamins, provitamins) |
| Ciba Company | (Dr. Murphy)(Progesterone) |
| Schering Company | (Cortisone, oestrogens) |
| Hoffman-La Roche Co. | (Vitamin K) |
| Lederle Company | (Chlortetracycline) |

E. G. C.
July, 1957.

TABLE OF CONTENTS

Page

## TABLE OF ILLUSTRATIONS



### Photomicrographs

Page

Page

Page

# INTRODUCTION

Gallbladder disease has doubtless been present for as long as man and animals have existed, and known for as long as cadavers have been opened. There is on record a case of gallstones in an Egyptian mummy of the 21st Dynasty. (British Museum).

Since the first report of gallstones in the 14th Century, extensive studies have been carried out in an effort to determine the ætiology of the condition. Probably, prior to this date, and subsequently for some time, most thought on the subject was purely philosophical or speculative.

In spite of the fact that the earlier original work was carried out with instruments, materials and methods which by modern standards seem inadequate and inaccurate, nevertheless, the results were remarkable and although values were not correct, the relative changes produced experimentally, and carefully observed, were significant.

Since the subject of gallbladder disease is as complex as is the human body, even with modern methods and an elaborate array of new materials and equipment, the complete answer has not yet been found. The diseased gallbladder and biliary tract produces not a single stone for identification and explanation, but seemingly as many varieties as the pebbles on the beach, at least when they are examined macroscopically.

The problem investigated from all angles, physiological, anatomical, clinical, morbid, bacteriological, biological, biochemical and experimental has yeilded an impressive collection of literature, and as always the quantity is proof of the complexity of the problem.

## RATIONALE OF THE EXPERIMENTS

Research workers in other fields have noted the incidental production of gallstones in certain laboratory animals. Dam and Christensen (1952) noted during dietary experiments on hamsters, that feeding of low fat cholesterol free diet induced cholelithiasis. The original studies were concerned with muscular dystrophy, however these were changed to studies on cholelithiasis. They succeeded in producing suggestive evidence, that by alteration of diet the cholesterol portion of the calculi could be made to disappear. Several factors remain to be explained and much more detail can be abstracted from suitably modified experiments.

Cook et al (1954) had noted during atherogenic experiments, that 2 rabbits fed 3-beta cholestanol (dihydrocholesterol) developed cholelithiasis. Mosbach and Bevans (1956) confirmed their results and carried the work a little further so that they reported the histopathology at the conclusion of the experiments plus accurately identifying an apparently new type of gallbladder concretion. It is felt that the work of the above authors can be modified and enlargedupon so as to show the detailed development of biliary tract disease as well as deducing the exact manner of formation of the calculi produced.

The effects of the administration of oral and parenteral drugs, such as, oestrogens, progesterone, aureomycin and cortisone will be studied, provided a high incidence of biliary tract disease is obtained by dietary means. We feel that the present day classification of gallstones loosely describes their chemical composition, therefore an attempt will be made to use geological techniques to ascertain the minute physical structure of human calculi and those obtained as a result of the experimental work.

It is believed that in most cases the liver is primarily responsible for the production of biliary tract disease. There may be other processes producing disease for there is more than one type of pathological gallbladder and many types of gallstones. The answers to the questions: why does gallbladder disease occur?; why does it not occur more frequently?; and how does it occur? will probably be found in 1) accurate analysis of bile and 2) the factors modifying the liver secretion of bile, viz:- diet, infection, hormones, and the entero-hepatic circulation of the sterols and bile-salts are of prime importance. Secondarily, the effect of group 2 on the end organ, the gallbladder, with its reaction to abnormal bile, toxic by-products, plus its great potential for stasis and infection as a result of abnormal neuromuscular, hormonal or higher central nervous system effect.

Little has been written concerning the action of intestinal and liver bacteria on bile, and cholesterol in humans nor is much more known in experimental animals. It is proposed to carry out bacteriological studies at certain stages of the experiments. We are of the opinion, that although herbivorous animals are being used in these experiments, they will afford results which may be compared in some respect to those seen in human disease. Their cholesterol metabolism differs from that in humans, but this does not detract from their value in experimental work. The simple methods being used to produce disease have much to commend them, for complicating factors are kept at a minimum. An attempt will be made to cover the subject as completely as possible within the limits of the time and materials available.

## OBJECT

To investigate the following:

1) The effects of a lithogenic diet on hamsters and rabbits.

2) Administration of oestrogen, progesterone, cortisone, chlortetracycline and dietary changes in hamsters on the diet.

3) The feeding of 3-beta-cholestanol to rabbits, guinea pigs, and rats.

4) Cholecystectomy performed in rabbits subsequently fed 3 beta-cholestanol.

5) Petrographic methods used to study varied human biliary

calculi with reference to the textural relationship of their constituents.

6) Comparison of these calculi with those produced experimentally.

7) Histopathological sections, bile chemistry and blood cholesterol levels will be done were possible.

8) Random bacteriological sampling of bile, gallbladders and calculi.

It is hoped some information clarifying certain aspects of the pathogenesis of biliary tract disease will be obtained.

The work will be directed particularly towards determining the actual structure of calculi and the different stages they pass through in the bile, from their birth to the mature state. Sacrifice of the animals will be carried out at frequent intervals to obtain calculi (or pre-calculons bodies) of known age.

## ANATOMY AND APPLIED ANATOMY

The excretory apparatus of the liver consists of:

1) Common hepatic duct formed by the junction of the right and left hepatic ducts which leave the liver at the porta hepatis.

2) Gallbladder: which serves as a reservoir for bile.

3) Cystic duct: or the duct of the gallbladder.

4) Common bile duct formed by the junction of the cystic and hepatic ducts.

The gallbladder is a pear-shaped sac lodged in a fossa on the under surface of the right lobe of the liver. Its upper surface is attached to the liver by connective tissue and the under surface and sides are covered by peritoneum. The fundus is directed downwards to the right and projects below the liver margin. The body is directed upwards to the left. A small pouch may project from the right wall of the neck, towards the duodenum, called Hartmann's pouch. (Broca originally described it). A recent connection with pathological dilatation has been demonstrated. The cystic duct from 3-4 cms. long passes backwards, downwards and to the left, from the neck of the gallbladder, and joins the common hepatic duct to form the common bile duct. The cystic duct runs parallel with and adheres to the common hepatic duct for a short distance before joining with it. The mucous

membrane of the cystic duct is thrown into a series of crescentic folds from 5-12 in number and usually known as the valves of Heister. Similar valves are supposed to occur in the neck of the gallbladder commonly known as the sphincter of Lutkens. The ductus choledochus is about 7 cms. long and is formed near the porta hepatis. It passes behind the first part of the duodenum with the gastro-duodenal artery on its left and then runs in a groove on the upper and lateral part of the posterior surface of the duodenum. At the left side of the second part of the duodenum it comes into contact with the pancreatic duct and accompanies it into the wall of this part of the gut. There it unites to form the ampulla of the bile duct. The distal constricted end of this ampulla opens into the second part of the duodenum, on the summit of the duodenal papilla 8-10 cms. from the pylorus. The method of union of these ducts is extremely variable and may contribute towards the development of disease in the biliary tree or pancreas. (Fig. 1).

BLOOD SUPPLY: The cystic artery is usually a branch of the right hepatic artery and divides into two branches, a lower one which passes to the under surface of the gallbladder, and an upper one which runs in the areolar tissue between the upper surface of the gallbladder and the visceral surface of the liver, to both of which it gives branches. The cystic vein formed by the union of the tributaries which accompany the cystic artery ends in the right branch of the portal vein. There are also small venous branches going directly into the liver substance. Accessory bile ducts are

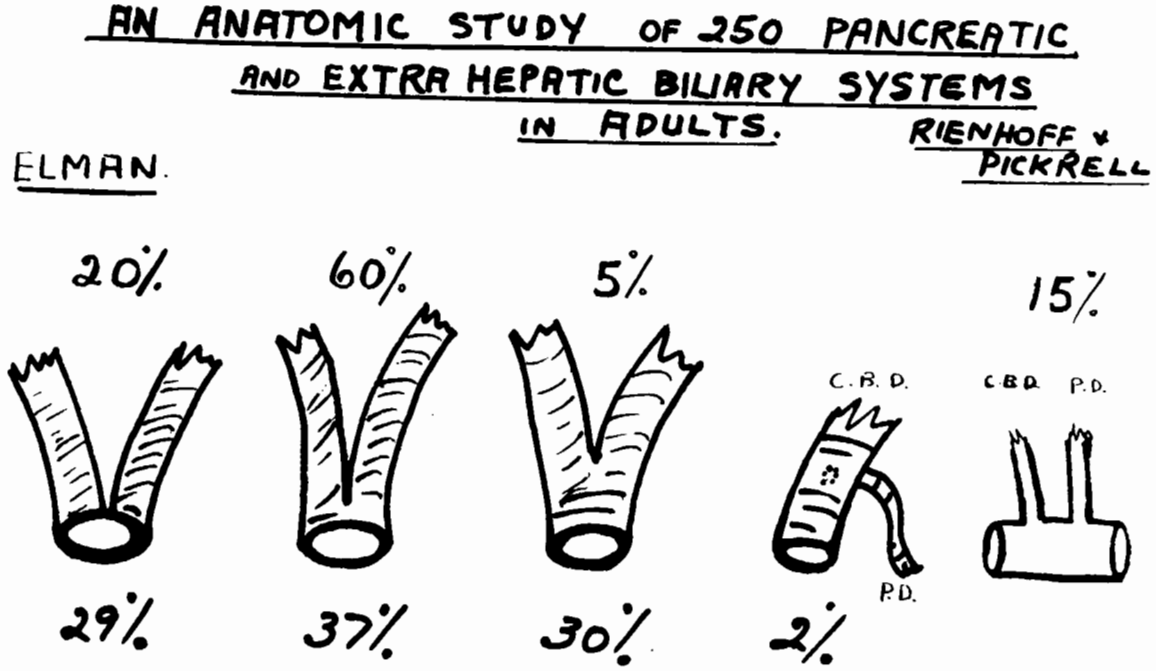



FIGURE 1.

Anatomic study of union of pancreatic and common bile ducts.

occasionally found in this region. The lymph vessels pass to the cystic gland and the hepatic glands, those from the bile ducts end in the hepatic glands alongside the bile ducts and in the upper pancreatico-splenic glands. The lymphatic drainage is complex and extensive.

NERVE SUPPLY: Mainly sympathetic from the coeliac plexus and passes along the hepatic artery and its branches. Fibres from the right phrenic nerve through communications between the phrenic and coeliac plexuses also appear to reach the gallbladder in the hepatic plexus. This is frequently evidence by referred right shoulder pain in cases of gallbladder disease. The vagus is the motor and secretory nerve, it inhibits the sphincter. The sympathetic is sensory to the gallbladder and motor to the sphincter.

STRUCTURE: The gallbladder has three coats; serous, derived from the peritoneal covering, fibromuscular and an internal or mucous coat. The fibromuscular coat, a thin but strong layer, consists of dense fibrous tissue mixed with plain muscle fibres which are disposed chiefly in a longitudinal direction, a few running transversely. The mucous coat is loosely connected with the fibrous layer. It is generally of yellowish brown colour and is elevated into minute ruggae which give it a honeycomb appearance. It is deeply stained with bile after death. The mucous coat of the gall-bladder is continuous through the common hepatic duct with that of

the ducts of the liver and through the bile duct with that of the duodenum.

The epithelium is columnar and actively absorbs water and electrolytes, rendering the bile more concentrated and altering the pH. Mucus is also secreted. The coats of the large biliary ducts are an external or fibrous, and an internal or mucous coat. The fibrous coat is composed of strong fibro-areolar tissue, with a certain amount of muscular tissue arranged for the most part in a circular manner around the ducts. The mucous layer is continuous with that of the duodenum and the epithelium is of a columnar variety. Large mucous glands are scattered irregularly in the larger ducts. The circular muscle around the lower part of the common duct and the ampulla including the main pancreatic duct is called the sphincter of Oddi.

There are cases in the literature of congenitalabsence of the gallbladder (Dixon, 1945) in humans and in herbivorous animals. By cholecystography the gallbladder has been shown to vary in position according to the build of the individual. In broad types (hypersthenic), the gallbladder is broad and lies high up and far laterally opposite the first lumbar vertebra. In the asthenic types, it is narrow, lies nearer the vertebral column and may reach as low as the 4th lumbar vertebra. By its position and structure the ballbladder favours stasis of its contents which

partly fulfils a physiological function.

Naunyn (1869) and Babkin (1914) separately observed that bile is stored in the gallbladder between meals, concentrated, voided and replaced with fresh liver bile when digestion of a meal takes place.

## PHYSIOLOGY AND APPLIED PHYSIO-PATHOLOGY

There is approximately the same amount of agreement between various authorities concerningthe physiology of the gallbladder as there is concerning the etiology of gallstones. Certain aspects of the function of the gallbladder ducts and liver secretion of bile have however been generally accepted: bile is secreted by the liver at a varying rate; during digestion bile from the liver as well as the gallblader empties into the duodenum; if sphincters are present in the gallbladder and the ductus choledochus, then they must be open if flow is to occur; since bile is stored between meals then the sphincter of Oddi must be closed to divert bile into the gallbladder for concentration.

The gallbladder is very distensible and generally can store 48 hours secretion of liver bile. It is usually concentrated down to 30-50 ccs. The viscus has been known to hold 500 ccs. of bile. With normal function and diet the bile is changed over, fairly frequently, so that stasis should normally be rare. In the normal vesicle water, chlorides and carbonates are absorbed by the mucus membrane. This permits a concentration of bile from 4-10 times that of liver bile.

Bile is a complex secretion, the chemistry is still in the process of being clarified. (Fig. 2). Because of the relative increase of bile salts and base, the bile has a pH of less than 7.

BILE COMPOSITION (HUMAN)

Handbook of Biological Data, 1957.

| | LIVER mgms/100/ml | GALLBLADDER |
|---|---|---|
| PH | 7.5 (6.2-8.5) | 6.0 (5.6-8.0) |
| SOLIDS | 2660 (1000-4000) | 11,140 (4,700-16,500) |
| DRY MATTER | 2330-3300 | 18,000 |
| INORGANIC MATTER | 200-900 | 500-1100 |
| CALCIUM M/eg/L | 2.0-4.5 | 5.0-7.0 |
| CHLORIDE M/eg/L | 75-110 | 15-30 |
| PHOSPHORUS, TOTAL | 9-22.3 | |
| POTASSIUM M/eg/L | 4.9 | |
| NITROGEN, TOTAL | 67-92 | 67-92 |
| BASE, TOTAL M/eg/L | 150-180 | |
| PROTEIN, TOTAL | 275 | 315-450 |
| UREA | 23.6 | 20-45 |
| BILE ACIDS | 200-1830 | 1500-10,000 |
| BILE PIGMENT | 50-170 | 200-1500 |
| BILE SALTS | 650-1400 | 11,500 |
| BILIRUBIN | 20-200 | 1,000 |
| MUCIN & PIGMENT | 610-930 | 3420-4300 |
| GLUCIDES | 35-91 | 240 |
| CHOLESTEROL | 120-170 | 630-930 |
| FAT NEUTRAL | 110-300 | 370-560 |
| FATTY ACIDS | 110-300 | 150-1090 |
| LECITHINS | 100-575 | 3,500 |
| PHOSPHOLIPIDS | 60 | 200 |
| CHOLINE, TOTAL | 35-89 | 550 |

FIGURE 2.

Table of Bile Composition

The liver bile pH is normally over 7.5. Claims have been made that the damaged gallbladder reverses this process and the bile tends to become alkaline. This is due to an increase in the water, chlorides, bicarbonate, cholesterol and mucus. There is a decrease in bile acids and pigments. Alteration of the bile salt, cholesterol ratio below the so-called 'critical level' 13 (Andrews, 1932), may play an important part in the precipitation of cholesterol to form calculi.

Bile is an aqueous solution yet the chief constituents are insoluble in water (Elton and Deutsch, 1933):

1) Free bile acids are not water soluble.

2) Bile salts however from the action of alkali on the acids are freely soluble in water.

3) Cholesterol is insoluble in water, but is soluble in bile salt solution.

4) Bile salt cholesterol complex prevents precipitation of cholesterol in the bile. The cholesterol is in a colloidal state.

4) Pure bilirubin likewise is insoluble in water, but since it is acidic in character it is sparingly soluble in alkali, but freely soluble in an alkaline solution of pH11.

The flow of bile from the hepatic ducts into the gallbladder and duodenum encounters many influences which assist or retard its

progress. They are:

1) The secretory pressure of the liver. This must be greater than that of the gallbladder while the latter is filling, and less than the pressure of the gallbladder when it is emptying.

2) Anatomic kinks in the passageway of the bile into and out of the gallbladder. At the neck, the collum cysticum and the spiral valve in the cystic duct.

3) The ability of the gallbladder to store bile.

4) The ability of the gallbladder to evacuate in response to stimulation by hormones, reflex nervous mechanisms, food or by virtue of its own intrinsic musculature and elastic recoil.

5) The resistance of the sphincter-like mechanism at the ampulla.

6) Duodenal tone and peristalsis.

7) Maintenence of the reciprocal relationship between the gallbladder and the choledocho-duodenal mechanism.

Most of these influences are active in maintaining one of the most important functions of the gallbladder, that is, the functional activity of the extrabiliary ducts. This function may be lost following cholecystectomy and may explain many of the cases of post cholecystectomy-syndrome. It appears that the body attempts to restore this mechanism in some cases by compensatory growth and dilatation of the cystic duct stump. This does not

appear to be the complete story for no compensatory changes have been noted in cases of congenital absence of the gallbladder in humans and animals. Furthermore, many of the herbivorous animals have no gallbladder and no exceptional abnormality in the anatomy has ever been noticed. There must be a different relationship between liver secretion, the sphincter of Oddi, and the quality of the bile in the horse, rat, elephant, pigeon, deer and gopher, as the gallbladder is absent.

The major role of the physiologic emptying of the gallbladder is enacted by a humoral and reflex nervous mechanism. The entrance of acid chyme from the stomach into the duodenum induces the release of cholecystokinin (Ivy, 1934) from the duodenal and small intestine mucosa. The gallbladder contracts in response to the hormone after it is absorbed in the blood stream. The ingestion of eggs, cream, fats are among the most potent stimuli causing gallbladder evacuation. (Graham and Cole, 1928).

The gallbladder is evidently not necessary for life, but has an important bearing on digestion as a whole and acts as a pressure chamber regulating intraductal tension. It has been observed that the removal of a diseased contracted gallbladder requires little or no readjustment on the part of the patient.

The removal of a normal or doubtfully diseased gallbladder often initiates a train of symptoms much worse than the status quo. The known functions of the bile are as follows:

1) The bile salts as well as the digestive juices facilitate the digestive action of all the pancreatic enzymes, lipase, amylase and trypsin. By their hydrotropic action and perhaps by modifying the permeability of the intestinal epithelium, they enable the fatty acids formed during digestion to be absorbed. The bile acids unite with fatty acids to form loose chemical compounds which are water soluble and diffusible. This bile acid-fatty acid complex is stable in a slightly acid solution and is unaffected by trypsin. The 'complex' is believed to diffuse freely into the lining cells of the intestinal villi where it breaks up once more into its constituent parts; i.e. fatty acids and bile acids with cholesterol in solution. When the common bile duct is obstructed 25-75% of the fat intake is lost in the faeces, mainly in the form of fatty acids. The absence of bile does not interfere with the digestion of fat but absorption is impaired. Digestion is disturbed as shown by the increase in the absolute amount of neutral fat excreted.

2) Bile salts promote the absorption of vitamins D, K, iron and other substances needed for haemoglobin formation and secretion.

3) Bile has a laxative effect on the bowel.

4) The bile salts are absorbed with cholesterol via the intestine and act as a chloretic on the liver. This is shown by diminished secretion of bile if it is being drained totally to the exterior and also by increased secretion of the bile when bile salts are given by mouth.

Cholesterol is a sterol, one of the many important members of the cyclo-penteno-phenanthrene system. (4 carbon rings). $C_{27}H_{46}O$. Some 19 zoo-sterols have been identified in vertebrates. Cholesterol is present in all animal cells in varying concentrations. Cholesterol in the diet is not an essential for life in most vertebrates. The endogenous production covers the deficit in the diet. Four varieties of cholesterol are released into the blood stream: (a) Synthesized (endogenous), (b) Originally ingested (exogenous, now non-esterified), (c) Esterfied cholesterol-protein complex,(d( giant molecular cholesterol-protein complex. Some of this cholesterol is returned to the liver to be secreted in the bile directly from the liver. Thus the circuit, intestine-liver-blood stream-bile-intestine is complete, and shows an entero-hepatic circulation of cholesterol in different forms.

The liver action on steroids is:

1) Excretion of cholesterol in bile.
2) The formation and excretion of cholic acid
3) The destruction or inactivation by conjugation of steroid

sex hormones.

4) The liver synthesizes, esterifies and destroys cholesterol. (Goldbloom, 1952).

The other related steroids in the 4 carbon ring group are: (a) Oestrogens (oestradiol), (b) Corpus luteum group (progesterone), (c) Male sex hormones (androgens), (d) Adrenal cortex hormone (corticosterone and related substances), (e) From cholesterol (coprosterol, ergosterol, stigmasterol), (f) Bile acids (cholic), and (g) Cardiac glucosides of the digitalis group (digitoxigenin).

Cholesterol occurs in the serum mainly in the free form or in the esterified form in the ratio of 1/3 to 2/3. On an adequate diet lacking cholesterol little change is noted either in the bile or blood levels in the healthy animal. Lecithins are usually associated with cholesterol.

Conn (1950) demonstrated that after 4 days treatment with A.C.T.H. there was an abrupt fall in the cholesterol ester fraction of the total blood cholesterol level. He interpreted this as showing (a) that the ester could be a precursor for the steroidal hormones (depleted cortex reserves of cholesterol) or (b) that it was due to the physiological activity of one or more of the cortical steroids.

Long (1949) noted that epinephrine and stress caused a sharp fall in total adrenal cholesterol. Block (1946) stated that cholesterol could be converted into pregnanedial, another steroidal hormone. Isaksson (1954) found that cholic acid seemed to participate in the formation of the cholesterol-dissolving lecithin-bile system to a greater extent than did chenodesoxycholic and desoxycholic acids in normal human. He noted that in cases of cholesterol calculi in 53 cases, 70% showed that the weight ratio of lecithin-bile salts system to cholesterol was lower than 11-1 or 12-1.

BILE PIGMENTS: These are bilirubin and biliverdin. Bilirubin is formed by the scatterd macrophages all over the body from the haemoglobin of destroyed red blood cells. It circulates in the blood stream and is excreted by the liver cells from the vascular capillaries into the bile canaliculi. (McNee 1913). Biliverdin is formed in the bile passages as the oxidation product of bilirubin. They serve no digestive purpose and are partly reabsorbed, and partly excreted in the faeces and urine.

BILE SALTS: are formed in the liver and mainly consist of sodium glycocholate and sodium taurocholate. Glycine (of glycocholic acid) is readily formed in the body. Taurine (of taurocholic acid) is a sulphur containing substance derived from cystine present in the body proteins or food. The 2 salts are present in equal amounts in the bile. During fasting the supply of taurine is

limited and this lowers the bile salt secretion to a minimum. On a full diet it is the limited amount of cholic acid which controls the amount of bile salt formed. The table shows most of the recognized elements in bile. (Fig. 2). Generally, most substances found in the blood stream are present in bile and with some exceptions in almost the same concentration. This includes some products of excretion, plus enzymes, vitamins and hormones. There is a wide range of all the inorganic mineral salts in small amounts.

The total daily secretion of bile varies from 350-1000 ccs. Gallbladder bile is generally darker than normal golden-yellow liver bile, and it is not miscible with duct bile probably because of its higher mucus content. Bacteria injected into the blood stream can be recovered from the bile in from 2-30 minutes. (Bockus, 1946). Bile is usually considered sterile, but not bactericidal. Many of the Salmonella-intestinal bacteria flourish well in bile media.

Birch and Spong (1887) observed 2 cases of cholecystostomy whose cystic ducts were obstructed. About 20 ccs. of fluid was secreted from the gallbladder every 24 hours. The fluid closely resembled that seen in a gallbladder affected with hydrops. It was slightly alkaline, mucoid, almost clear and opalescent or

straw coloured. This mucoid substance was a nucleo-protein.

There is as yet no general agreement whether or not the gall-bladder can secrete or absorb colesterol through the mucosa. The occurrence of cholesterolosis is usually cited as proof that there must be a passage of cholesterol to afford the heavy cholesterol deposits seen in this condition.

Wilkie and Doubilet (1933) concluded that there was a 2-way flow of cholesterol through the gallbladder in dogs.

Whipple (1922) has shown that total drainage externally of bile will cause early death in dogs.

Siperstein et al (1952) writing on the nature of the metabolic products of $C^{14}$ cholesterol excreted in bile in faeces, showed that 80% of the faecal $C^{14}$ and 90% of the bile $C^{14}$ are present in the form of bile acids.

Gerdes and Boyden (1938) noted that in pregnancy, the female gallbladder which normally empties more rapidly than in the male, empties more slowly. The gallbladder bile in the duodenum was more concentrated than usual. Cholesterol crystals were found in the bile. They concluded that this was due to hormonal action on the gallbladder mechanism. (Relaxin, progesterone). They also concluded that there was marked slowing of flow in the 2nd and 3rd trimesters. This persisted for a short while after delivery. At

term they found the gallbladder contents to be thick and tarry. They felt that this pregnancy stasis could predispose to stone formation later.

Potter (1936) observed large distended gallbladders in 390 sections. 58 analyses of bile were carried out from this group. (bile obtained by needle aspiration). The cholesterol level was raised and the bile salt concentration was low. No calculi were noted. He postulated therefore that with stasis and increased concentration of bile salts damage occurred in the wall. This altered mucosal concentrating power and so changes in the normal ratio of bile constituents. Then if metabolic factors or infection were superimposed, precipitation of stones might follow.

Much work remains to be done in the fields of bile chemistry and gallbladder function physiology. Progress has been made in the analysis of bile salts and acids using the newer procedures of column-partition chromatography and ultraviolet absorption spectrometry. (Mosbach et al, 1954). The use of radioactive isotopes for the labelling of certain basic body elements is contributing vital information regarding particularly the behaviour of the liver in cholesterol metabolism.

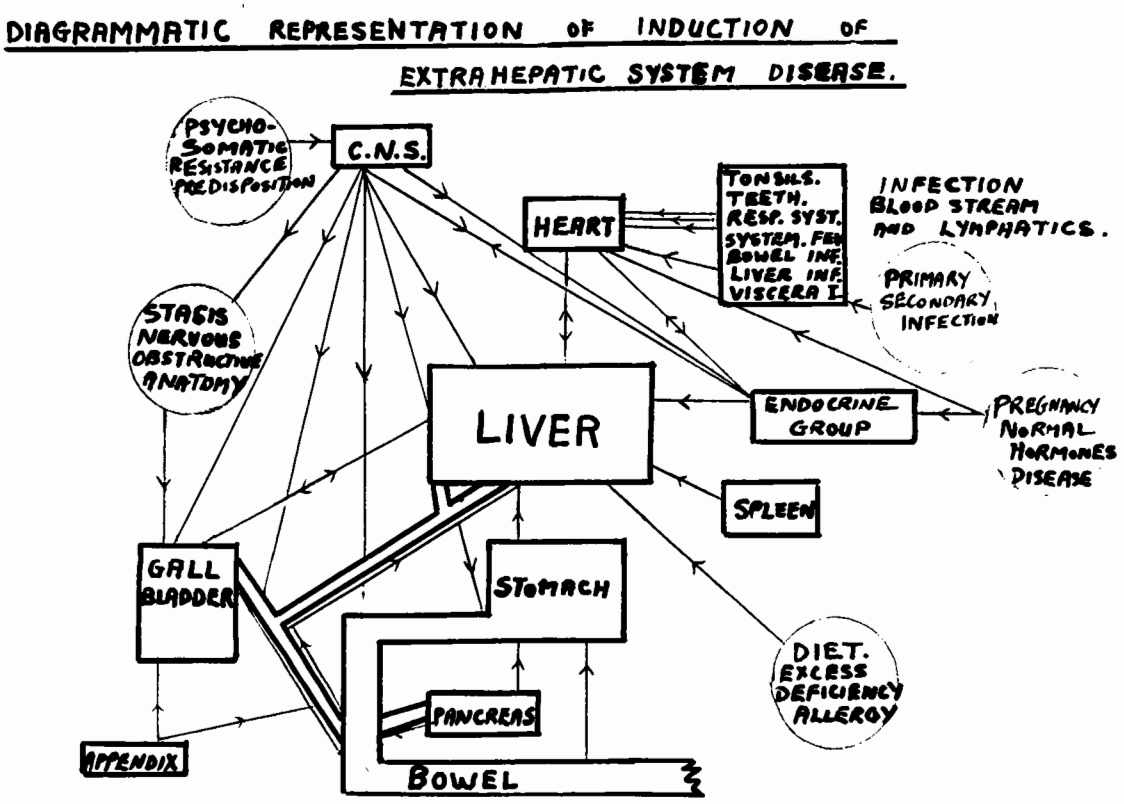



FIGURE 3.

Factors in gallbladder disease production.

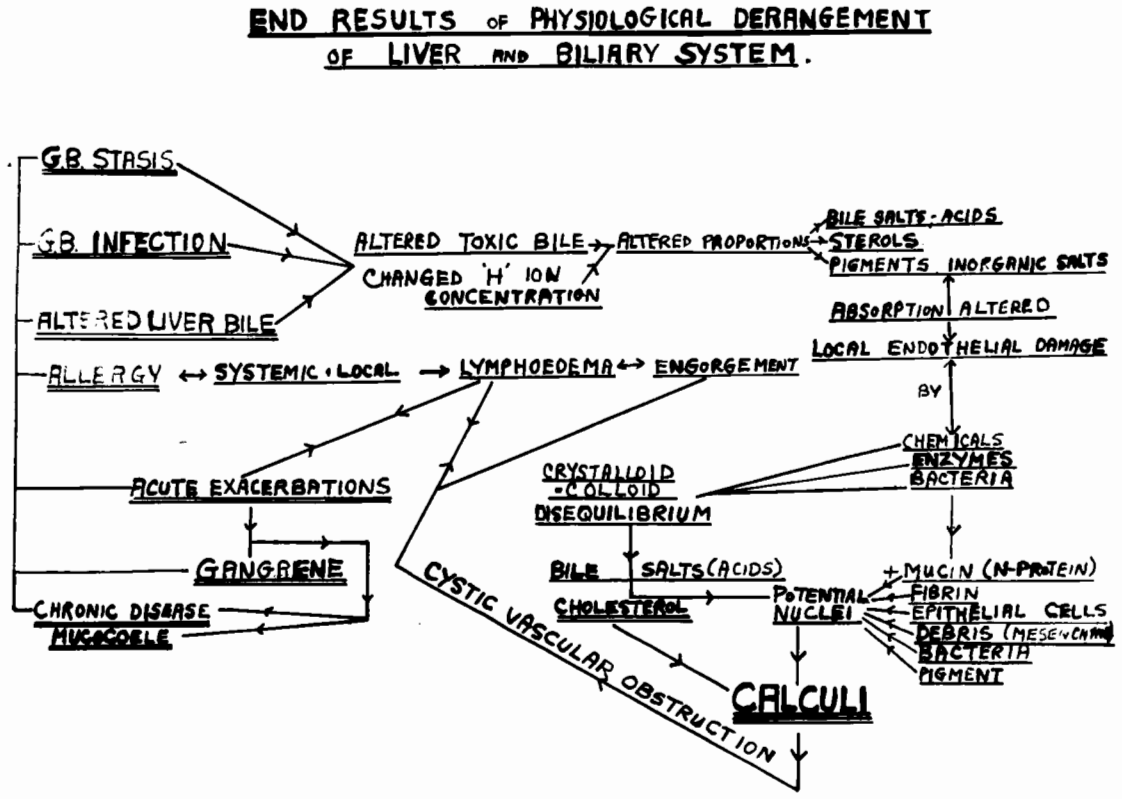



Figure 3a.

Mechanism of biliary tract calculi.

## PATHOLOGY

### Cholecystitis:

Inflammation of the gallbladder may be acute or chronic and may be due to bacteria, elements of the bile, allergy and unknown toxins. There is still much debate on the probable route of infecting bacteria and whether or not they are primary or secondary in the condition. The diagram demonstrates all the possible routes of infection whether proved or otherwise. (Fig. 3). The specific responses induced experimentally by bacteria, chemo-toxic substances and allergy will be discussed under the appropriate headings.

When inflammation of the gallbladder is compared with inflammation in other hollow abdominal organs, such as, the appendix and the urinary bladder, some striking differences are readily apparent. In the acutely inflamed appendix there is close correlation between the pathological changes and the clinical symptoms, and bacteria are present in enormous numbers. In acute cholecystitis, the histological picture is rarely one of acute suppuration and there is little difference between the bacterial content of the acute and quiescent cases. Moreover, it is not uncommon to find bacteria derived from the liver in cultures of the wall of the normal gallbladder. There is therefore

much to be said for the view that in the ordinary case of gall-bladder inflammation bacterial infection plays a minor role.

Occlusion of the cystic duct by inflammatory oedema, debris or calculi is probably the most important single factor as well as deciding the initial and final composition of the contained bile. Denton (1927) has pointed out a fact which is well known to observant clinicians, namely, that the condition appears to be one of infarction rather than inflammation. It is equally true that the former condition could be the end result of the work of the later condition plus calculi to interrupt blood supply. The veins of the gallbladder are much more closely incorporated with the cystic duct than is the cystic artery so that a large stone impacted in the duct may close the veins before the artery and thus lead to a vicious circule of oedema, congestion, anoxia and gangrene.

Calculi may or may not be present in the acutely inflamed gallbladder. In children calculi are commonly absent in acute cholecystitis cases. The fact that acutely inflamed gallbladders are found without calculi and with no bacteria or specific body infection response clearly demonstrates that a chemical or toxic cholecystitis does exist and occurs quite frequently. In chronic cholecystitis, acute exacerbations do occur, and a history of recurrent attacks is usual. Calculi are more often associated

with long standing disease whether known or hidden.

Acute Cholecystitis:

The pathological features are: the gallbladder wall is thickened, the serous surface is congested and may be covered by fibrinous exudate, the mucosa is a bright red colour. When obstruction is present the lumen of the viscus may be distended with what appears to be purulent fluid so that it is known as empyema of the gallbladder. When the contents, however, are examined microscopically, they may be found to consist of an emulsion of cholesterol crystals, calcium carbonate and mucus. True empyema does occur, infrequently, demonstrating the part that infection plays in cholecystitis. The microscopic picture shows a remarkable absence of polymorphonuclear cells and the exudate is most marked in the outer layers of the wall. Round cells have been found in normal gallbladder tissue. There is a marked inflammatory oedema and yet the epithelial lining may be totally intact.

Chronic Cholecystitis:

The external surface may be opaque and yellowish due to an accumulation of sub-serous fat. The gallbladder is usually small and contracted, the thickness of the wall depends on the relative balance of obstruction and inflammation. The mucosa

may be oedematous and swollen. The villi may be thick and swollen or if debased may be non existent. When gallstones are present the surface may be eroded, but it is remarkably intact even in the worst looking gallbladders containing many calculi. The stroma of the mucus membrane is infiltrated with inflammatory cells. These are mostly mononuclear lymphocytes plasma cells, with polymorphonuclear cells present if there was an acute exacerbation. In the later stages there may be an abundant formation of granulation tissue with numerous fibroblasts and other fixed connective tissue cells. Congestion may be marked, but haemorrhages as a rule are not common.

As a result of the scarring the inner surface becomes reticulated and honeycombed, presenting an interlacing network of fine bands but eventually these also disappear and the lining becomes perfectly smooth. The thickening of the walls may be extreme in degree and the cavity is almost obliterated in some cases. If obstruction of the cystic duct has occurred early, then the still distensible walls dilate, and the viscus becomes filled with clear colourless watery fluid which has been secreted by the remnants of the mucosa. It is assumed that all the bile constituents present at the time of obstruction, and trapped in the viscus, are absorbed totally by the gallbladder or in the presense of disease some may permeate through to the surrounding tissues.

This condition is known as hydrops of the gallbladder.

Cholesterolosis or Lipoid Gallbladder

First described by Moynihan (1909) and later called "Strawberry Gallbladder" by MacCarty (1919). The reddened mucosa is studded with tiny yellow specs suggesting the seeds of a ripe strawberry. Sometimes the entire gallbladder is involved, at other times only a portion. The yellow material gives the usual reactions for fat, but differs from ordinary fat in some important particulars. The most noteworthy is its appearance when viewed in frozen section by polarized light under crossed Nicols prisms. It displays the properties of an ester of cholesterol and is similar to the lipoid in the adrenal cortex and ovaries.

The cholesterol content of the lipoid gallbladder is enormously in excess of that found in the normal viscus. It has been reported as being sixty timesnormal. (Boyd). The distribution of the lipoid varies and it is usually confined to the surface epithelium at the base of the cells. In some cases it is scattered throughout the stroma, both free, and contained in the wandering cells. It is claimed that this cholesterol had its origin in the bile or was excreted from the mucosa. There is no absolute agreement as yet on the functions of the gallbladder mucosa. Elman and Graham (1932) believed it is excreted by the

gallbladder. Blaisdell (1927) obtained deposits of cholesterol in the mucosa and submucosa by feeding an animal cholesterol and producing a condition of hypercholesterolaemia, but not when the cystic duct was first tied. Payne (1950), feeding cholesterol to rabbits in experimental atherosclerosis studies, obtained cholesterol blood levels of up to 2790 mgms. for 6 days. There were no instances of cholesterol deposition in any of the gallbladder layers. Patey (1933) could not repeat Blaisdell's experiment. Wilkie and Doubilet (1933) demonstrated in dogs that the flow of cholesterol through the gallbladder depended on the blood-bile cholesterol levels. They maintained that there was a fixed but variable ratio between these which balanced itself on occasion by excreting or absorbing cholesterol through the gallbladder mucosa.

Two facts however somewhat detract from the surgical interest of cholesterolosis of the gallbladder. The first is well marked lipoid deposits can occur in a gallbladder which is otherwise normal. The second is that there is no convincing proof that these deposits can cause symptoms or that they are of clinical significance. Furthermore, it has been noted that removal of the strawberry gallbladder does not always cure the symptoms complained of by the patient. Where gallstones are present, chronic cholecystitis usually accompanies the condition.

The Stasis Gallbladder:

German observers have called attention to the condition of stauungsblase, or stasis gallbladder. Patients with symptoms are frequently found at operation to have neither cholecystitis or calculi. (Westphal, 1923; Aschoff, 1909). Atonic and hypertonic varieties have been described. In the atonic variety the gallbladder wall is thin and lax; in the hypertonic it is thick and the bladder is distended. In both there is stasis of bile and a tendency to the formation of stones. Westphal (1923) has shown that the hypertonic, or as he calls it, the hyperkinetic variety, is due to vagal spasm of the neck of the gallbladder and the sphincter of Oddi; a condition which is frequently present in the early stages of pregnancy. This may be related to the apparent higher incidence of gallstones in multiparous women.

Cholecystitis Glandularis Proliferans:

Due to inflammation, possibly because of increased intracystic pressure, outpouchings of the mucosa into or through the muscularis may occur. These were first noted by Rokitansky (1842) and Aschoff (1909) who called them sinuses. Sometimes there is a proliferation of the epithelium and new gland formation. Papillary projections or papilloma may also be formed.

Carcinoma:

The gallbladder is peculiarly liable to malignant disease. There is a high incidence of cholelithiasis in malignant tumors of the gallbladder. (Fig. 4). The carcinogen methyl colanthrene has been isolated from bile constituents. This substance along with the chronic irritation of calculi and predisposition by the individual would seem to explain the liability of the organ to malignant disease, especially in females.

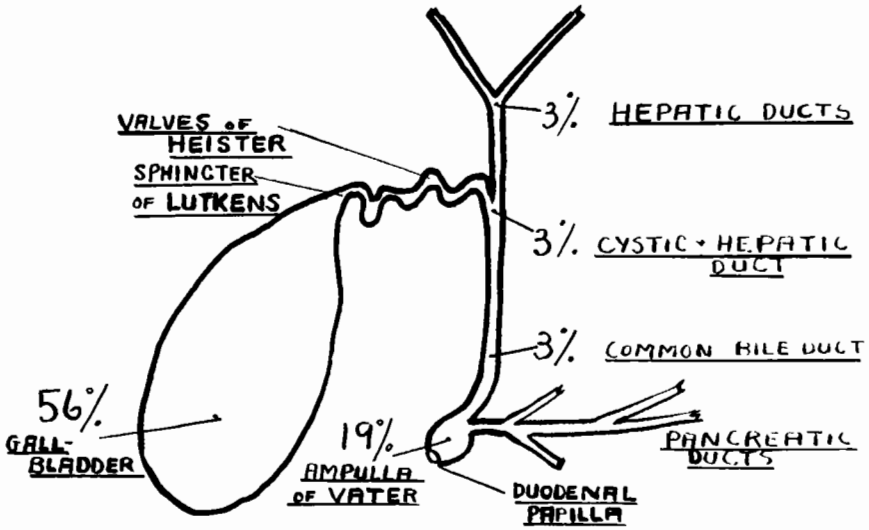
Congenital Anomalies:

The gallbladder or any of the ducts can be involved. Aberrant ducts, absence or double gallbladder, cysts, etc. have been noted at birth and in later life. The occurrence of disease of the biliary tract will depend upon the abnormality present, as will the time of onset of the disease. The type of calculi formed, if any, might similarly be so determined.

Porcelain Gallbladder:

There are reports in the literature of calcification in toto of the gallbladder (Biocca, 1947). This is explained as a sclerosis of the tissues due to vascular impairment, a lipoid infiltration with associated hypercholesterolaemia. This accentuates the degenerative processes which lead to necrosis. The lipid infiltration produces (induces) a precipitation of

## COMMON SITES OF OCCURRENCE OF LITHIASIS AND TUMOUR IN THE BILIARY SYSTEM.




| | |
|---|---|
| GALL BLADDER TUMOURS & LITHIASIS | = 82·2% (Gu Authors) |
| DUCT TUMOURS & LITHIASIS | = 21-55% |
| DUCT CALCULI & CHOLELITHIASIS | = 11-19·8% |
| DEATHS FROM CA. OF BILIARY SYSTEM | = 6·0% |
| CA OF GB. IN 206 098 AUTOPSIES | = 908 CASES |
| PATIENTS & G.B CALCULI OVER 69 YRS. AGE = CA. PROBABILITY | 5·0% |

FIGURE 4.

Sites of carcinoma of biliary system.

calcium salts.

Cohrs and Nieberle "Textbook of Veterinary Medicine," state that empyema of the gallbladder occurs rarely in domestic animals and obstruction of the cystic duct is also rare. Diastomatosis in cattle is responsible for calcium plaque deposits in the chronically inflamed mucosa, and may result in tubular casts. There are several references in the literature to cholelithiasis and choledocholithiasis in cats and dogs. Gallstones are much less common in animals than in man. They occur most frequently in cattle compared to other animals.

Calculi are common in the ducts probably because of Diastomatosis. The deposits vary from gravel to large solitary stones and their composition is similar to the varied types found in man. A major difference is that cholesterol calcium or pure cholesterol stones have not been found in animals. Pure calcium stones are not common. The mixed type with pigment occur most frequently in cattle. Inspissation deposits of soft consistency are often found as casts in pig's gallbladders, in the absence of cystic duct obstruction. The Veterinary College of Toronto reports very few cases of cholelithiasis and at present there are no specimens of cholelithiasis in the Pathology Museum. The incidence of acute cholecystitis and chronic forms of the disease is reported as being very low. A study of normal slaughter-house

domestic animals would contribute many details in this respect.

Gallstones:

They may be found in the gallbladder, bile ducts or liver. (not frequently diagnosed). They have been detected in the foetus and at birth. Congenital blood diseases with increased blood pigment production is the usual cause in these cases. Thereafter, they occur at all ages, more frequently in the aged, and not usually in the under 25 year group. They occur in females twice as frequently as in males and usually at a slightly younger age. Pregnancy is supposed by some to be the main factor causing the difference in incidence. Solitary gallstones appear more frequently in males. The frequency-incidence ratio of 2 to 1 seems also to hold for the occurrence of carcinoma in females and males, irrespective of the age group.

Gallstones may be single or multiple, large or small. Their final shape is dependant upon the duration of the disease, the numbers present and the size of the gallbladder. Different families of stones may be found in the same gallbladder of different size and constituency. From the appearance of many calculi it appears as if the calculi do change coats as it were, dependant upon the surrounding milieu. Gallbladder disease is most often diagnosed because of the presence of stones and the symptoms they produce. The large solitary calculus may be "silent" and produce few symptoms. (Robertson, 1945). It is

reported that there is a very high incidence of gallbladder disease and that 50% of cases are undiagnosed. The heaviest and largest calculus reported (Ritter) weighed 110 grams and was 6 inches long. The largest number of calculi reported in one gallbladder was 36,329. They occurred in a white female, aged 37 who had cholecystitis. The stones were carefully counted in small broups by a medical student (Schenken, 1945) to avoid error. Little is known about the time required for stone formation in humans and until recently little concerning calculus formation in laboratory animals. Cameron (1923) had a confirmed case of new stone formation in 8 weeks. Mentzner (1926) reported a case where 2 stones were removed from the gallbladder. (Cholecystotomy?). Several days later at autopsy a large cholesterol cast was found in the gallbladder.

Gallstone recurrence after cholecystotolithotomy has been reported at 30% especially where performed on young patients with relatively healthy looking gallbladders. (504 cases). New stone formation does occur in the common bile duct following cholecystectomy. There are reports of spontaneous disappearance or dissolution of gallstones. (Miller, 1956). Autopsy reports on 63,270 cases show an incidence of calculi of 15.6%.

Gallstone Classification:

The present day classification of calculi is an arbitrary

physico-chemical appraisal.

The usual categories are as follows:

1. Radiate cholesterol
2. Pigment stones
3. Combination, mixed or infected calculi. (Cholesterol, pigment calcium).
4. Calcium carbonate calculi (rare).
5. Pigment-calcium calculi.

Organic debris, nucleoprotein, fibrin, pus, bacteria may be found admixed in any of the above calculi. It is obvious that calculi consist of elements derived from the bile. The variations of these elements that can occur in the calculi formed are certainly legion. The physical formation of these stones has also provided material for debate and dissension. Liesegang (1929) stated that if diffusion of a substance occurs into a colloidal mass containing a second substance which reacts with the outside material, then visible zones of precipitation will be seen. For example, the calcium diffuses away from colloid cholesterol and crystalline cholesterol is left. Where the calcium going towards the periphery meets pigment, visible "tree" rings develop.

Ord (1879) in a paper entitled "On the influence of colloids upon crystalline form and cohesion" suggested a mechanism based on

the presence of colloids for the production of calculi. Wells (1925), Schade (1910), Lichwitz (1907), Joly (1929), Weiser (1933) accepted this theory.

Meckel von Hemsbach (1856) regarded the successive layers of gallstones as examples of a universal tendency in nature to successive and alternating deposits of contrasting substances, the process being controlled by definite physico-chemical laws. He found application of this tendency in many formations, including biliary, and urinary calculi as well as pearls, shells, agates and even the geologic layers of the earth's surface. Delario (1935) concluded along similar lines that calculi were formed as in nature by 2 main methods:

(1) Pectolite crystals - simply formed of crystalline material radiating from a central focus.

(2) Geode as in quartz. Crystallization proceeding from the periphery of the mass (i.e. through colloid), imprisoning salts within. The exterior crystals are small and the inner slowly formed crystals are in large plate form. Flattening of crystallization at varying ring zones is seen. Calcium bilirubinate is pushed outwards. If crystallization is rapid, the crystals are small and more pigment is trapped.

Sweet (1935) firmly supported a colloidal theory and

maintained that the pigments in the mass were pushed centrally to form the so-called nucleus. He also pointed out that cavities did exist in the centre of fresh calculi. This could be explained by evaporation of water at the centre of the mass. The stellate fissuring seen on X-ray could be so caused, or possibly by simple fissuring due to irregular contraction of the mass (earth's crust) or by gas production of anaerobic bacteria.

None of these authors have been able to demonstrate conclusively the exact mode of calculi construction in either humans or experimental animals, but have offered suggestive evidence, mainly chemico-physical in character.

## HISTORICAL REVIEW OF EXPERIMENTAL CHOLECYSTITIS AND CHOLELITHIASIS.

### STASIS:

Most of the experimental work recorded on Gallbladder disease begins in the late 19th Century. However, many of the earliest physicians and philosopher practitioners had noted Biliary tract pathology prior to this.

Hippocrates, c. 400 B.C., knew of liver disease and right upper quadrant pain. Galen, c. 200 A.D., considered stasis of the bile (coagulation) as the cause of gallbladder disease. Alexander of Trallanius, 400 A.D., noted the occurence of gallstones in an ox. Sylvaticus first described gallstones in humans in 1314 A.D. Benevenius described gallstones and wrote a treatise on the hidden causes of disease (1420).

Fernelius (1554) noted that some gallstones float in water, and that occlusion by stones of the cystic duct frequently occurred causing stasis. Kentman (1565) attributed stones to liver heat burning up the bile. Paracelsus (1616) stated that because of diet and destruction of tissues bile may precipitate as tartarus. This is the first reference to a metabolic theory explaining the occurrence of calculi. Aschoff may be considered his first disciple.

Vallismiri (1730) noted that gallstones dissolved in turpentine. Coe (1757) published the first monograph on gallstones in the English language. Morgagni (1767) found glands in the gallbladder and emphasized the importance age, sedentary habits and irritation of the glands or the gallbladder as factors in stone formation. He was first to suggest an inflammatory theory.

Rokitansky (1842) concluded that stones were the result of stagnation and the holding back of bile. Meckel von Hemsbach (1856) made important observations on both origin and structure of gallstones. He strongly supported Morgagni's view that catarrh of the mucus membrane of the gallbladder was a leading factor. Naunyn (1892) was later associated with this theory. Meckel, a geologist, studied the secondary changes that occur in gallstones and pointed out that by re-crystallization of cholesterol the structure of a stone may change and he noted the tendency for the crystals to assume a radiate arrangement.

Thudicum (1863) wrote an excellent treatise on cholelithiasis and concluded that a "bad chyme aberration of the digestive system was the cause."

Kraus (1881) stated that an intermediate cause of calculi was a slowing of bile into the intestine with resultant accumulation of bile in the gallbladder.

Cohnheim (1882) referred to gallstones as being due to a difficulty in emptying of the gallbladder. Marchand (1888) made the broad generalization that everything which led to thickening of the bile, obstruction of the bile ducts or insufficient emptying resulted in granular deposits (Koerngen) from which larger deposits might readily form.

Klebs (1889) asserted that the preliminary condition for gallstones was stagnation of bile. Naunyn agreed that stasis was the decisive factor. Luetkens (1892), Weltz (1894), Aschoff and Bacmeister (1909) and Meltzer (1917) reached the same conclusion that stasis was the essential factor.

Courvoisier (1890) noted that in cases of obstructive jaundice due to causes other than stone, the gallbladder was distended. If due to stone in the duct, there was a small contracted gallbladder due to previous inflammation in over 80% of the cases. This law is still applicable.

The founder of surgery of the gallbladder was probably Jean Louis Petit, 1733, who suggested that if a diagnosis of a large inflamed gallbladder adherent to the abdominal wall was made, then the treatment was to cut down and evacuate the pus and calculi. The recommendation was not implemented at that time because of the fear of opening into the peritoneal cavity.

Bobbs (1867) evacuated gallstones from a human patient. Lawson Tait (1879) performed many cholecystotomies. Langenbuch (1882) is credited with having performed the first cholecystectomy on a patient. Stadelmann, Blackstein and Welch all published experimental work in 1891. Buxbaum (1898) is credited with first demonstrating gallstones by X-ray.

Foster (1880) proposed the disturbance of the law of contrary innervation as a pathogenic factor in disease of the bile ducts and gallbladder. Meltzer (1917) agreed with him and was first to suggest non-surgical drainage of the gallbladder. Graham and Cole (1924) pioneered oral cholecystography. Breuer (1931) reported the presence of gass in gallstones by X-ray.

Hein (1846) made the shrewd observation that rest alone was not sufficient to produce gallstones, as the bile was normally at rest whilst the intestine was inactive.

Schade (1910) minimized the role of stasis in gallstones by stating that although stasis was a very important factor in the precipitation of cholesterol from bile, this precipitated cholesterol was not always associated with calculi formation, and that other influences seemed to be necessary. Lichwitz (1914) asserted that the idea of stasis as a factor in the production of gallstones was not surely established. Rous and

McMaster (1921) asserted that intermittent biliary stasis is admittedly the principal predisposing cause of cholelithiasis.

Chauffard (1922) was certain that stasis no matter what the cause favoured changes in solubility due to over concentration of cholesterol with consequent precipitation.

Westphal (1923) laid much stress on functional disorders of motility of the gallbladder and biliary passages. Stasis was the result of either hypotonicity of the gallbladder or hypertonicity of the sphincter.

Rousing (1923) noted that Aschoff had accepted stasis as an "irrefutable fact" and that from this one would conclude that there was a large amount of convincing proof behind it. "Unfortunately," he added, one seeks in vain for any such evidence." Rousing observed that acquired obstructing biliary lesions in man did not necessarily produce gallstones.

Wheelon (1926) found an involvement of biliary structures in 75% of 74 patients with duodenal ileus; cholecystitis was present in 38 cases; hepatitis in 14 cases and peribiliary adhesions in 48 cases.

Shapiro and Kasbach (1927) produced duodenal ileus by passing a strip of rectus sheath under the duodenum, proximal

to the ligament of Trietz, suturing it to the base so as to form a sling. The result was most effective with definite increase in gallbladder evacuation time. Freeing the sling gave a return to normal function.

Whitaker (1927) reported soft masses resembling blood clot in the gallbladders of 4 of 16 cats after cutting and dilating the sphincter of the common bile duct. In 1929 he reported masses resembling calculi after inducing stasis and hyperconcentration of bile through fasting and dehydration for 5-15 days whilst the animals were under barbital anaesthesia. No analysis was reported.

Copher and Illingworth (1928) could not produce Whitaker's results and so they agreed with Pavel (1935) and Furth (1937) that stasis was not a necessary factor.

Rous, McMaster and Drury (1924) had discovered incidentally that small calculi of calcium carbonate and calcium bilirubinate were formed along the glass and rubber tubings after intubating dog's gallbladders for the collection of bile aseptically.

Aoyama (1914) had tied the cystic duct in rabbits and guinea pigs after injecting cholesterol 4 times subcutaneously. Most of the animals formed cholesterol concretions.

Ordnoff (1929) suggested a relationship between disturbance of duodenal motility so frequently seen in pregnancy and the cholecystic symptoms and disease which may attend and follow it.

Bockus (1929) believed that duodenal ileus in some way renders the biliary tract more liable to infection.

Walsh and Ivy (1930) observed stricture of the common bile duct alone produced in a series of dogs 50-100 mgms. of calcium pigment and carbonate sediment in the gallbladders. Chronic cholecystitis was caused by placing a stone in the gallbladder and then introducing biliary stasis.

Westphal et al (1931) narrowed the cystic duct in dogs for varying periods of time combined with cholesterol feeding. Charcoal and sodium were introduced also in the series. Biliary concretions 1 mm. in size were produced. One was 1 cm. in diameter.

Schaefer (1932) stated the principal factor in the precipitation of cholesterol is concentration by absorption of fluid and stasis is the usual cause of this. Osler's textbook (1938) stated that all causes and conditions favouring stasis predisposed to gallstones.

Andrews et al (1932) quoted the alleged sedentary habits of women, obesity and old age as causes of stasis. Newman (1933)

said, stasis in itself is no explanation for anything. He also stated the magic word, stasis, on analysis disappears from the list of primary causal factors of gallstones. Andrews (1933) adopted this opinion by stating there is no evidence that stasis alone is a factor. Greene et al (1936) agreed with Andrews that biliary stasis alone is insufficient to produce calculi. Von Babarczy (1938) supported the theory of stasis as did Halpert (1928), Walters (1936) and Moore (1937).

Phemister and Aronsohn (1939) reported precipitation of calcium salts takes place in the gallbladder, only when complete obstruction and low grade infection are present. They concluded calcium salts represented a specific response on the part of the wall of an inflamed gallbladder.

Bisgard and Biker (1940) asserted that neither biliary stasis nor stasis in the pancreas is a single factor producing gallstones.

Carter (1939): In 25% of cases in which gallstones were present, stasis was the only one of the accepted usual factors implicated which could be demonstrated. There is therefore presumptive evidence that stasis is important, but other factors (unknown) must also have been in operation for 86% of the non-calculous gallbladders also showed evidence of stasis. In 24 cases there were gallstones unassociated with any bacteriologic or pathologic evidence of infection or inflammation. He concluded

that this group of cases showed infection was not necessary for stone formation and presumptive evidence that in many cases stones may precede infection of the gallbladder.

Robertson (1945) concluded that stasis so useful in the laboratory test tube experiment is not the cause of gallstones.

In 1947 Calzolari observed 2 types of calcium carbonate stones in humans, soft and large, and hard and small. He concluded that the small concretions were dependant on obstruction of the cystic duct with virulent infection also being present.

Macgregor (1952) studied complete biliary obstruction in dogs, by ligation and division of the common bile duct. He observed that the liver stands biliary obstruction far better than hepatocellular toxins. Signs of failure were noted at 4-7 weeks. The gallbladder was thin and distended with no other pathology or concrements. The liver showed signs of biliary cirrhosis with fatty infiltration.

D. P. D. Wilkie (1928) summarized the work of the pioneers to that time and stated, "the work of the future must be to discover how to prevent metabolic disturbances which together with infection and stasis lead to gallstone formation." This excellent summary was indeed accurate for most of the present day experimental work revolves around these 3 factors. The above statement plus the

studies of Carter expresses our opinion of the role of stasis in the production of gallstones.

The work of Popper and Szanto (1956) confirms in part the opinion we have regarding the role of the liver in biliary tract disease. In their observations on intrahepatic cholestasis (cholangiolitis) i.e. bile stasis within the liver not due to extrahepatic obstruction, they noted the occurrence of increased deposition of bile. In the acute stages no inflammation was observed. They assumed that the process started with increased permeability of the ductules, leading to bile regurgitation as well as transient inflammatory exudation in case of drug sensitivity. Inspissation of bile in the ductules adds an obstructive component to the leakage. The latter incites periductular inflammation which is a result rather than the cause of cholestasis. The inflammation finally stimulates periductular fibrosis as an additional obstructive factor in the late stages. They observed <u>microcalculi</u> in 22% of cases of viral hepatitis; 67% in postnecrotic cirrhosis and 100% in septal cirrhosis (nutritional). In conclusion they stated that intrahepatic cholestasis was one of the liver responses to injury which may or may not be combined with other responses, e.g. liver cell damage. The cholestatic response was not specific for any type of hepatic injury. It may occur to equal degree in drug hypersensitivity, e.g. (chlorpromazine), viral infections (hepatitis), nutritional deficiency

(cirrhosis) and can occur without any apparent cause.

It may be deduced from this work that liver lesions occur more frequently than diagnosed, that they can easily explain the presence of bile thrombi in the bile and biliary tract and more important, afford an explanation for the secretion of abnormal or toxic bile.

STUDIES ON METABOLISM:

Plater (1536-1614) compared gallstones to stalactites and spoke of a foreign substance which modified the constitution of bile.

Thenard (1821) thought precipitation of bile pigment came from diminution of Natron (alkaline salts) and cholesterol was involved.

Seifert (1851) inferred that an increase of lime in the drinking water was important.

Thudicum (1863) is usually given credit for being the first to express a more rational idea, namely that fermentation of bile caused a precipitation by splitting the bile acids and alkalies.

Klebs (1889) probably expressed the matter far better than anyone else by stating that although the presence of gallstones furnishes many perplexities, nevertheless, one thing is sure, namely, that they arise from a metamorphosis of the normal constituents of the bile. The cholesterin is held in solution by the bile salts and can only be precipitated when these salts are diminished.

De Fourcroy (1789) made known the discovery of cholesterol and asserted that all human stones contain it.

Chevreul (1815) gave the substance its present name.

Bile acids were discovered in the middle of the 19th Century and many investigators sought to show the role they played in keeping cholesterol in solution. Andrews (1932a) confirmed the earlier work and showed by dialysis in vitro that bile salts will pass through a semipermeable membrane and leave cholesterol deposits behind. They also stated that no substance other than bile salts was capable of holding cholesterol in solution in the bile.

Naunyn (1892) and his followers complicated the picture, by asserting that cholesterol was a secretion of gallbladder and bile ducts.

Aschoff since 1906 insisted that cholesterol was absorbed by the gallbladder.

Tourinimi (1924) showed by injecting a measured quantity of bile into animal gallbladders, with the cystic duct tied, that Aschoff's statement had some truth experimentally.

Dostal and Andrews (1933), in a review of the literature, concluded that the effect of diet or hypercholesterolaemia on biliary cholesterol as a factor in gallstone formation cannot be said to be settled, although the weight of evidence lies on the negative side.

1) Clinically they observed that bile from a fistula in a diabetic patient showed no changes in concentration or in the content of cholesterol in feeding experiments.

2) They noted that variations in fats and proteins had no effect on bile excretion.

3) Feeding cholesterol even when accompanied by bile acids likewise had no effect.

4) No relation between cholesterol content and volume of bile excreted was noted.

5) No correlation between cholesterol and total solids of bile could be found.

6) The constancy of bile cholesterol was remarkable and varied only between 55 and 75 mgms. per 100 ccs. In dogs the range of fluctuation was much greater than in man. They concluded that the responsibility for gallstone formation seems to lie in the gallbladder rather than in the liver. It has been proved conclusively that cholesterol is synthesized and stored mainly in the liver.

Tennent et al (1956) have demonstrated by animal experiments using tagged acetates that the liver is the essential organ in cholesterol production.

Hanel et al (1954a) using slices of liver from hamsters which had gallstones showed no significant difference in the

rate of cholesterol synthesis either in the controls or these animals. Radioactive $C^{14}$ acetate was used. Hanel et al (1954b) also demonstrated no significant difference in the rate of liver cholesterol synthesis in hamsters with and without gallstones.

Peters and Van Slyke (1946) emphasized that cholesterol was not only synthesized and destroyed in the body, but absorbed and possibly excreted in the intestine. It was also excreted by the liver to be partly reabsorbed by the intestine again.

Friedman et al (1952) observed on good evidence that cholesterol was synthesized in the liver, adrenals, skin, testes and intestine. They added that there was no generally accepted quantitative information available concerning the origin of cholesterol found in either the plasma or bile. This was in agreement with the findings of Block (1946) and Srere (1948).

Long (1949) reported that in Addisonian's with diabetes, the administration of A.C.T.H. raised the serum cholesterol, but quoted that A.C.T.H. was not necessary for adrenal synthesis of cholesterol. (Proved in vitro).

Vollmer (1949) found a very low lipid content in hamster's adrenals, no cholesterol was present. Hamsters infected with Leishmania had raised blood cholesterols.

Altschul (1952) agreed with Payne that there was no lipid infiltration of the gallbladder wall in rabbits on high cholesterol feeding. He stated that the gallbladder is an important organ in the excretion of cholesterol and in spite of this, or because of this, there was no damage of any of the wall layers by cholesterol. Some foam cells were noted in the outermost layer. In prairie gophers (ground squirrels) he reported weight gain and calculi in 4 out of 7 fed a high cholesterol diet from 2-7 months. The calculi contained 90% cholesterol. He quoted Kennedy and Okey as demonstrating gallstones occurring in guinea pigs after splenectomy and on a high cholesterol diet. One rabbit on cholesterol orally demonstrated a concretion. Another rabbit painted with 7/Ketocholesterol also showed a concretion. (No analysis).

Littler and Ellis (1952) in a critical survey of 100 cases of cholelithiasis postulated an underlying disturbance of steroid metabolism as the predisposing factor in etiology. The cholesterol metabolism seemed more likely to become unbalanced in females as a result of either pregnancy or advancing years.

Henegar and Turner (1950) stated that single cholesterol stones are a result of disordered metabolism causing decrease in ratio of bile salts to cholesterol in the bile. Normally the gallbladder does not absorb or excrete bile salts or cholesterol. The normal gallbladder absorbing fluids did not change

the ratio but infection or inflammation allows it to absorb bile salts and so cholesterol crystals are deposited. Pure cholesterol stones may develop in the absence of infection, but stasis is present. In patients with altered cholesterol metabolism the gallbladder showed no pathological changes and was able to concentrate. They assumed mixed stones started as pure cholesterol stones. Obstruction by a stone occurs, calcium salts, pigment more cholesterol are deposted in layers on the other stones in the gallbladder and to a lesser extent on the stone in the cystic duct. Stones in the common bile duct are usually preceded by calculi in the gallbladder, although primary stones do occur in cirrhosis of the liver or obstruction at the sphincter. In cholecystitis with cholelithiasis, but with no obstruction of the outflow and no complicating infections of the biliary tree, there is usually no change in the value for serum cholesterol. During pregnancy some degree of hypercholesterolaemia develops, the reason for which is unknown.

Wilkie and Doubilet (1933), in their experiments on cholesterol flow, noted that by feeding dogs 1 gm. of cholesterol daily they were able to raise the serum values in 2 cases from 77 mgms.% to 155 mgms.% and 99 mgms.% to 208 mgms.%.

Pibram (1923) had shown by duodenal intubation of pregnant women that the bile cholesterol levels during the first 3 months

were low but later reached exceesively high levels which persisted for a period after delivery.

Campbell (1924) conclusively demonstrated as did Andrews that the average patients with cholelithiasis had normal blood cholesterol levels.

Goodman (1907) showed that in vivo destruction of red cells raised the cholesterol level.

Kusumoto (1908) using tolylenediamine poisoned animals so as to cause haemolysis and demonstrated a large increase in bile cholesterol content.

Peirce (1912) showed that in the bile the cholesterol esters were much lower than the free cholesterol.

D'Amato quoted by Andrews demonstrated that liver damage by injections of alcohol-acetic acid and B coli caused less cholesterol to be excreted in the bile.

Rothschild (1915) significantly showed that starvation causes increases of cholesterol in the blood, liver and bile.

McMaster (1924) confirmed this and obtained tripling of bile cholesterol in his experiments.

Fox (1927) in carefully controlled experiments was not able

to demonstrate any dietary influence on cholesterol levels.

Gardner et al (1930), after a review of the literature on cholesterol metabolism, stated that they did not believe that biliary cholesterol could be affected by diet.

Gainsborough (1930) commenting on so-called 'lipoid nephrosis' observed that although blood cholesterol levels were high, bile levels were normal.

Rous (1924) in a scientific manner using the 2-way fistula of McMaster sampled bile and returned it to the intestine. He found a slight increase in bile cholesterol on a rich cholesterol diet, but a very great rise from starvation which overshadowed all other influences.

Voit (1892) showed that most faecal sterols were not biliary in origin and that the intestine secreted lipoid material. It was proved by side tracking bile and noted that the faecal sterols did not diminish in amount but increased.

Sperry (1926) in classical experiments confirmed this by showing that upon external drainage of bile the sterols increased in the bowel by amounts from 1-4 times normal.

Carter et al (1939b) and Rehfuss and Nelson (1935) advised a low cholesterol diet as a routine treatment for people with gall-

bladder disease. However, the increase or decrease of cholesterol in the bile was relatively unimportant as long as the proper ratio of bile salts to cholesterol could be maintained.

Mayer et al (1953) reported hypercholesterolaemia as an integral part of the "Hereditary obese hyperglycaemic syndrome" in mice. The levels in these mice were double those of their litter mates who were not obese. Cholesterol levels were further increased by 2 weeks treatment with high protein and high carbohydrate diet, by fasting and by growth hormone. Decreased by thyroxine. In non-obese animals A.C.T.H. and growth hormone increased the cholesterol levels, whereas high carbohydrate, high protein diets and thyroxine lowered the levels.

Swell and Flick (1953) noted that in rats fed 25% lard, oleic acid, stearic acid, with or without cholesterol, the blood cholesterol rose sharply with lard, usually in the ester fraction. They suggested that incomplete absorption of cholesterol takes place when a high percentage of saturated fatty acids is present in the diet. The body can synthesize large amounts of cholesterol without exogenous supply. Levels in humans seem to be independent of intake.

Byers and Friedman (1952b) studied the effect of various bile acids on hypercholesterolaemia following biliary obstruction in the rat. Cholic acid was the only one of 4 acids studied,

(desoxycholic, dehydrocholic, glycocholic) which appeared to be effective. Cholic acid fed to the animals markedly increased the hypercholesterolaemia associated with the obstruction.

Byers (1952a) in further studies noted that the rate of biliary excretion of cholesterol in the rat was found to be a function dependant upon the rate of hepatic synthesis of cholesterol. They suggested that the bile cholesterol represents an increment of the cholesterol synthesized by the hepatic cells. The measurement of biliary cholesterol excretion may be employed as a means of assaying the hepatic rate of cholesterol synthesis. They concluded that the accumulation of bile acid occurring in the plasma after biliary obstruction in the rat appears to be responsible for the raised cholesterol blood levels.

Byers and Friedman (1954) showed that in rabbits and dogs the reticulo-endothelial system plays a role in the disposition of cholesterol of dietary origin. When interfered with, cholesterol lipid and chylomicra accumulate in excess in the plasma of the animal. They stated that the cholesterol from the intestine absorbed into the blood travels as an <u>insoluble</u> portion of a lipid rich particle (chylomicron) and differs from cholesterol synthesized by the normal animal, as the latter is in <u>soluble</u> form. Thus in one case the body is subjected to a colloidal suspension and in the other to a solution of this sterol.

A portion of the cholesterol synthesized by the nephrotic rat travels in particulate form in the plasma. There are 2 processes by which suspensoids are rendered soluble:

1) Mainly by the reticulo-endothelial system and then transferred to the hepatic cells and stored.

2) They may be converted into cholic acid by the liver.

Virchow (1857) noted cholesterol deposits in the gallbladder. McCarty (1936) reported the presence of cholesterolosis in 18% of 5,000 autopsy gallbladders. Mayo (1921) noted an incidence of 39% in 1254 cases of disease of the gallbladder. Mentzner (1926) in autopsy reports on 612 cases found 38% cholesterolosis. Illingworth (1928) found 21 cases in 100 consecutively operated cases. Cholesterol stones in these cases varied from 20-50%.

Womack and Haffner (1944) writing on the significance of cholesterolosis stated that few could deny that it was a chemical cholecystitis with mild inflammation and as a result of metabolic disease. They noted that bile is damaging to tissue and is capable of producing a condition resembling cholecystitis, and produces marked capillary permeability in the region in which it is placed. This is first evidence by oedema but later there is ecchymosis and diapedesis of red cells. They showed that inflammation occurs in dogs if the cystic duct is obstructed. 354 cases were studied and in 116 or 32.8% some histological evidence of lipid or bile reaction was found in the wall. It was

suggested that the crystals of cholesterol in the gallbladder wall enters the intercellular spaces in fluid form.

Feldman (1954) studied 165 cases in autopsies. Noted that cholesterolosis is a disease predominant in the 5th and 7th decades of life. He found a major difference from most series in that it occurred more frequently in males. Blood cholesterols were no aid to diagnosis. Excessive amounts of cholesterol crystals in duodenal bile were highly suggestive. He stated that despite the role of the liver in cholesterol metabolism that there was no causal relationship between the liver and cholesterolosis. Contrary to the accepted belief, autopsy study showed that cholecystitis and gallstones are not very common findings associated with cholesterolosis. Most cases of cholesterolosis which were associated with stones also showed coexisting chronic cholecystitis. In 1,319 adult autopsies, cholesterolosis of the gallbladder occurred in 12.5% cases. There was a marked discrepancy in incidence between autopsy and surgical cases.

Mentzner (1926) showed that in 100 women with gallbladder disease 64% had cholesterolosis and had been pregnant at the same time.

Mackey (1937) reviewed the literature on cholesterolosis and supplemented it with personal observations in 87 cases. He noted that:

1) Advanced inflammatory or degenerative changes are rare in this condition.

2) Probably the gallbladder can absorb bile, and when cholesterol is excessive the process becomes vizualized.

3) The presence of organisms is rare in simple cholesterolosis.

4) In 1/3 of cases cholesterol stones occur which he deduced were parallel results of supersaturation of bile with cholesterol. They were not cause and effect.

5) Cholesterolosis per se did not produce clinical symptoms consequently mere presence of lipid infiltration did not justify cholecystectomy nor did it support a prediction that the clinical result of this operation would be gratifying.

Okey (1942) cholesterol fed guinea pigs developed gallstones on diets containing excessive amounts of riboflavin. Irritation of the gallbladder and biliary passages and occasional duct impaction was observed.

Lam (1954) reported an incidence of gallbladder disease higher than average in Indians (S. Dakota). There were 52 operations on the biliary tract out of a total of 227 major operations. He attributed this to the fact that their food was usually soaked in grease.

Magee et al (1952) noted that certain dietary factors influenced the output of bile acids. They concluded that a cholate

production of less than 100 mgm/Kg. (in dogs) indicated hepatic disturbance due perhaps to hepatitis or extra hepatic obstruction.

CLINICAL AND EXPERIMENTAL STUDIES ON INFECTION:

The research workers who do not support the theory that infection is the primary cause of cholecystitis and cholelithiasis, frequently make the following observations:

(a) Bacteria are seldom found in the stained sections of gall-bladder wall and the bile is found to be sterile in a high percentage of cases of diseased gallbladder.

(b) Clinically, sepsis seems to be a rare complication of cholecystitis and the gallbladder is rarely involved in cases of pyaemia.

(c) Where organisms are found in the bile it is equally possible that they are passing through, or are harmless and not the cause of the disease present.

(d) Results after local insertion of streptococci from acute human infection showed little tendency for acute infection of the gallbladder to occur from any of the contents of the organ. Bacteria present in bile did not cause any reaction unless extremely virulent, present in overwhelming dosage and even then not frequently.

Aronsohn and Andrews (1938) observed that almost 9 out of 10 publications considered bacterial infection to be a likely agent.

John Hunter (Q) was one of the first to associate gallbladder disease with infection. (Typhoid fever cholecystitis).

Moynihan (1909) stated that each calculus was a monument to

the bacteria entombed therein.

Gilbert and Girode (Q) proved Hunter's statement by bacteriological studies.

Osler (Q.1938) reported 19 cases of acute cholecystitis in 1,500 cases of typhoid fever. Typhoid fever is more prevalent in uncivilized areas yet the incidence of gallbladder disease in civilized areas continues to increase.

Rehfuss and Nelson (1945) reviewed 4,395 cases of cholecystitis. Bacteriological studies revealed a variable percentage of organisms and infection. The organisms isolated were b. coli, b. typhosum, staphylococcus, streptococcus, gram positive bacilli, streptococcus faecalis, b. welchii and enterococcus.

Burden (1938) stated cholecystitis is not usually the result of an acute process, but rather a result of mild infection slowly affecting different layers of the gallbladder. Chronicity is favoured by the anatomy and histology of the organ which predisposes to stasis and obstruction.

Deaver (Q.1938) quoted the modern opinion that a primary hepatitis preceded by acute appendicitis is the mechanism. Other authors state that no information has been presented to support any theory of liver infection, whether by virus or bacteria.

Feinblatt (Q.1938): A pathological study leads one to believe that the role of infection in the causation of cholecystitis has been greatly overestimated. The importance of metabolic and mechanical factors have not received sufficient consideration.

Naunyn (1892) suggested infection with resultant desquamation of the gallbladder epithelium, diapedesis of leucocytes, exudation of fibrin with precipitation of cholesterol to form the nucleus of a stone. He later agree that a colloid-disturbance-mechanism would more likely explain stone formation.

Cushing (1899) and others produced cholelithiasis and cholecystitis by finger tip trauma to the gallbladder plus local infection.

Copher and Illingworth (1928) observed that many workers were unable to produce cholelithiasis in experimental animals, either by stasis alone or combined with infection of the bile even in the presence of varied foreign bodies in the viscus. Other factors seemed to be involved. A likely factor might be intramural infection present in cholelithiasis even when the bile was sterile.

Blackstein (1891) injected B. coli and B. typhosum intravenously in rabbits with doubtful results, debris and clumps of

bacteria were noted.

Welch (1891) could not find this debris after 4 months injection with B. typhosum.

Marcantonio (1892) infected dog's gallbladders with no results after 6 months. A foreign body in the gallbladder resulted in some pigment deposition.

Gilbert and Dominici (1893) produced cholecystitis in a rabbit by intravesical injection of B. typhosum and obtained a small concretion. Gilbert (Q.1938) found a stone in the gallbladder of a dog previously inoculated with B. coli communis.

Mignot (1898) was unable to produce stones in dogs and guinea pigs by introducing foreign bodies. However, injection of attenuated strains of B. coli resulted in 3 small biliary calculi in a guinea pig.

Richardson (1898) injected agglutinated cultures of B. typhosum into gallbladders of rabbits and produced a firm brown calculus. No analysis was made.

Miyake (1900) ligating the cystic duct in the presence of an infection with B. coli, in a dog, resulted in formation of 2 facetted stones after 9 months. Foreign bodies, plus cystic duct ligation and infection led to the formation of small stones of calcium, cholesterol and pigment in 2 dogs. One year after

infecting the gallbladder of a rabbit and narrowing the cystic duct by placing a piece of gauze under it, he obtained a calculus reported to be rich in cholesterol with pigment and calcium.

Italia (1901) reported production of concretions by intra vesical injection of attenuated cultures of B. typhosum and B. coli into the gallbladders of dogs and rabbits. They resembled human calculi, and chemically were shown to contain all the elements found in human stones.

Rosenow (1914) reported that dogs and rabbits surviving for a long time the intra-venous injection of streptococci isolated from human patients with cholecystitis not infrequently showed the beginning of formation of gallstones. He reported later that 6 rabbits and 3 dogs out of 80 animals showed formation of minute black gallstones with cholecystitis found constantly. No detailed description of stones was given.

Greig (1915) found that 18 rabbits dying after a long course of intravenous injections of vibrio cholera, 9 showed gallstones which were mainly of cholesterol composition.

Emmerich and Wagner (1916) injected 0.5 ccs. of a 24 hour culture of B. typhosum in sodium chloride solution from 2-3 weeks after immunizing them with 3 doses of serum (typhoid). The animals lived from 100-341 days after operation. Cholecystitis

resulted in almost every case and calculi measuring from pin-head size to 0.5 cms. in diameter were found in 5 of 16 rabbits. These concretions were considered to be of organic material only since they burned without residue, and showed only slight cholesterol reaction and gave no pigment reaction.

Sotti and Torri (1920) reported that in 1913, they found sterile concretions of definite morphology in the gallbladder of rabbits after splenectomy and ligation of the ductus choledochus. The chemical composition of the calculi was not known.

Meyer et al (1921) found calculi in the gallbladders of rabbits in all animals which survived over 100 days after I.V. and cystic duct injection of typhoid bacilli.

Badile (1923) produced cholecystitis but no calculi by narrowing the common bile duct in dogs after inoculating the gallbladder with B. coli and B. typhosum.

Agrifoglio (1924) loosely ligated the common bile duct and injected colon and typhoid bacilli I.V. or into the gallbladder. Concretions formed only in the cases with attenuated bacilli placed in the gallbladder.

A. L. Wilkie (1928) injected streptococci from human cases into the lumen of the gallbladder intravenously and into the wall

of the gallbladder. Negative results were obtained in the intra vesical method. The intra-mural method, after 3 months, resulted in cholecystitis with pinhead sized calculi composed of large amounts of cholesterol, but no calcium. The intravenous method was fruitless until by repeated injections chronic cholecystitis was produced occasionally accompanied by cholesterol calculi formation. Injecting the streptococci intra-murally with the cystic duct ligated produced stones in some cases. These stones contained a large amount of calcium as well as cholesterol. Repeated I. V. injections with the cystic duct ligated resulted in stone formation, mainly composed of calcium but with little cholesterol. Cystic gland culture yielded streptococci in 2 cases and in 1 case cl. welchii. B. coli were cultured from 2 calculi.

Illingworth (1928) studying strawberry gallbladder reported production of a large semi-solid concretion in a rabbit's gall-bladder after feeding 0.2 gms. cholesterol daily for 13 weeks and making an intra-mural injection of streptococci, 1 week after the beginning of feeding. No analysis was reported.

Hospers (1932) in an excellent review of the literature, reported his work on hypercholesterolaemia and infection. Rabbits were given 10 ccs. of 1% emulsion of cholesterol twice weekly up to 9 months. Cholesterol blood levels were done and when they were raised, the abdomen was opened, 1 cc. of bile was aspirated

and 1 cc. of a 24 hour culture of typhoid suspension was injected into the gallbladder. After 2 weeks rest cholesterol treatment was resumed for 5-32 weeks after infection was produced. A few animals were killed, some died from peritonitis, the remainder died naturally. Deaths occurred from 1-8 months after the cessation of injections.

Results: of 7 rabbits on cholesterol injections, only 4 showed no bile changes; 1 showed fine cholesterol crystals in the bile; 2 showed calcium bilirubinate black crystals, from pinhead to 1 mm. in size. No cholesterol was present. The gallbladder wall was normal in 7 cases. 2 rabbits with intra-vesical injection of typhoid bacilli showed marked chronic cholecystitis, the wall was thick, opaque and white. 1 rabbit, living 5 months after infection, showed a white plug of cellular debris in the gall-bladder; no bile was present. 1 rabbit killed after 16 months had a wall measuring 1 mm. thick and the gallbladder lumen was almost obliterated. The remaining rabbits with cholesterol and infection showed varying results. 4 showed cholesterol crystals in bile, not seen in rabbits without infection.

Tonelli (1931) proposed that where malaria was endemic that pigment calculi were found in the frequently occurring cases of cholelithiasis.

Narita (1932) injected suspensions of staphylococci, streptococci, b. coli, pyocaneus, Kartoffel bacillus and b. subtilis, by ear vein, portal vein and hepatic artery. He noted that where bacteria had to pass through the liver before entering the general circulation, the venous blood bacterial count was less than when injected via an ear vein. The defense mechanism of the liver appeared to be more active, if the bacterial invasion was by way of the hepatic artery, than if by portal vein. When the reticuloendothelial system was blocked by India ink emulsion the ability of the animal to withstand infection of the blood stream was diminished.

Rousing (1923) opposed the views on an infective etiology because no organisms were found in calculi at the time of operations. He also held that if they were found, they were secondary invaders.

Aschoff (1909) from autopsy studies tried to reconcile conflicting views by claiming that there were 2 main modes of calculi formation, one caused by infection and the other by upset mechanism.

Williams and McLachlan (1930) repeated the work of Rosenow and Wilkie and were unable to obtain the same results.

Gordon, Taylor and Whitby (1930) from collected statistics, claimed an infective agent was responsible and quoted, the gallbladder wall was infected in 70% of cases, the fluid contents in

40% and the gallstones in 30%. They found anaerobes to be not infrequent invaders.

Andrews et al (1933b) stated that if the gallbladder was inflamed, it not only absorbed water as shown by Rous and McMaster, but also bile acids. They maintained a diminution of bile acid gave precipitation of cholesterol. This would explain the role of infection in gallstone formation.

Aronsohn and Andrews (1938) noted most strains of bacteria, even when injected in overshelming numbers did not cause cholecystitis, however when trauma was added, they did cause cholecystitis.

Hartmann (1903) claimed that an infection of bile was present in a considerable majority of cases (clinical).

Johnson (1925) found it to occur in only 32% of cases and Drennan (1922) found an infection in 19% of cases.

Huntemueller (1924) in his series found that staphylococcus headed the list of isolated organisms and were twice as common as B. coli.

Illingworth (1928) in a study of 100 post-operative cases stated that infection would travel to the liver by 3 routes:

(1) Systemic circulation or by portal vein and hence excreted in the bile.

(2) From liver by low grade lymphangitis and directly to the liver (Graham). From stomach, duodenum, and appendix the liver could be involved.

(3) In systemic blood from teeth, tonsils, (Rosenow) and so intramural infection and hence bile infection. In this series 7 varieties of streptococci were isolated. There were more organisms present in acute cases (10 out of 12) than in cases of chronic cholecystitis (41 out of 65).

Crile (1935) agreed with Aschoff, Bacmeister and Rovsing, and said that infection had been shown by experimental and clinical studies, to be the consequence, and not the cause of gallstones.

McCarty (1936) studied the statistics of 21,523 cases of gallbladder disease and was unable to reach any definite conclusion as to the primary etiological factor.

Ravdin and Johnston (1932) gave the opinion that either stone or infection could primarily occur. Walters (1936) agreed with them.

Phemister and Aronsohn et al (1939) insisted that the deposition of calcium is evidence of inflammatory changes in the wall of the gallbladder. They stated that when a gallstone consists of a

central nucleus of cholesterol with several rings of calcium and pigment this structure is evidence of the occurrence of successive attacks of obstruction and active cholecystitis in the gallbladder which initially contains a stone of pure cholesterol of metabolic origin.

Mayo (1921) noted that chronic pancreatitis was very common in infective cholecystitis and he advised surgery in these cases.

Eustis (1923) expressed belief that infection of the gallbladder and ductus extended to the pancreas and caused inflammatory changes which could lead to diabetes.

Ottoman and Baker (1945) reported 2 cases of empyema of the gallbladder occurring as a complication of simple superficial skin staphylococcal infection. The organism was isolated from the gallbladder in each case. 1 case had calculi.

Clavel and Dumas (1946) noted in 102 cases of gallbladder disease in children that systemic and upper respiratory infections were importantly related. They quoted typhoid fever, scarlatina, grippe, pneumonia, gastro-intestinal illness, intestinal parasitis and specifically appendicitis. They found cholecystitis with calculi associated in some cases.

Bustos (1949) in over 40 cases found the following parasites

as primary and secondary invaders of the gallbladder: lambilia, fasciola hepatica, ascaris, entamoeba histolytica, hydatid, taenia solium, ankylostoma duodenale, necator americanus. There was much speculation as to the exact mode and route of invasion.

Rehfuss and Nelson (1945) intravenous injection of a viable strain of non-haemolytic streptococcus from a patient with cholecystitis was carried out in experimental animals. Three series were done using vaccine and filtrate therapy in 2 series. Results: cholecystitis with positive culture recovery was obtained. The evidence of gallbladder damage was markedly constant in all 3 groups, attesting to the fact that the viable organism was responsible for the changes.

Lester (1947) found in 109 cases of clinical cholecystitis, positive cultures twice as high when the gallbladder was gangrenous at operation as compared to the acute cholecystitis series. There was frequent correlation between severity of the inflammatory process in the gallbladder and the febril and leucocytic response.

Meleney (1948) in 244 cases of biliary tract disease found positive cultures in 58% of cases of acute cholecystitis and 33% recovery in the chronic cases. B. coli eas found in 50% of the cases, non-haemolytic streptococci in approximately 50% of cultures. Gram negative rods were common and bl. welchii were also isolated. 60% were pure cultures, the remainder were a mixed flora.

Anderson and Priestley (1951) cultured choledochal bile in 100 cases. Whether stone was present or not, b. coli was most commonly found alone or associated with aerobacter aerogenes, streptococcus faecalis and protens, frequently occurred. Previous operations on the biliary tract especially if on the common bile duct seemed to predispose to bacterial infection. Infection in choledochal bile was found in 87.8% of cases where chills and fever occurred in the presence of diseased biliary tract.

Schwegman - DeMuth (Q) reported 17 cases of post-operative cholecystitis (for other diseases) with 8 perforations of the gall-bladder occurring in the group. There was a high incidence of acute cholecystitis in the absence of stone. Sparkman also reported similar cases.

Glenn and Wantz (1956) reported 18 personal cases of acute cholecystitis following surgical treatment for unrelated disease. The total number to date in the literature was 62. The patients disease had lasted from 1-17 days. Acute cholecystitis occurred from 2-18 days after feeding had begun. 10% of the cases had long standing biliary tract disease. 9 had calculi at operation (50%). 22.2% of the total had no calculi at operation. 2/3 of the cases had a gallbladder history or evidence of gallbladder disease. The contents of the gallbladders were varied, empyema in one, green bile in some and milky in others. Cultures in 16 cases showed

9 positive for B. coli, B. pyocaneus, Aerobacter aerogenes, Streptococcus haemolyticus and Stophylococcus. There were more males than females. The males were the older group.

Jemerin (1949) stated that cholecystitis emphysematosa was an inflammation of the gallbladder by gas producing organisms. He quoted Stalz (1901) as reporting the first case. 14 cases were found in the literature some associated with lithiasis. Cl. oedematiens in pure culture was isolated from the pus in the gallbladder in the case reported.

Rehfuss (1945) injected bacteria I.V. into animals and obtained 68% infection in all or certain layers of the gallbladder. He had made a 10 years study and concluded that there was no particular indication that any specific bacterium be incriminated.

Brown and Milch (1948) reported 39 patients who had died following surgery. 6 of these cases were ascribed to cl. welchii infection.

Thomas and Womach (1952) in a series of experiments on dogs involving ligation of the various elements of the cystic pedicle concluded that secondary bacterial infection was occasionally present and superimposed on chemically damaged tissue.

Bonta and Longwood (1952) reported a case of acute cholecystitis in a 4 year old girl. Salmonella Orianenberg was isolated from the gallbladder pus and the stool. They concluded

that infection of the gallbladder in children is usually preceded by an acute infective process elsewhere, most commonly respiratory tract.

Elfving (1949) stated only 25 cases of T.B. of the gallbladder were found in the literature to that date. 23 of these cases were in females. This case revealed an enlarged inflamed gallbladder filled with small stones. The wall was 5 mm. thick. The diagnosis of tuberculosis of the viscus was made by histological examination. Culture was negative.

Weitz (1955) quoted 34 cases of tuberculosis of the gallbladder in his country to that date. They had all been confirmed and 27 cases had cholelithiasis. He reported 2 cases and believed that infection was by the blood stream or from other acute T. B. foci in the body.

Vallejo (1950) reported the case of a 27 year old male with T.B. (pulmonary and laryngeal). At operation he had a T. B. peritonitis and Koch's bacillus was isolated from the bile. There had been no previous gallbladder trouble.

Twiss et al (1951) reviewed 259 operative cases with post-operative drainage studies. Cultures at operation were positive in 28% of cases and sterile in all parts of the biliary tract in 72% of the cases. They believed infection was superimposed and

was more common where achlorhydria was present. They concluded that pathological changes in the gallbladder may be due to mechanical or chemical irritations, such as, excessive bile concentrations, or the presence of activated pancreatic ferments. Allergic reactions could also result in similar changes causing an inflammatory response.

Kilman and Herman (1954) reported the isolation of an agent causing Bilirubinaemia and jaundice in raccoons. Ferrets were also susceptible to the infection. There was no haemolytic anaemia, and no apparent liver dysfunction.

Potter 1938 investigating cholecystitis in childhood found: (a) 270 cases up to 1927 and (b) 162 cases in the following 10 years.

| Ages | (a) | (b) |
|---|---|---|
| Foetal | 2 | 0 |
| Neonatal | 20 | 4 |
| Infancy | 37 | 28 |
| 1 - 5 yrs. | 26 | 30 |
| 5 - 10 yrs. | 59 | 50 |
| 10 - 15 yrs. | 84 | 52 |
| Cholelithiasis | 141 | 65 |

The ratio of female to males was 2:1.

Estrada (1952) stated the liver is a storehouse for bacteria and the same organisms grown from liver biopsies were found in "T" tube bile. Amylase producing bacteria were found such as, B. subtilis, Cl. perfringens, Cl. butyricum, Cl. chauvei and non amylase forming, B. coli, haemolytic Streptococcus, streptococcus viridans, Cl. aerofoetidum. It would seem that bacteria grow more

readily and are more easily idientified after operation than before. It was also noted that gelatine was liquefied by P. vulgaris, B. coli, Cl. chauvei and was not due to trypsin from the pancreas. In a series of dogs he found that division of the sphincter of Oddi did not cause lesions in the liver or pancreas.

Marshall et al (1935) noted that acute cholecystitis is much more common in the over 50 years old group. In 74 cases, 5 had no calculi and 12 had 1 calculus only. Cultures were positive in 8% of the quiescent cases and 6% positive in the active cases. They concluded infection was not important and that the disease was not due to vascular upset with lymphoedema plus stone.

(Marshall, 1948). It has been noted that gangrenous cholecystitis is quite common after the 5th decade of life. Calculi are present in 93% of cases with impaction and obstruction. Secondary infection is found, and a history of previous attacks can be elicited. He concluded that the lesion was usually vascular due to impacted stone.

Bartlett (1956) reviewed 224 cases of gallbladder disease. (27.2% had acute cholecystitis; 7 without stone). 20% had common bile duct stone. 2 cases of acute gangrenous gallbladder in males had occurred following operations for spinal fusion and disc removal.

Mentzner (1934) did autopsies on normal slaughtered animals. He found in 964 pigs, 100 calves, 400 sheep, 37 cows varied biliary tract disease. 9 animals only had calculi. 1 had adenocarcinoma of the gallbladder. Cholesterolosis was very common in pigs and sheep. Acute cholecystitis was present as follows: 0.31% pigs; 3.2% sheep and 1.2% cattle. It appears that biliary tract disease is more common in "healthy" animals than generally reported in the veterinary literature.

Chau (1950) in a McGill thesis on deprivation of arterial supply to the liver in dogs showed that Cl. welchii are normal liver inhabitants and that ligation of the artery resulted in an overwhelming infection locally which very rapidly becomes systemic.

Comment: It would seem that primary and secondary bacterial infection with disease can occur. Bacteria whether saprophytic, symbiotic or pathogenic can be present without being responsible for the disease process found. The bile isusually sterile but is not bacteriostatic. Many of the intestinal organisms can grow in bile as would be expected. Certain anerobes use bile in their metabolism, the end products might be of some importance in the pathogenesis of biliary tract disease.

OTHER ETIOLOGICAL FACTORS:

There is a fairly large group of other causes which seem to have a definite bearing on the production of biliary tract disease. Experimental and clinical studies have substantiated the findings reported.

Notwithstanding the fact that many of these factors might have only a slight influence and may not ever be prime agents, it is entirely probable that on occasion they may occur combined with the presence of other main causative agents and accelerate the disease process, acting as it were, as a catalyst does in a chemical reaction. More and more evidence is being accumulated showing that acute or chronic pancreatitis is more frequently present in biliary tract diesase than is generally suspected. Conversely the exact relationship of secondary biliary tract pathology developing after pancreatic disease occurs, has not yet been determined satisfactorily.

Fisher et al (1953) repeated the work of many others and injected bile into the accessory pancreatic duct in dogs. He concluded from their preliminary results, that there is substantiation to reports in the literature that bile in the pancreas without increased pressure and acinar rupture is entirely innocuous, and that pancreatic juice flowing retrograde into the

common duct may produce hepatic damage similar to that reported in acute pancreatitis.

Dragstedt (1934), Lewison (1940), Jones (1943) and Walters (1941) and others support the theory that the presence of bile in the pancreas is an etiological factor.

Mann (1923), Rich (1936) and Lium (1948) are in opposition to the common channel theory and bile as an important factor in the pathogenesis of pancreatitis.

Bernard first produced acute haemorrhagic pancreatitis by injecting bile in 1856.

Reid (1949) studying the effect of pancreatic juice on the gallbladder in dogs, concluded that pancreatic juice as a single factor has no effect on the gallbladder. However, pancreatic juice plus stasis (tied cystic duct) causes a pathologic condition in some instances. This work corroborated that of Hjworth in rabbits, who found that activated pancreatic juice (trypsin), caused chronic cholecystitis with 10 cases of cholelithiasis where the cystic duct was simultaneously occluded at the time of injection of trypsin into the gallbladder. He observed that the changes are in all stages as found in the human viscus with chronic cholecystitis, and that stones do not spontaneously occur in the rabbit, the reason for which might be that the common bile duct and the pancreatic duct have

separate openings in this animal. The fact that in the human female the duct of Santorini is quoted as opening separately in 14% of cases while in the male it opens in 44% of cases was advanced as a possible explanation for the increased incidence of biliary tract disease in the female (approximately 3:1).

Grey et al (1949) reported their results on division of the sphincter of Oddi in cats, concluded that regurgitation does occur and that chronic inflammatory changes in the common duct and gallbladder were most marked where greatest reflux had occurred. It was not apparent whether inflammatory changes were due to activated enzymes or to bacterial action or both. The gallbladder was analysed for diastase. They stated that some cases of chronic cholecystitis may be due to incompetent sphincter mechanism.

Lancereaux (1899) was the first to comment on and infer that pancreatitis was related to diabetes and an infected gallbladder. Many other as already quoted had noted similar relationships subsequently.

Murphy (1913) showed that drainage of an infected gallbladder led to permanent disappearance of glycosuria when the gallbladder was drained.

Rabinovitch (1923) made a study of blood sugars in biliary tract didease and found that 88% had hyperglycaemia when

cholelithiasis was present.

Joslin (1936) reported a 5.4% incidence of gallbladder calculi in 5,400 diabetics.

Wilder (1940) reported a 5.4% incidence of cholelithiasis in 2584 diabetic patients.

John (1951) found an incidence of 5.08% in 6000 diabetics. The incidence of females to males was 4:1. He claimed that an upset of carbohydrate metabolism was responsible for cholecystitis. Observations have been made in portal cirrhosis that cholelithiasis is present more frequently in both sexes and the ratio of females to males is still maintained at approximately 2:1. One series was reported calculi in 33% of females and 16% in male cases.

HYDROGEN ION CONCENTRATION:

Changes in the pH of the bile of the liver and the gallbladder have been frequently blamed for initiating biliary tract disease.

Lichwitz (1907) asserted that acid bile favoured the precipitation of cholesterol and bilirubin, but Andrews concluded that their work did not show that alteration of pH had any specific relationship to gallbladder disease.

Bronner (1934), however, claimed that the reaction of the liver bile is a true index of the current diet and had an im-

portant bearing on diseaseproduction.

Feldman et al (1954) asserted that change in pH was one of the most important factors in the formation of gallstones. The pH of bile in the presence of gallbladder calculi is usually alkaline in both humans and animals. It seems probably that the liver function will be all important in deciding the hydrogen ion concentration of the secreted bile even although the concentrating power of the gallbladder can reduce the degree of alkalinity of liver bile.

Rehfuss (1945) in his remarks on the etiology of cholecystitis, mentioned among other things that bacteria or their toxins could initiate a stage of sensitization. Subsequent recurrent attacks would then result in an allergic response locally. Many studies on bacterial antigens were made over a 10 year period. The results were not specific and he concluded that protoplasmic poisoning, necrosis of the cells, a profound disturbance of cellular chemistry or enzymatic activity could occur. Bacterial sensitization could playa part in any of these processes.

Stockinger (1948) in studies on allergic cholecystopathy and secondary cholecystitis following dysentery, made these observations. There was little in the literature to suggest any connection between bacillary dysentery and gallbladder disease. In 426 cases, there was a positive history of 125

cases of dysentery, that is 29.3%. He believed in an indirect invasion of the gallbladder. Allergic manifestations had been observed in bacillary dysentery, particularly in the mucus membrane which shows the effects of an allergic reaction. Such a gallbladder would be more susceptible to secondary bacterial infection. Once such an infection occurred, the process was the same as in the ordinary case of gallbladder disease of bacterial nature.

Graham (1928) in "Diseases of the Gallbladder," proposed that intestinal allergy be seriously considered as a cause of chronic cholecystitis.

Singer (Q) stated that an allergy to egg yolk could cause biliary colic.

Aronshohn and Andrews (1938) showed experimentally that injection of egg white set up an active acute inflammation whilst animals which had been previously sensitized demonstrated an allergic condition shown by oedema of the gallbladder walls, when intravenous injection of egg white was carried out. No reactions occurred in control animals. They concluded that changes in the pH of bile rarely brings about changes in reaction in the gallbladder wall unless they are extreme; Less than 3 or greater than 10. Such changes are not likely to arise in man. The toxic effect of bile salts is not due to a change

in 'H'-ion concentration since adjustment of the bile salts to the pH of bile before introduction did not diminish their toxic effect. A marked oedema of the gallbladder wall was produced by the intravenous injection of bile salts. The shortest time in which this reaction took place was 3 minutes after injection. They made the following pertinent remarks on the toxicity of bile acids and secretion. An increase in their amount could occur due to (1) simple oversecretion; (2) excretion of bile by the liver which was too concentrated in the first place; or (3) excretion of bile which contained substances which might become toxic on further concentration in the gallbladder could also occur. Number one was thought to be unlikely unless stasis and absorption of salts were shown to take place in which case cholesterol might be precipitated. Increased liver secretion was improbable, because toxic levels would have been noted earlier and reported. It is not easy to dispose of the qualitative difference in the bile salts excreted by the liver. The unconjugated bile acids occur in large amounts in the liver bile of diabetics and it is not unlikely that this commonly occurs. Bile salt chemistry is still limited and we are unable to identify even in large amounts of bile certain bile acids. Thus a chemical mechanism seems likely in view of the haemorrhagic pathological picture described by Denton. Afebrile cases of disease of the gall-bladder without demonstrable infection could be due to a chemical

irritation. The low values for bile acids from cases of closed actue gallbladders is explained on the assumption that the original high concentration causes an oedema which permits rapid leaking out of the contents by osmosis, as can be seen by staining of the local peritoneum experimentally or at surgery. It is suggested that a temporary increase in bile salt concentration in the gallbladder brings about human cholecystitis.

Liaw (1955) reported an incidence of calculi in 62% of rabbits by injecting a mixture of a sodium salt of a free bile acid and a fatty acid into the gallbladder. Chronic damage of the biliary system was caused.

It has been conclusively demonstrated by many modern workers that certain concentrations of bile salts or related metabolites do cause biliary tract pathology.

Boyden and Layne (1945) studied 105 cases of Pernicious Anaemia from 31,311 autopsies. The incidence of gallstones singly or with cholecystitis was 32%. They noted clinically that delay in emptying and non-visualization was fairly frequent in Pernicious Anaemia. They concluded that this condition may cause narrowing of the choledochoduodenal junction.

Pemberton (1931) in a report on 188 cases of splenic anaemia, haemolytic jaundice and haemorrhagic purpura, found disease of

the gallbladder with and without calculi in 68.6% of cases. The biliary tract disease occurred as a secondary complication. Splenectomy was done for the original disease.

Weens (1945) reporting on cholelithiasis in a sickle-cell anaemia, found in a review of the literature that 12 cases in 44 autopsies had stone present. He recommended that in the Negro a careful search be made for sickle cells if cholelithiasis be present.

Bates and Brown (1952) commenting on the incidence of gall-bladder disease in chronic haemolytic anaemia, found in 152 unselected cases that 43% had gallbladder disease. Biliary abnormalities were present in patients over the age of 40 in 51%. The incidence of cholelithiasis increased as age advanced to 40 and was higher than the quoted figures in the general population. They observed that early splenectomy might prevent gallbladder disease. All young people presenting with symptoms of gallbladder trouble should be considered as suffering from haemolytic anaemia until proved otherwise.

Smyth (1949) reported a case of congenital absence of the gallbladder in a 72 year old female. The common bile duct was distended with a large calculus present. The interesting aspect of the case was why the symptoms did not occur earlier and when had the calculus formed. No analysis of the stone was made.

Mann (1918) was one of the original workers on a chemical

theory as an explanation of acute cholecystitis. He injected freshly prepared Dakin's solution intravenously in animals using 8-10 ccs. per Kg. An acute type of cholecystitis as well as other organ changes was produced.

Nakashima and Saheki (1929,1931) found that gallstones from cattle decreased in size when placed in dog's gallbladders provided a diet rich in Vitamin A was fed. The same authors found that this held true also for human stones in dog's gallbladders. This work was not confirmed by other workers. The influence of associated diseases on biliary tract pathology has been noted. At this point the question of constitutional susceptibility must be considered.

Chauffard (1922) quoted Dienlafoy as stating that the diathetic state dominates the pathogenesis of biliary lithiasis. Many other authors have made statements, such as, "cholesterol diathesis," familial tendency," "predisposition," "pregnancy-cholesterol diathesis," "racial tendency," and so on. At first sight many series of observations seem to substantiate these possibilities. Strict and unbiased analysis of the cases presented in support do not fully take into account the factors of coincidence and change occurrence. At the same time, it is not unlikely that biliary tract disease will occur in certain individuals more commonly than in others.

This is simply because as in any other disease they are born with more factors present in their homeostatic mechanism which would tend to make them susceptible to disease of the biliary tract. That the pace of life, environment, occupation, sex, diet, (as external factors) could exert a profound influence cannot be gainsayed. (High rate of modern "stress" diseases). It is claimed that we are living longer, developing more gall-bladder disease and that 50% of cases of gallbladder disease are not diagnosed. Presumably, mainly because of lack of symptoms. That the statement has a factual basis is clearly demonstrated by the results of the increasing number of routine autopsies being performed today. Crump (1931) calimed that in 1,000 autopsies, 77.9% of bodies over 40 years of age and 87.1% of bodies over 30 years of age showed evidence of biliary tract pathology.

Since gallbladder disease can occur without symptoms, as can calculi without causing colic or trauma, it is probable that a larger number of people pass small calculi or seedlings 'normally' via the biliary ducts to the intestine. To prove this would be of little value, but it must be considered when gall-stone formation is being discussed. Minute biliary concretions or bile thrombi might well be "normal" and equally well could initiate cholelithiasis under suitable conditions. Routine

microscopic examination of bile after surgery and autopsies might contribute interesting data which might help unravel the mystery of gallstone formation.

CLINICAL OBSERVATIONS:

From the current literature it is apparent that cholecystectomy is the second most frequently performed abdominal operation. Colcock et al (1955) and Strohl et al (1953) reported that biliary tract disease is the most common condition requiring abdominal surgery in the patient over 70 years old.

Several significant clinical factors which cannot be evaluated experimentally have been noted. In a series of 1,356 cases of cholecystitis and cholelithiasis, 39% had been or were overweight, 21% gave a history of weight. Of those with common bile duct stone, 50% had weight loss. Fever was present in only 12.1% cases, and of this group there was a higher percentage with fever than in those with common bile duct stone. The fever incidence was 35.9% to 32.1% in patients with acute or subacute cholecystitis. 16 patients of this series had acute or chronic cholecystitis. 11 patients had associated cholangitis or hepatitis. In 65 cases of cholesterolosis calculi were present in 59 patients. Calculi have long been known to be associated with carcinoma of the biliary tract. The figures quoted by various authorities vary from 80-100%. (Fig. 4). It seems probably that certain people will have a greater tendency to develop malignant tumors under these conditions. In view of the

modern discovery that bile contains the material for the synthesis of the potent carcinogen methyl-colanthrene, it would appear that herein lies the major factor in the production of carcinoma of the biliary tract.

Edlund and Olsson (1956) in a 15 year study and review of 8,368 cholecystectomies, concluded that there was a 30% increase in the number of cases requiring operation in the last 5 years period. The sex incidence ratio was females to males 2.5:1. They attributed the increase in the post war period to 'better' nutrition, that is, more fats and food was now available. The ratio of chronic cholecystitis with stone to acute cholecystitis without stone was 6:1.

Bartlett (1956) reviewed the results of 224 consecutive operative cases. 7 cases only had no calculi present. The total number of cases of acute cholecystitis was 27.2%. 20% of cases had common bile duct stone. 2 cases of acute gangrenous gallbladder occurred after operative intervention for unrelated cases.

Radakovich et al (1951) published an interesting paper demonstrating that in dogs successful ligation and division of the pancreatic duct inhibits the visualization of the gallbladder by cholecystographic methods whether by oral or intravenous route. This failure of visualization appears to be due to the development

of physiological stasis of the viscus. The experiments demonstrate a close relationship of the two organs and suggest that the pancreas may control the tone of the gallbladder.

Pibram (1947) reported a method of the dissolution of common bile duct stones using ether by drop method via an indwelling catheter in the common bile duct. Disappearance of the calculi as shown by cholangiography was demonstrated in 51 cases. Pibram, commenting on the post cholecystectomy syndrome, stated that it could be due to 3 main causes:

1) Pressure alteration syndromes due to the missing tension bulb function of the gallbladder and then the secondary complications of cholangiohepatis and pancreatitis.

2) The lowered tolerance for food especially fatty food due to the missing digestive aid of the concentrated cystic bile and the hormone secreted from the gallbladder wall.

3) Paralysis of the sphincter leading to diarrhoea and enteritis.

Goldman et al (1945) did not find instillation of ether effective, but stated that solution "G" - Citric acid (mono.) 32.25; Mag. oxid. (anhyd) 3.84; Sod. carb. 4.37 and Aqua Q.S. 1,000 ccs. theoretically, since calcium bilirubinate stones were common should be effective.

Albright (1939) et al first used this solution successfully. Prevet's "Gomenol" reduces cholesterol and cholesterol pigment

calculi in vitro. This consists of: Oil of cajeput 20%, oil of olives 80%, usually the Melaleuca viridiflora oil (species of cajeput) is used. They concluded that the action of solution "G" might be due to irritation of the common bile duct and sphincter because it had a pH of 4. The solution resembles Liquor Mag. cit. (U.S.P.) which is a cathartic, this also could be the reason for its efficacy.

McCall et al (1945) reporting their work on the clinical significance of serum amylase and lipase, concluded that the serum enzyme activity in cases of calculous common duct obstruction appears to be increased only in those cases in which the pancreas has been damaged.

Zaslow et al (1950) in their work on the excretion and concentration of aureomycin in the abnormal human biliary tract observed that in 12 cases where the cystic duct was obstructed no antibiotic was found the gallbladder. They concluded that the route was via the bile after liver secretion. Inflammation did not alter the mode of entrance into the gallbladder lumen when the cystic duct was obstructed. They made the observation that the intra luminal pressure of the gallbladder can progressively increase until a point is reached where the blood supply instead of increasing decreases because of the pressure necrosis of the wall, so that perforation or gangrene can occur.

The process cannot reverse itself unless the obstructing agent is passed or cystostomy is performed. Aureomycin would be of no value in these cases, but has a marked value in cases of non calculous hepatitis or duct infection.

Pulaski et al (1955) working on the gallbladder bile concentrations of the major antibiotics following intravenous administration decided that oxytetracycline and chlortetracycline were the drugs of choice. The average concentration in the bile was 4 times that of the blood level. In some cases the levels were as high as 8 times that in the blood. It is quite probable that the widespread use of these antibiotics for other diseases may have a beneficial effect in latent biliary tract disease to an extent that will never be known. There is, however, a definite possibility that as an end result of antibiotic broad-spectrum treatment symbiotic bacteria in the bowel, concerned with cholesterol and fat metabolism may be killed off. Whether or not this would result in faulty cholesterol and fat absorption remains to be investigated.

## MATERIALS AND METHODS

A wide range pilot group of experiments was studied. The results from these determined the species, age, time intervals for sacrifice and methods used in the definitive work. The experiments were divided into 3 main series.

Series No. I.

Group A: 50 golden hamsters of both sexes, approximately 30 days old, were placed on the lithogenic diet of Dam and Christensen, 1952. Their diet was modified by the exclusion of vitamins A, D, E, k and "Antabuse" (Tetra-ethyl-thiuram-disulfide). The diet and water was given ad libitum. Animals were kept on the diet for 30 days. 10 animals were kept on the diet for 60 days, the remainder were sacrificed in batches of 4 after 7 days on the diet at intervals of 6 days thereafter. A small group was kept for periods up to 114 days. (See Table V).

Diet

| | |
|---|---|
| Casein (low vitamin content - cholesterol free, by analysis) | 20.0 gms. |
| Sucrose | 74.3 gms. |
| Salt mixture U.S.P. 2 | 5.0 gms. |
| Choline chloride | 0.2 gms. |
| Vitamin mixture | 0.5 gms. |

Vitamin mixture: Biotin 0.050 mgms., folic acid 0.050 mgms., ascorbic acid 5.000 mgms., thiamine hcl. 5.000 mgms., riboflavin 5.000 mgms., pyrodoxine hcl. 5.000 mgms., calcium pantothanate

5.000 mgms., nicotinic acid 8.000 mgms., inositol 15.000 mgms., P/aminobenzoic acid 35.000 mgms., vitamin K 3.000 mgms., sugar to 5000.0 mgms.

Group B. 10 hamsters of each sex on "L" diet received intraperitoneal injections 0.150 mgms. of oestradiol benzoate twice weekly for 30 days and then sacrificed.

Group C. 10 hamsters of each sex on "L" diet were given intraperitoneal of progesterone (long acting) 2 mgms. once weekly. Sacrificed at 30 days.

Group D. 10 hamsters of each sex on "L" diet received twice weekly injections of cortisone acetate intraperitoneally dose 1 mgm. All sacrificed at 30 days.

Group E. 10 hamsters of each sex given 2 mgms. of chlortetracycline admixed with the diet daily. Sacrificed at 30 days.

Group F. 10 hamsters of each sex on the "L" diet for 30 days and then placed on a mixture of "L" diet plus dried baker's yeast, (1 gm. per 20 gms. of diet) given ad libitum.

Group G. 20 New Zealand white rabbits of both sexes weighing approximately 2 kilos were placed on the lithogenic diet for 30 days, then sacrificed, as per Table VI.

A series of blood and bile cholesterol estimations were done

on these animals using modifications of (1) Burchard-Liebermann and (2) Schonheimer Sperry Methods. Random samples of bile were sent for bacteriological study. pH studies were done on all bile specimens using short range Alkacid test papers. This method was checked by Beckmann photoelectricmeter controls and found to be accurate within 0.2 - 0.4 range. The minute amount of bile in hamsters seriously limited the examinations that could be carried out. Hamsters were sacrificed using ether, rabbits received intravenous Nembutal.

Series II.

Group H. 20 New Zealand rabbits, weighing approximately 3 kilos of both sexes were fed ½ gm. of 3 beta-cholestanol daily for periods up to 21 days. Water was given ad libitum. The diet of Mosbach and Bevans 1956 was prepared by dissolving ½ gm. of 3 beta-cholestanol in absolute ethyl ether and pouring it over 40 gms. of stock Purina rabbit pellets. The ether evaporated and left 3 beta-cholestanol coated pellets. Each rabbit was fed 40 gms. of this diet daily and when totally consumed, 60 gms. of ordinary pellets added.

In this Group 3 animals were sacrificed at periods of 4 days subsequent to the 5th day of diet administration. After 21 days of the diet the remaining animals were placed on 100 gms.

of stock Purina pellets daily. 2 animals were sacrificed at 10 days and 3 after 20 days.

Group I. 20 guinea pigs of both sexes, weighing approximately 600 gms. were fed ¼ gm. of 3 beta-cholestanol daily, administered in rabbit pellets. (Method as in Group H - 15 gms. of pellets per dose). The animals were sacrificed at the same time intervals as in Group H and the subsequent handling was identical.

Group J. 20 rats of both sexes, weighing approximately 250 gms., R. V. H. strain were fed 1/8 gm. of 3 beta-cholestanol daily. (Method as in Group I - ½ dose given). The same procedure was followed thereafter, as in Group H.

Using the methods in Series I 10 samples of blood and bile were taken for analysis from rabbits and guinea pigs. Bile samples and calculi were sent for culture. Histopathological specimens were taken from all animals. Blood was taken from 10 rats for cholesterol estimations. No bile cholesterol estimations could be carried out. The bile pH of all animals was done.

Group K. 14 rabbits of each sex weighing approximately 4 kilos were cholecystectomized. The technique was as follows:

Intravenous Nembutal (1 gr. per 5 lbs. of body weight) diluted 5-fold was carefully injected into the central ear vein.

The abdomen was clipped using an electric clipper, then prepared with tincture of metaphen. A central upper abdominal incision was made and the peritoneal cavity entered. Bleeding vessels where encountered were ligated with fine cotton. The gallbladder was delivered into the wound by applying a haemostat to the fundus and using a little traction. Two ligatures were applied low down on the cystic duct after dissecting the gallbladder out of the liver. The gallbladder was removed, and the abdomen closed in two layers using continuous sutures of heavy cotton. The wound was painted with tincture and no dressings applied. All animals survived the operation and were placed on the 3 beta-cholestanol diet, one week after surgery.

The procedure followed was exactly as in Group H, except that all 14 rabbits were kept for 21 days on the diet and then sacrificed. Blood was taken for cholesterol estimations, and sections of common bile duct were obtained for histopathological study.

Because of the small size of the components of the biliary tract, a binocular loupe or dissecting microscope were used to obtain the specimens. In the smaller animals the gallbladder was dissected free, intact, using sterile technique where cultures were being taken. The gallbladder was placed upon a glass slide

illuminated from below. This rendered detailed examination of the contents of the viscus possible, in all cases. The calculi or concrements were carefully collected and after photography, weighed in groups and analyses subsequently using the methods in Hawk's Practical Physiological Chemistry. Although all carcases were carefully autopsied only biliary tract specimens were taken for pathology. All blood specimens were taken from the inferior vena cava. Brewer's meat broth was used where cultures were desired. The maximum amount of bile obtained from hamsters was in the region of .02 ccs.

The routine analysis of representative calculi in the laboratory entailed examination for cholesterol, bile salts, calcium, bilirubin, biliverdin, iron, phosphates. Quantitative examination could only be carried out for cholesterol and calcium.

Series III.

Group L. A large number of gallstones were collected from the major hospitals in the Montreal area. To this group were added calculi removed in British Guiana (S. America) and Europe. These calculi were grossly examined and innumerable microscopic and chemical analysis done. Finally, 19 calculi were chosen as being representative of most of the classes of gallstones described in the pathology textbooks.

The selected material was sent to the geology laboratory for petrographic slide section. The method used, as follows: The specimen was carefully bisected, to preserve the nuclear area, imbedded in No. 30 thermoplastic cement and then ground down to a very thin section. These sections were then examined by ordinary and polarizing microscopes, to identify the crystalline and amorphous elements and their physical inter-relationship. Microphotographs were taken by the usual technique. The remaining half-calculus was subjected to the same chemical analyses as the experimentally produced calculi.

Group M. Some human "sister" calculi (from the same gall-bladder) were placed in ether for 48 hours and the results noted and photographed. Chemical examinations of the debris were carried out.

Series I, cont.

Group AA: 20 hamsters of each sex were given the cholesterol free, low fat diet plus all vitamins, (i.e. vitamins A, D, E, K, were administered in the diet twice weekly) for 30 days and then sacrificed.

Group BB. 20 hamsters of each sex were given the same diet as above with the exception of vitamins E and K, for 30 days and then sacrificed.

## RESULTS

### Series I.

Group A. In the group of 50 hamsters, 12 died and were discarded. 29 out of 38 developed cholelithiasis, 17 females and 12 males. The earliest case occurred at 12 days, the group average was 28 days. No difference was noted in the type or incidence of calculi in the animals kept on the diet for maximum periods. (Table V). The young calculi were composed of almost pure cholesterol in some cases and in others pigment with small amounts of calcium and iron was found. (Figures 1 and 2). The animals gained little weight; however, their condition generally was good, and fur, mucosae and epidermis did not show the deficiency changes found by Dam, 1952 in their experiments. Two animals developed a keratitis however, and diarrhoea without deaths was noted in some.

The gallbladders in the majority of cases were filled, but not distended, with golden yellow bile. One hamster was infected with Eimeria Stiedae (Figure 3). This is rather a rare occurrence apparently. The histopathological sections revealed no significant pathology.

No calculi were obtained over 0.75 mm. in diameter, the smaller concrements would be capable of passing through the

cystic duct. The calculi were crystalline in structure, composed of irregular small snowball-like clusters of black and white material. In several cases the white material was blood red in parts. (?Blood). (Figure 4). These young calculi in many cases appeared to have enlarged by the coalescing of several smaller calculi. Greenish black smooth rounded calculi not unlike small versions of human were observed. (Figure 5).

Group B. The 20 hamsters receiving L diet and oestradrol benzoate developed mixed calculi in 5 cases, 2 male and 3 female animals. (Figures 6 and 7). The calculi were essentially the same as in Group A. The females thrived on this regime more than the males and gained more weight. The effect on bile and blood cholesterol levels is shown in Table VI. No significant pathology was seen in the sections.

Group C. The hamsters receiving progesterone plus L diet developed small mixed calculi in 4 cases, 2 in females and 2 in males. Both sexes did well and gained weight slowly. The calculi were essentially the same as in Group A. (Figure 8). The chemical analyses are shown in Table VI. No significant pathology was noted in the sections.

Group D. The animals receiving cortisone with L diet developed almost pure cholesterol stones in 5 cases, 3 in males and 2 in females. (Figure 9). The general condition of these

animals was good and they gained approximately 10 gms. more in weight than in Group C. (Salt and water retention?). The results of chemical analyses are shown in Table V. No significant pathology was observed in the sections.

Group E. 20 hamsters receiving L diet and chlortetracycline daily, developed mixed dark green rounded calculi in slightly larger aggregates in 3 animals. (2 female, 1 male). These hamsters showed an average weight gain of up to 30 grams. These animals were apparently in excellent condition. The results of chemical analysis are shown in Table VI. No significant pathology was observed in the sections taken from autopsies.

Group F. 20 hamsters were fed L diet for 30 days, then placed on the diet plus yeast for 30 days. 8 animals developed cholelithiasis, 5 were females, 3 males. These calculi (Figure 10) were significantly different from any of the other groups. They were mainly composed of pigment, plus traces only of cholesterol. These animals thrived on the second stage of the experiment to a marked degree, the average weight gain was 20 gms. The few blood and bile cholesterol examinations are shown in Table V.

Group G. The 20 rabbits on L diet, thrived poorly and gained very little weight. Thinning of the fur and skin lesions

were common. One rabbit developed a pigment calculus. (Figure 11). 14 other rabbits showed visible soft concretions (gels), some with minute black pigment specks. The gallbladders were filled with dark or light green bile, 1 with reddish brown bile. The gel forms dissolved in ether into fatty globules, with high cholesterol content. 8 rabbits were infected with Eimeria Stiedae. The controls with the same infection showed no deposits, nor any of the deficiency lesions. The chemical analyses are shown in Table VII.

Group AA. 8 females out of 20 developed pigmented concrements of varying consistency and amount, after 30 days on the diet. Only 1 male out of 20 developed small firm green concrements after 30 days.

Group BB. 6 females out of 20 developed green, and green and white concrements of varying consistency after 30 days on the diet. One male in 20 hamsters developed 4 small yellow white calculi after 30 days.

These animals thrived very well and averaged a weight gain of 30 gms. in the 30 day period. All the gallbladders were found to be small and contracted at autopsy. Contrary to the findings of Dam et al, the incisor teeth were a normal brown colour. The

fur, epidermis and mucosae were all healthy, no eye disease was found in these two groups.

Series II.

Group H. Rabbits on daily 3 beta-cholestanol in diet. In all cases after the 9th day cholelithiasis developed and 3 rabbits showed common bile duct concrements. The quantity of concrements varied directly with the length of time on the diet. At 21 days the gallbladder was packed solid so that a soft whitish green cast was formed. (Figure 12). The rabbits thrived on the 3 B diet and almost doubled their weight. 1 rabbit showed congenital absence of the gallbladder, the common bile was of normal size and anatomy. The concrements found in the earlier sacrificed animals were somewhat gelatinous and opaque whitish green in colour. At 21 days a mixture of these concrements with light crystalline green calculi was found. (Figure 13). Aggregates of both types were noted. The calculi appeared to increase in size by coalescing rather in the manner of butter fat globules in a churn. Their average size was not greater than 1.25 mm. Some crystalline forms were stained with blood pigment. (Figure 14). The quantity of bile was markedly limited by the presence of the calculi, and where present was thick and gelatinous. (gel?). The pathological changes in gallbladder and common bile duct became more pronounced the longer the animals were on the diet.

Oedema of all layers and round cell infiltration was common.

Rabbit No. 20 after 41 days (21 days diet) showed the gallbladder imbedded in the liver tissue. Oedema, 4 plus round cell infiltration, extensive scarring fibrosis in the subserosa and marked inflammatory reaction out to the adjacent liver tissue was observed. Duct changes were similar, and the liver showed perilobular fibrosis, and numerous lipophages. (Figure 17). One rabbit showed a grossly haemorrhagic gallbladder at autopsy, and marked inflammatory response microscopically. The calculi were blood stained in this case. (Figure 14). The chemical analyses Table VIII, show a significant depression of blood and bile cholesterol levels.

Group I. 20 guinea pigs on $\frac{1}{4}$ gm. of 3 beta-cholestanol grew at normal rate, only 2 animals showed calculi at 17 and 31 days. These calculi were small but more closely resembled human stones, in that they were light and dark brown (Figure 18), and were a mixture of a soft yellow gelatinous material and brown and yellow crystals. The bile was always fluid, yellow in colour, and ample was present for analyses (Table IX). There were no significant alterations of blood and bile cholesterol levels. The pathology was not remarkable in all cases, sections of gallbladder and bile duct revealed, plus one oedema, minimal

round cells, and occassional fatty changes in the liver. (Figure 19).

Group J. 20 rats on 1/8 gm of 3 beta-cholestanol daily, thrived well, gaining weight at normal rate. No calculi were found in the common bile duct, and no pathological changes were found in the sections examined. Laboratory studies are shown in Table IX. No alteration of blood cholesterol was found. 20 random cultures of bile, gallbladder and calculi, were negative in Groups H, I and J. The calculi in these first two groups contained little cholesterol and were mainly composed of the bile acids, glycodeoxycholic and glycocholic acids (Mosbach and Bevans, 1956). The bacteriology studies showed the presence of the following organisms: E coli, p. mirabilis, streptococcus viridans, streptococcus thermophilus, gaffkya tetragena, b. subtilis in various combinations in 4 bile specimens. These were considered partly contaminant, however, further studies on a large scale to determine normal flora are needed.

Group K. 14 rabbits were cholecystectomized and 1 week later started on the 3 beta-cholestanol regime for 21 days. 2 rabbits showed common bile ducts almost packed solid with concrements, and 2 others showed fewer concrements. The ducts showed a marked

inflammatory response. (Figure 16). The results of analyses of the blood cholesterol are shown in Table VIII. Depression of the levels is again demonstrated. It was accidentally noted that the young gelatinous forms of these concrements will become crystalline and green in color if they are left to dry out, and when returned to water they again, in a short period of time, become gelatinous and opaque, and greenish white in colour.

Series III.

Group L. The petrographic sections of selected human gallbladder calculi revealed as was expected, that there was a large organic amorphous component in all calculi except the cholesterol "solitaires." In these the pigmented debris was present in small amounts at ring surfaces. The mixed calculi all demonstrated a colloform structure and 2 or more "nuclei" which frequently had coalesced. These "nuclei" were all nearer the centre than the periphery of the calculus, but were generally eccentric in position. The "nuclei" had apparently existed and grown individually, then coalesced and formed the basic structure for a larger calculus, which in turn acting as a single unit had coalesced with one or more smaller units and so enlarged further. This probably occurring most frequently when these are firm but jelly like masses with entrapped bile constituents.

The position of the crystalline fractions show an interesting relationship to these so-called "nuclei." Plates of large cholesterol crystals, or groups of small crystals are seen extending from the nuclei both peripherally and centrally. Some large plate crystals by pass the nuclear mass and extend proximally and distally. The larger crystals are usually more central in the calculus proper, and the small crystals are seen peripherally. This is in keeping with the theory of Delario, 1935 that calculi are formed as in geode crystals in quartz seen in nature. The large crystals are slowly formed, the small ones rapidly formed. Widely admixed with the central cholesterol crystals are desoxycholic acid, sodium glycocholate and sodium taurocholate crystals. Several unidentified crystalline forms which probably are derivatives of cholic acid were noted. The cholesterol "solitaire" showed that an aggregation of variously coloured spherical "nuclei" occupying a sixth of the central zone had coalesced to form a pigmented mass. In the peripheral zones pigment which cannot be detected with the naked eye was noted. The large cholesterol plate crystals extended from the periphery of the calculus through the "nuclear" mass. It had the appearance of an excess cholesterol crystal colonization of a large aggregate gel mass, with the admixed pigment present having being pushed ahead of slowly invading crystals. Alternatively that an aggregate (colloidal)

"gel" had aged by a process of crystallization and that part of the contained bile constituents had combined with some of the external bile elements and pigment which had diffused inwards and interacted with them. Open spaces were frequently noted between coalescing nuclei, possibly formed by contraction of the material or by evaporation of contained moisture. The chemical analyses performed on the remaining half of the sectioned calculi showed that all the stones analyses contained from 79.4 - 95.5 gms. % of cholesterol. The content of calcium found ranged from 6.4 - 0.11 gms. %. Iron, pigments, carbonates and bile acids were commonly present. Subjecting the calculi to a simple flotation test was found to be an excellent method of choosing the calculi with high cholesterol and low mineral content. (In the selection of calculi for petrography).

Group M. The "sister" calculi did not all disintegrate in ether after 48 hours. (Figure 30). A fairly large core was obtained in 3 cases, which was ether resistant. When the outer coat was broken, however, they did dissolve in ether in 48 hours. This fact may account for the failure of ether to dissolve some calculi when instilled into common bile ducts for this purpose.

## DISCUSSION

The importance of metabolic factors in the pathogenesis of cholelithiasis has been demonstrated. By eliminating bacteria as a causal agent, and by cholecystectomy, it has also been shown that the hepatic bile contains the responsible ingredients for the production of calculi in rabbits in the experiments with 3 Beta-cholestanol. This chemical has been metabolized by the liver into (1) bile which forms aggregates very rapidly or (2) the agent may have been metabolized into an innocuous metabolite, but in so doing the liver bile was secreted in a toxic altered form. The hepatic bile in these cases apparently causes an inflammatory response in the gallbladder and ductal system before concrements are formed. (Mosbach and Bevans). Using smaller doses we have found few traces of this process occurring as would be expected. These calculi are essentially composed of bile acids and these have been shown by other workers to be most effective in causing an acute inflammatory response in the gallbladder when injected locally. The feeding of 3 beta-cholestanol therefore is a way of proving the efficacy of their method, by administering a related sterol orally.

The hamsters and rabbits on the cholesterol-free diet appear to confirm the theory that the liver is primarily responsible for concrement formation. The alteration of the original diet of

Dam by withholding vitamins has had 2 effects on results, one quite surprising, being the fact that our hamsters showed none of the signs of deficiency diseases found in Dam's series. The second effect, has been that of speeding up the production of cholelithiasis. This may have clinical application; however, the rabbits show a different sterol metabolism from hamsters, did poorly on the diet and formed only one hard calculus.

For the first time in the experiments a marked sex difference in the incidence of cholelithiasis has been demonstrated. The animals given vitamins A and D had a lower incidence of stone formation, being 30% in the females and 5% in the males. These figures are statistically significant and lower than in the groups <u>not</u> given the vitamins A, D or A, D, E, and K. The effect of the vitamins A and D especially, on the liver conjugation, and the production or inhibition of the sex steroid hormones should be studied further.

A diet low in cholesterol and fats with vitamin A, D, E, and K deficiency might equally well have totally different and more serious effects in humans. (Groups AA and BB).

These two methods, however, have been most valuable in facilitating close study of concrements of two major types at very early and later stages of their formation. One is justified

in concluding that calculi can form (1) by precipitation of cholesterol and bile salts out of ionic solution onto organic debris in hamsters on the L diet, and (2) that in rabbits fed 3 beta-cholestanol the calculi developed from "sol" to "gel" form and aged by a process of crystallization: The latter a common process occurring in colloidal chemistry.

These forms have never been looked for in humans, because of some obvious but not insurmountable problems. The examination of bile obtained by aspiration during surgery for diseases other than gallbladder pathology would possibly reveal more accurately what is the 'normal' state of bile contained in the gallbladder. Potter performed this procedure during section in over 300 women without any ill effect apparently. The bile was not examined microscopically.

The petrographic and microscopic studies of human calculi had many characteristics in common with the experimental calculi. Unfortunately, because of size and their composition none of the experimentally produced calculi could be sectioned for petrographic study. The exact role of mucus in the normal and abnormal gallbladder as a peptizing agent (protective colloid) preventing the coalescence of particles (in solution) out of

solution as a flocculate is not known. Conceivably, it could prevent in certain cases the "gel"-form formation as has been postulated.

During the macroscopic examination of the large batch of human calculi, several large calculi were noted to have one or more daughter calculi of up to 1 cm. in size adherent and completely fused to the outer coat. This is in keeping with the theory of aggregation, and we visualize that this take place internally, as has been shown in the petrographic studies. Other large mixed calculi revealed a soft centre of black tarry bile when sectioned, this would appear to be contrary evidence to the theory that calculi generally form by accretion; that is, by the deposition of layer upon layer around a firm starting nucleus. One large calculus when sectioned at different levels showed 18 small internal "nuclei" which were in effect individual calculi gathered into the parent stone as it grew in size. Section of the smaller stones, showed soft pigmented material in some, and candle grease like material in others.

Close examination of large calculi or the typical mulberry type shows that the outer coat has been formed by the deposition of small spherical lobules of white crystals, which later coalesced. We were able to emulate these structures simply by allowing a solution of (1) "normal" calculus, (2) 3 beta-cholestanol or (3) cholesterol in ether, to crystallize out on some prepared organic

debris from human calculi, or on the surface of a sectioned human calculus. The result obtained is nearly identical with the forms shown in hamster calculi; this is seen by comparing Figures 6,29,31.. The constituents of bile and the metabolism of the sterol-steroid group are not yet totally known.

The use of radioactive isotopes, paper chromatography, ultra violet absorption spectrometry will probably add the key stones to our total understanding of the pathogenesis of cholelithiasis in the future.

Miller and Armour, 1956 report a case of spontaneous disappearance of gallstones. This could happen in two ways; one, by part disintegration of the calculi and passage into the intestine (absorption of the usual constituents of calculi has not been proved). Two, by a change in character of the secreted hepatic bile, and hence, gallbladder bile,the stones could have dissolved in toto. The results with the hamsters fed yeast, would seem to support a hypothesis that experimentally produced calculi can be changed by alteration of diet and the homeostatic mechanism. The possible effects of the relatively large quantities of protein and carbohydrate must be considered when assessing the end results of the "L" cholesterol-free diet.

Research workers in athersclerosis (unpublished) have shown that the chronic dietary level of protein and carbohydrate affects the cholesterol levels in humans. This confirms the hypothesis that an upset of liver function and the homeostatic mechanism can be induced by dietary variations.

## CLINICAL CORRELATION

The delicacy of the liver balance in sterol metabolism has been shown in experimental animals. Considering the comparatively normal uncomplicated life and diet of these animals, it seems remarkable that the incidence of gallbladder disease in humans is not much higher than is diagnosed. 3 beta-cholestanol should not be administered to humans suffering from atherosclerosis without the knowledge that an acute cholecystitis can develop as a minimal side-effect.

Excesses of diet, particularly high carbohydrate, low fat, deficient in vitamins by causing gallbladder stasis and alteration of hepatic bile could easily be responsible for an earlier onset of cholelithiasis in susceptible females. Dieting may be a contributing factor in cholelithiasis.

It will be noted that the vitamins withheld from the hamster diet "L", A, D, E, K are fat soluble. In humans a low fat diet could result in a non-apparent vitamin A, D, E, K deficiency due to lack of absorption, and so influence the hepatic metabolism of fats and sterols. (Reference Groups AA and BB - Series I).

The administration of chlortetracycline would seem to be

a useful adjunct to therapy in all cases of gallbladder disease in spite of the fact that it does not gain access to the gallbladder in obstruction of the cystic duct. The effect on the liver is difficult to assess. The quality of the hepatic bile has been shown to be responsible for gallstone formation in animals. One might postulate that gallstones in young people (not causing symptoms) should be followed by X-ray for a reasonable length of time, and not subjected to cholecystectomy, because of the obvious possibility of early common bile-duct stone formation. Where the condition was acute, cholecystostomy could be performed, leaving the gallbladder as a tension bulb, which may or may not function. Should the calculi reform than a second operation might be necessary, just as it would be if common bile duct stones developed after the first operation for gallbladder removal.

Whether or not obvious liver disease, haemolytic jaundice ( spherocytosis) existed should be influencing factors. The real danger of carcinoma in the presence of cholelithiasis would not be forgotten in the older patients. A dietary regime, or a method of oral ingestion of a detergent might in the interval be discovered that would be "litho-lytic." Cholecystectomy might conceivably lead to a higher percentage of post-cholecystectomy syndrome in the patients discussed.

The paradoxical diminution of calculus-yield, when steroids are given to the experimental animals is difficult to explain. It is possible that large doses of adreno-corticoids may cause cholesterol stone formation in humans. The raised blood levels of cholesterol caused by oestrogens and progesterone might indicate that large doses of these substances should not be administered to atherosclerotic patients, or young people.

The effect of the steroids on the biliary tree may be responsible for the occurrence of acute cholecystitis immediately following operations for unrelated disease. The state of dehydration, fasting, recumbency, drugs administered may cause stasis by (1) food stimulus lack or (2) by interference with biliary mechanism of contraction, or (3) quality of the hepatic bile secreted, might be other etiological factors. Similarly, if previous gallbladder infection, especially with cholelithiasis had been present then the occurrence of an acute exacerbation can easily be understood. Where previous disease in the gallbladder was not present, poses a different set of circumstances difficult to understand. A close study from all aspects of these cases of post-operative secondary biliary tract disease might well add valuable knowledge to our understanding of gallbladder disease.

Thus, the effect of stress with its accompanying corticoid release might be a more significant factor than is generally realized. Popper and Szanto, 1956 have shown in their studies on intra-hepatic cholestasis that hitherto unsuspected factors can cause bile changes, such as, thrombi, or micro-calculi in the hepatic biliary system. They stated that it could be due to a viral hepatitis, drug allergy or other non established etiology.

Here again, another aspect of liver upset and altered bile is shown, which is difficult to detect except by liver biopsy or in the post-mortem specimen.

## CONCLUSIONS

1. Golden hamsters fed a low fat, cholesterol-free diet, deficient in vitamins A, D, E, K develop cholelithiasis in a high percentage of cases. The addition of vitamins A, D, E, K to the cholesterol-free diet markedly lowers the incidence of biliary concrements in hamsters.

2. Injections of oestrogen, progesterone, and cortisone to animals on the diet results in a marked fall in the incidence of cholelithiasis. Bile cholesterol levels are raised, and female hamsters tolerate the steroids much better than males.

3. Feeding chlortetracycline with the diet also lowered the incidence of calculus formation. A marked weight gain and general improvement of the condition of the hamsters was noted.

4. The feeding of yeast results in less calculi plus a change from mixed cholesterol to pigmented stones.

5. The incidence of calculi in these immature animals is slightly higher in the females.

6. The pH of the bile in all cases is alkaline as is the normal bile of the species used.

7. The plasma and bile cholesterol levels in these animals

is very labile. There is no fixed significant correlation between the plasma and bile cholesterol levels in cases of cholelithiasis in these animals. The presence of unknown or unidentified sterol fractions in the liver and gallbladder bile may reflect changes in cases of cholelithiasis.

8. No significant pathological changes were found in the gallbladder and liver histological sections of hamsters on the lithogenic diet.

9. (a) A marked derangement of the liver synthesis and excretion of the sterol-steroid group would appear to be the prime factor in the pathogenesis of cholelithiasis.

(b) There is a marked difference in the response to a cholesterol-free diet between rabbits and hamsters. The former do not thrive on the absence of cholesterol from exogenous sources.

10. There is a definite species difference in the metabolism of 3 beta-cholestanol. Rabbits fed this substance for 9 days or more develop gallbladder and common bile duct calculi in a very high percentage of cases. Guinea pigs produce fewer calculi and rats do not show choledocholithiasis when fed 3 beta-cholestanol. Human subjects suffering from

atherosclerosis should be given this chemical with reserve, until more information regarding the end result is known in humans.

11. The administration of 3 beta-cholestanol to rabbits results in an appreciable lowering in blood cholesterol levels in some animals.

12. Cholecystectomized rabbits developed choledocholithiasis when fed 3 beta-cholestanol in 28% of cases.

13. Varied pathological changes were found in the gall-bladder and common bile duct of the animals fed 3 beta-cholestanol. This substance or its metabolites causes a marked inflammatory response with oedema and round cell infiltration in the gall-bladder and common bile duct of guinea pigs and rabbits, provided the dose is adequate.

14. Some of these soft pre-concrement "gel" forms may pass easily and frequently from gallbladder to duodenum provided there is no dyskinesia present.

15. The petrographic examination of human calculi commonly showed the mixed aggregate centres seen in the experimentally produced concrements. Several apparently pure pigmented and pure cholesterol calculi were seen to be admixed with several other bile constituents when examined with the polarizing microscope.

This was confirmed by chemical analysis.

16. Ether may dissolve certain human stones, but some have been shown to contain an inner calculus which does not dissolve in ether.

17. The negative bacteriological studies done in the hamsters in Series I and rabbits and guinea pigs in Series II suggests that metabolic factors were responsible for the cholelithiasis produced.

18. The Eimeria stiedae (coccidial) infection of the experimental group of rabbits was not responsible for the depositions found in the gallbladder. The control rabbits were also infected with this parasite and did not show the presence of any depositions in the gallbladder.

19. These experiments have demonstrated 2 definite methods of the formation of gallstones in the animals used. (1) The precipitation of cholesterol and bile acids out of ionic solution on to pigmented organic debris. (2) The development of a colloidal solution from "sol" to a "gel" state. The aggregation of this "gel"-form in clusters, finally the "ageing" of this colloidal mass by a process of crystallization, In the process various constituents of bile are trapped, or by a process of diffusion invade the mass. This supports the theories of Liesegang, and Sweet, on the physico-chemical mode of formation of biliary calculi.

## SUMMARY

A collective review of the literature concerning experimental cholelithiasis has been made. The materials and methods in this investigation have been described in detail. To illustrate the results of each group of experiments, tables, photographs and photomicrographs have been included in large numbers.

Using simple dietary methods, biliary tract calculi have been produced in hamsters, rabbits, and guinea pigs. The calculi contained, cholesterol, pigment, bile acids. In one group a low fat, cholesterol-free diet, deficient in vitamins A, D, E, K was used, and in the other 3 beta-cholestanol in varying doses was fed daily with normal diet to rabbits, guinea pigs and rats. The effect of chlortetracycline, cortisone, oestradiol benzoate and progesterone was studied in the hamsters on the cholesterol-free diet. Cholecystectomized rabbits fed 3 beta-cholestanol developed choledocholithiasis.

Blood and bile cholesterol assays were carried out to determine if there was any significant relationship between these levels and the formation of calculi in the experiments.

Bacteriological studies were done to exclude the possibility of bacteria being responsible for the stone production.

Histopathological findings are reported. The relationship of the results obtained to the pathogenesis of cholelithiasis in humans is discussed. Using a new approach, the method of petrography commonly used in geology was applied to human calculi.

Evidence has been presented that experimental calculi formed by 2 main methods: (1) By precipitation out of ionic solution onto organic debris. (2) By the "ageing" into crystalline form of a colloidal "gel."

## BIBLIOGRAPHY

1. Adams, T. W., Mussleman, M. M.
Pancreatic venous thrombosis as an etiological factor in Acute Necrotizing Haemorrhagic pancreatitis.
Surg. Forum. IV: 401, 1953.

2. Agrifoglio, M.
Clin. Med. Ital. 55: 89, 1924.

3. Altschul, R.
Selected studies on atherosclerosis.
Publ. Univ. Sask., 1952.

4. Albright, F., Sulkowitch, H. W., Chute, R.
J. A. M. A. 113: 2049, 1939.

5. Anderson, R. E., Priestley, J. T.
Observations on the bacteriology of choledochal bile.
Ann. Surg. 133: 486, 1951.

6. Anderson, W. A. D.
Textbook of Pathology.

7. Andrews, E., Dostal, L. E., Goff, M., Hrdina, L.
Ann. Surg. 96: 615, 1932. (a)

8. Andrews, E.
Etiology of gallstones.
Arch. Surg. 25: 796, 1932. (b)

9. Andrews, E.
S. G. O. 57: 36, 1933. (a)

10. Andrews, E., Dostal, L. E., Hrdina, L.
Arch. Surg. 26: 382, 1933 (b)

11. Aoyama, T.
Deutsche Ztschr. f. Chir. 132: 234, 1914.

12. Armour, J.
Verbal Communication, Royal Victoria Hospital, Montreal, 1957.

13. Aronsohn, H. G., Andrews, E.
Experimental Cholelithiasis.
S. G. O. 66: 748, 1938.

14. Aschoff, L., Bacmeister, A.
Die cholelithiasis.
Jena. Gustav Fischer, 1909. (Lectures on Pathology, New York, P. B. Hoeber, 1924).

15. Babkin, B. P.
Die äussere Sekretion der verdauungsdrüsen.
Berlin, 344, 1914.

16. Badile, L.
Pathologica, 15: 307, 1923.

17. Bartlett, W. C.
Review 224 cases.
S. G. O. 103: 4, 1956.

18. Bates, G. C., Brown, C. H.
Incidence of gallbladder disease in chronic hemolytic anemia (Spherocytosis).
G. E. 21: 104, 1952.

19. Beer, E.
Med. News. IXXXV: 202, 1904.

20. Bernard, C.
Quoted by Opie, E. L. in "Disease of the Pancreas," Phil. Lippincott and Company, 1903.

21. Biocca, P.
Considerazioni sulla patogenesi della colecisti di porcellana. 54: 173, 1947.

22. Birch, De B., Spong, H.
The Secretion of the gallbladder.
J. Physio. 8: 378, 1887.

23. Bjorn, Isaksson.
Studies on bile acids.
Acta Soc. Med. Upsaliensis, 59: 307, 1954.

24. Blackstein, A. G.
Bull. J. Hopkins Hospital, 2: 96, 1891.

25. Blaisdel, F. E., Chandler, L. R.
Am. J. Med. Sc. 174: 492, 1927.

26. Block, K., Borek, E., Rittenberg, D.
J. Biol. Chem. 162: 441, 1946.

27. Bockus, H. L.
Intermittent arteriomesenteric occlusion of the duodenum with dilatation and stasis.
Penn. Med. J. 32: 618, 1929.

28. Bockus, H. L.
Gastroenterology. Vol. III.
W. B. Saunders & Company, New York, 1946.

29. Bonta, J. A., Lovingood, C. G.
Surgery. 31: 309, 1952.

30. Boyden, E. A., Layne, J. A.
A study of the non-vizualization and rate of emptying of the gallbladder in patients with pernicious anemia.
G. E. 4: 121, 1945.

31. Boyd, W.
Surgical Pathology.

32. Idem.
Pathology of Internal Disease.

33. Idem.
Textbook of Pathology.
Lea and Febiger, Philadelphia.

34. Brown, R. K., Milch, E.
Gallbladder gas gangrene.
G. E. 10: 626, 1948.

35. Bronner, H.
Arch. klin. Chir. 180: 597, 1934.

36. Burchard, Liebermann.
Arch. Surg. 26: 113, 1933.

37. Burden.
Quoted Aronsohn et al.
S. G. O. 66: 748, 1938.

38. Bustos, J. O.
Parasitic cholecystitis.
Prensa Med. Argent. 36: 33, 1949.

39. Byers, S. O., Friedman, M.
Biliary cholesterol: increment and indicator of hepatic synthesis of cholesterol.
Am. J. Phy. 168: 297, 1952. (a)

40. Byers, S. O., Firedman, M.
Effect of various bile acids on the hypercholesteremia following biliary obstruction in the rat.
Am. J. Phy. 168: 138, 1952. (b)

41. Byers, S. O., Friedman, M.
Observations concerning the production and excretion of cholesterol in mammals.
Am. J. Phy. 179: 79, 1954.

42. Calzolari, T., Dolfini, G. E.
Calcareous cholelithiasis simulating a procelain gallbladder.
Policlinico sez. Chir. 54: 36, 1947.

43. Cameron, A. L.
Time element in stone formation.
J. A. M. A. 81: 1671, 1923.

44. Campbell, J. M. H.
Cholesterol in blood in cases of gallstones.
Quart. J. Med. 18: 123, 1924.

45. Carter, R. F., Greene, C. H., Twiss, J. R., Hotz, R.
Etiology of gallstones and critical survey of the literature and study of applicability of various theories in 239 operative cases.
Arch. Surg. 39: 5, 691, 1939. (a)

46. Carter, R. F., Greene, C. H., Twiss, J. R.
"Diagnosis and Management of Diseases of the Biliary Tract." pp. 432, 1939, Lea and Febiger, Phila. (b)

47. Carmichael, E. S.
J. Path. &. Bact. 8: 453, 1903.

48. Chabrol, E.
Introduction to the study of biliary lithiasis.
Rev. du Prat. 3: 867, 1953.

49. Chau, A. Y. S.
McGill thesis, 1950. (Experimental Surgery).

50. Chauffard, A. M. E.
Lecons sur la lithiase biliaire.
2nd Edition, 247 pp., Masson et Cie., Paris, 1922.

51. Chevreul, M. E.
Ann. de Chim. 95: 5, 1815.

52. Cohrs, Nieberle.
Vet. Med. Textbook, p. 408, 1949. Translated Cornell University.

53. Christensen, F., Dam, H., Prangle, I.
Alimentary production of gallstones in hamsters, II.
Acta Phys. Scand. 27: 315, 1952.

54. Christensen, F., Dam, H.
Alimentary production of gallstones in hamsters, III.
Acta. Phys. Scand. 31: 75, 1954.

55. Christensen, F., Dam, H., Kristensen, G.
Alimentary production of gallstones in hamsters, VI.
Acta. Phys. Scand. 36: 329, 1956.

56. Clavel, C., Dumas, J.
Acute calculous cholecystistis in children.
Lyon Chir. 41: 411, 1946.

57. Cleland, J. B.
Gallstones in 7000 post-mortem examinations.
Med. J. Aust. September 26, 1953.

58. Conn, J. W., et al.
Serum cholesterol, a probable precursor of adrenal cortical hormones.
J. Lab. Clin. Med. 35: 504, 1950.

59. Copher, G. H., Illingworth, C. F. W.
Experimental study of the factor of biliary stasis in the production of gallstones.
S. G. O. 46: 658, 1928.

60. Cook, R. P., Kliman, A., Fieser, L. F.
Arch. Biochem. and Biophysics. 52: 439, 1954.

61. Coe, T. A.
A treatise on biliary concretions. pp. 345. Text.
D. Wilson and T. Durham, London, 1757.

62. Colcock, B. P., McManus, J. E.
Experiences with 1356 cases of cholecystitis and cholelithiasis.
S. G. O. 101: 161, 1955.

63. Cohnheim, J.
Vorlesungen ueber allgemeine .
Pathologie. Vol. 2. 2nd Ed. 597 pp. Berlin Aug. Hirschwald,
1882.

64. Crile, G.
Energy background of genesis of gallstones.
S. G. O. 60: 818, 1935.

65. Crump, C.
Reports on 1000 autopsies.
S. G. O. 53: 447, 1931.

66. Cushing, H. et al.
Bull. Johns Hopkins Hospital. X: 166, 1899.

67. Dam, H., Christensen, F.
Alimentary production of gallstones in hamsters, I.
Acta. Path. et. Microbiol. Scand. 30: 236, 1952.

68. De Fourcroy, A. F.
Quoted by Sobotka, H., 1937.

69. Deaver, Quoted by Aronsohn et al, 1938.

70. Denton, Quoted by Aronsohn et al, 1938.

71. Denton, J.
Observations on infarction in cholecystitis.
Arch. Surg. 14: 1, 1927.

72. Dixon, C. F., Lichtman, A. 1.
Congenital absence of the gallbladder.
Surg. 17: 11, 1945.

73. Dostal, L. E., Andrews, E.
Etiology of gallstones: effect of diet on bile salt:
cholesterol ratio.
Arch. Surg. 26: 258, 1933.

74. Dragstedt, L., Haymond, H., Ellis, J.
Pathogenesis of acute pancreatitis.
Arch. Surg. 28: 232, 1934.

75. Drennan, J. G.
Ann. Surg. LXXVI: 482, 1922.

76. Edlund and Olsson (Gothenburg)
A study of 8368 cases of cholecystectomy
Acta Chir. Scand. Vol. III. 481, 1956.

77. Elfving, G.
A case of tuberculosis of the gallbladder
Ann. Chir. Gyn Fem. 38: 80, 1949.

78. Elman, R., Graham, E. A.
The pathogenesis of the strawberry gallbladder
Arch. Surg. 24: 14, 1932.

79. Elton. Deutsch.
Concentration and precipitation of bilirubin in the gallbladder and bile ducts.
Arch. Path. 15: 818, 1933.

80. Emmerich and Wagner,
Central Bl. f. allg. path u. path.
Anat. 27: 433, 1916.

81. Estrada, R. L.
Studies in source and variations of amylase in animals and human bile.
Surg. Forum. 29: 416, 1952.

82. Eustis, A.
Relation of gallbladder disease to diabetes.
New Orleans M. & S. J. 75: 449, 1923.

83. Feinblatt, Quoted by Aronsohn, 1938.

84. Feldman, M.
Cholesterolosis of the gallbladder: Autopsy study of 165 cases.
G. E. 27: 641, 1954.

85. Fernel (ius)
Quoted by Hoppe-Seyler. Nothnagel's encyclopedia of Practical Medicine. Trans. (Alfred Stengel, Am. Ed. p. 25, 1903).

86. Fisher, B., Fisher, E., Selker, R.
Further observations on the role of bile in the pathogenesis of pancreatitis.
Surg. Forum. IV: 406, 1953.

87. Fox, F. W.
The composition of human bile and its bearing upon sterol metabolism.
Quart. J. Med. 21: 107, 1927.

88. Friedman, M., Byers, S.
Bile acid accumulation in the production of hypercholesteremia occurring after biliary obstruction.
Am. J. Phys. 168: 292, 1952.

89. Fulton, H.
Gas containing gallstones.
G. E. 28: 862, 1955.

90. Furth, O.
Pathologische physiology der aufbau der gallensteinen.
Wien. Klin. Wchnschr. 50: 629, 1927.

91. Gainsborough, H.
A study of Lipoid nephrosis.
Quart. J. Med. 23: 101, 1929.

92. Galambosa.
Intra-hepatic lithiasis with multiple locations.
Am. J. Dig. Dis. 21: 95, 1954.

93. Gardner, J. A., Gainsborough, H.
Blood cholesterol studies in biliary and hepatic diseases.
Quart. J. Med. XXII: 465, 1930.

94. Gerdes, M. M., Boyden, E. A.
The rate of emptying of the human gallbladder in pregnancy.
S. G. O. 66: 145, 1938.

95. Gilbert, A.
Arch. Gen. de Med. 2: 257, 1898.

96. Gilbert, A., Dominici, S. A.
Compt. rend soc. de Biol. 23: 1033, 1893.

97. Gilbert, Girode, Quoted by Aronsohn, Andrews , 1938.

98. Glenn, F., Wantz, G. E.
Acute cholecystitis following the surgical treatment of unrelated disease.
S. G. O. 102: 145, 1956.

99. Glenn, F.
Acute cholecystitis following the surgical treatment of unrelated disease.
Ann. Surg. 126: 411, 1947.

100. Goldbloom, A. A.
Clinical studies of blood lipid metabolism.
Am. J. of Dig. Dis. 19: 9, 1952.

101. Goldman, B., Jackman, J., Eastman, R. H.
The management of post-operative choledocholithiasis.
S. G. O. 81: 521, 1945.

102. Goodman, E. H.
Beitrz. Ehcm. physiol. u. path. 9: 91, 1907.

103. Gordon-Taylor, G., Whitby, L. E. H.
Brit. J. Surg. 18: 78, 1930.

104. Graham, E. A., Cole, W. H., Copher, C. H., Moore, S.
Diseases of gallbladder and bile ducts.
Lea and Febiger, Phila., 1928).

105. Gray, S. H., Probstein, J. G., Sachar, L. A.
Chronic cholecystitis produced by division of the sphincter of Oddi.
Arch. Surg. 59: 1007, 1949.

106. Greene, C. H., Twiss, J. R., Carter, R. F.
Tr. Am. G. E. Association, pp. 6, 1936.

107. Greig, E. D, W.
Indian J. M. Research. 3: 259, 1915.

108. Halpert, B.
New aspects of formation and classification of gallstones.
Arch. Path. 6: 623, 1928.

109. Halpert, B.
Effects of cholecystectomy on the biliary system in dog s.
Arch. Surg. 26: 589, 1933.

110. Hanel, H. K., Christensen, F., Dam, H.
Cholesterol synthesis from 2 C14 acetate by the liver in vivo.
Acta Path. et Microb. Scand. 35: 423, 1954. (a).

111. Hanel, H. K., Christensen, F., Dam, H.
Cholesterol synthesis from 2 C-14 acetate by the liver in vitro.
Acta Path. et Microb. Scand. 35: 237, 1954 (b).

112. Hartmann, O.
Deut. Zeits. f. Chir. IXVIII: 207, 1903.

113. Hawk, P. B., Oser, B. L., Summerson, W. H.
Practical Physiological Chemistry. 12th Edition, Blakiston Company (Toronto).

114. Hein, J.
Zschr. Zeit. Med. 4: 293, 1846.

115. Henegar, G. C., Turner, J. L.
Medical progress in cholesterol metabolism in relation to disease of liver and biliary tract.
Calif. M. J. 73, 1950.

116. Hjorth, E.
Contribution to the knowledge of pancreatic reflux as an etiologic factor in chronic affections of the gallbladder.
Acta. Chir. Scand. 96: 134, 1947.

117. Hospers.
Arch. Path. 14: 66, 1932.

118. Huntemueller, Klin. Woch. 1: 349, 1924.

119. Hunter, J.
Cited by Aronsohn, Andrews, 1938.

120. Illingworth, C. F. W.
Types of gallbladder infection.
Brit. J. Surg. 15: 221, 1927.

121. Idem.
Strawberry gallbladder
Brit. J. Surg. 17: 203, 1929.

122. Isaksson, B.
The lipid constituents of bile from human gallbladder containing cholesterol stones.
Acta. Soc. Med. Upsaliensis. 59: 277, 1954.

123. Italia, F. E.
Policlinico, 1901.
Cited by Zeutralbl. f. Chir. 28: 693, 1901.

124. Ivy, A. C.
Factors concerned in the evacuation of the gallbladder.
Med. 11: 345, 1932.

125. Idem.
Phys. Rev. 14: 1, 1934.

126. Jemerin, E. E.
Cholecystitis emphysematosa.
Surg. 25: 237, 1949.

127. Jennings, W. K.
Relationship of post-cholecystectomy disturbances to bacterial sensitivity.
S. G. O. 95: 439, 1952.

128. John, H. J.
Am. J. Dig. Dis. 18: 109, 1951.

129. Johnson, W. O.
Am. J. Med. Sc. CIXX: 181, 1925.

130. Joly, J. S.
Stone and calculous disease of the urinary organs.
St. Louis, C. V. Mosby Company, 1929.

131. Jones, R.
The etiology and pathogenesis of acute haemorrhagic pancreatitis.
Am. J. M. Sc. 205: 277, 1943.

132. Joslin, E. P.
Treatment of diabetes mellitus.
Lea and Febiger, Phila. 5th Edition, 1936.

133. Kilham, L., Herman, C. M.
Isolation of an agent causing bilirubinemia and jaundice in raccoons.
Proc. Soc. Exp. Biol. Med. 85: 272, 1954.

134. Klebs, E.
Die allgemeine pathologie, Part 2: 836.
Jena. & Fischer, 1889.

135. Klinkert, D.
Berl. Klin. Wchnschr. 48: 335, 1911.

136. Kraus, J.
Prag. Med. Wschr. 6: 81, 1881.

137. Idem.
Prag. Med. Wschr. 9: 483, 1884.

138. Kusumoto, C.
Biochem. Ztschr. 13: 354, 1908.

139. Lam, R. B.
Gallbladder disease in American Indians.
Lancet. August, 1954: 305.

140. Lancereaux, E.
Traite de maladies de foie et eu pancreas.
O. Doin, pp. 1019, Paris, 1899.

141. Lester, L. J.
Acute cholecystitis.
Surgery, 21: 675, 1947.

142. Lewison, E. F.
Acute pancreatitis, etiologic review and report of 35 cases.
Arch. Surg. 41: 1008-1037, 1940.

143. Liaw, J.
Experimental formation of gallstone and changes in bile acids and fatty acids in the bile.
Fukuoka Acta. Med. 46: 222, 1955.

144. Lichtman.
Diseases of Liver, gallbladder and bile ducts.
2nd Edition, 1949. (Lea and Febiger) Phila.

145. Lichwitz, L.
Deut. Arch. Klin. Med. 92: 100, 1907.

146. Lichwitz, L.
Ergebn. Inn. Med. XII: 1, 1914.

147. Lieber, M. M.
Review of 34,665 consecutive autopsies.
Ann. Surg. 135: 394, 1952.

148. Liesegang, R. E.
Spezielle methoden der diffusion in gallerten.
Aberhalden E. Phys. Chem. Methoden. 3:33, 1929.

149. Littler, T. R., Ellis, G. R.
Gallstones, a critical survey.
B. M. J. 842, 1952.

150. Lium, R., Maddock, S.
Etiology of acute pancreatitis, an experimental study.
Surgery, 24: 593, 1948.

151. Long, E.
Trans. Adrenal cortex conference, 1949.
Joshua Macy Foundation, New York.

152. Lucas, O. C., Stevenson, L. S., Stevenson, J. S.
Petrologic study of a urinary calculus.
Trans. Roy. Soc. Canada. XLIV: 35, 1950.

153. Luetkens, U.
Vogel. Leipzig. pp. 205, 1926.

154. MacGregor, C. A.
A study of liver function in the dog with complete biliary obstruction.
Surg. Forum. 399: 81, 1952.

155. Mackey, W. A.
Cholesterolosis.
Brit. J. Surg. 24: 570, 1937.

156. Magee, D. F., Kim, K. S., Pressoa, V. C., Ivy, A. C.
Dietary Factors influencing output of bile acids.
Am. J. of Phys. 169: 309, 1952.

157. Mann, F. C., Foster, J. P.
Production of experimental cholecystitis.
Am. J. Phys. 47: 279, 1918.

158. Mann, F. C., Giordano, A. S.
The bile factor in pancreatitis.
Arch. Surg. 6: 1, 1923.

159. Marcantonio.
Gallbladder infection.
Riforma Med. 8: 1892. (Ital).

160. Marchand, F.
Deut. Med. Wschr. 14: 221, 1888.

161. Marshall, S. F., Phillips, E. S., Andrews, E.
Pathologic changes of diseased gallbladders, New Classification.
A. Surg. 31: 767, 1935.

162. Marshall, S. F.
Gangrenous cholecystitis.
Surg. Clin. N. Amer. 28: 979, 1948.

163. Mayer, J., Jones, Anne, K.
Hypercholesteremia in the hereditary obese hypoglycaemic syndrome of mice.
Am. J. Phys. 175: 339, 1953.

164. Mayo, W. J.
Cholecystitis without stones or jaundice, in its relation to chronic pancreatitis.
Am. J. M. Sc. 147: 469, 1914.

165. Mayo, C. H.
Minn. Med. J. 4: 1, 1921.

166. Mayo-Robson, A. W.
Surgical treatment of certain cases of glycosuria.
Brit. M. J. 1: 973, 1910.

167. McCall, Milton, L., Reinhold, J. G.
Clinical significance of serum amylase and lipase.
S. G. O. 80: 435, 1945.

168. McCarty, W. C.
The gallbladder and its diseases.
Proc. Staff Meeting, Mayo Clinic, 11: 805, 1936.

169. McCarty, W. C.
Frequency of strawberry gallbladders.
Ann. Surg. 69: 131, 1919.

170. McMaster, P. D., Elman, R.
On the expulsion of bile by the gallbladder and a reciprocal relationship with the sphincter activity.
J. Exp. Med. XLIV: 173, 1926.

171. McMaster, P. D.
Studies on total bile.
J. Exp. Med. 40: 25, 1924.

172. McKibbin, J. P., McDonald, J. R.
The significance of polymorphonuclear lencocytes in gallbladders.
Surgery. 1945, 17: 319.

173. Mechling, R. S., Watson, J. R.
Report on 387 consecutive cases of cholecystitis and cholelithiasis.
S. G. O. 91: 404, 1950.

174. Meleney, F. L.
Clinical aspects and treatment of surgical infections.
W. B. Saunders Company, 1948. Philadelphia.

175. Meltzer, S. J.
The disturbance of the Law of Contrary Innervation as a pathologic factor in disease of the bile ducts and the gallbladder.
Am. J. M. Sc. 153: 469, 1917.

175. Mentzner, S. H.
Cholecystic disease in pigs, sheep and cattle.
Cal. and West Med. 40: 333, 1934.

176. Mentzner, S. H.
Gallbladder stasis.
S. G. O. 42: 782, 1926.

177. Meyer, K. F., Neilson, N. M., Feusier, M. L.
Quoted Rous et al.
J. Inf. Dis. XXVIII: 456, 1921.

178. Meyer, Friedman, Byers, S. O., Michaelis, F.
Cholesterol excretion in mammals. II. Excretion in the rat.
Am. J. Phys. 162: 575, 1950.

179. Mignot, R.
Gallbladder infection.
Arch. Gen. de Med. (These 1896) 2: 257, 1898.

180. Miller, M. C.
Spontaneous disappearance of gallstones.
G. E. 31: 588, 1956.

181. Miyake, H.
Mitt. a.d. grenzgeb. d.
Med. u. Chir. 6: 479, 1900. (Quoted by Robertson, 1945).

182. Moore, S. W.
Intra-mural formation of calculi.
Arch. Surg. 34: 410, 1937.

183. Mosbach, E. H., Kalinsky, Helen J., Halpern, E.,
Kendall, F. E.
Determination of Deoxycholic and cholic acids in bile.
Arch. Bio. Biophy. 51: 402, 1954.

184. Mosbach, E. H., Bevans, M.
Formation of gallstones in rabbits fed 3 beta-cholestanol.
Arch. of Biochem. and Biophysics. 63: 1, 1956.

185. Moynihan, B.
Ann. Surg. 50: 1265, 1909.

186. Murphy, J. B.
Cholecystitis, Symptomatic diabetes due to gallbladder infection.
S. Clin. (J. B. Murphy) 2: 496, 1913.

187. Nakashima, Saheki.
Dissolution of gallstones with added vitamin A.
Jap. J. G. E. 1: 134, 1929.

188. Jap. J. G. E. 3: 27, 1931.

189. Narita, T.
The relation of the liver to infections of the blood stream.
Ztschr. F. d. ges. exper. Med. 80: 303, 1932.

190. Naunyn, B.
Function of gallbladder.
Arch. Anat. Physiol. u. Wissench. Med. 579, 1869.

191. Naunyn, B.
Treatise on cholelithiasis.
New Sydenham Society London, 1896.
Klinik der Cholelithiasis. (Liepzig) F. C. W. Vogel, 1892.

192. Newman, C.
Stasis in the gallbladder.
Lancet. (London) 1: 841, 1933.

193. Okey, R.
Gallstone formation and intake of "B" vitamins in cholesterol fed guinea pigs.
Proc. Soc. Exp. Biol. Med. 51: 349, 1942.

194. Ordnoff, B. H.
Reverse movements in the contents of the duodenum and its probable significance.
Ill. M. J. 55: 406, 1929.

195. Osler, W.
The Principles and Practice of Medicine.
Ed. B. D. Appleton, Century Company, New York, 1938.

196. Ottoman, R. E., Baker, J.
Metastatic staphylococcal infection of the gallbladder.
West. J. Surg. 53: 361, 1945.

197. Palmer, E. D.
Report on Saint's Triad.
Am. J. Digest. Dis. 22: 314, 1955.

198. Patey, D. H.
Modern views on the mechanism of formation of gallstones.
B. M. J. 1: 866, 1933.

199. Pavel, I.
Du role peu important de la stase dans la pathogenie de la lithiase biliare.
Presse Med. 43: 1565, 1935.

200. Payne, T. P.
Cholesterol studies in rabbits.
McGill Thesis, April, 1950.

201. Peirce, S. J. S.
Deutsches Arch. f. Klin Med. 106: 337, 1912.

202. Pemberton, J. de J.
Results of splenectomy in splenic anaemia, haemolytic jaundice, haemorrhagic purpura.
Ann. Surg. 94: 755, 1931.

203. Pers, M., Baden, H.
The frequency of recurrence of calculi in the gallbladder after cholecystolithotomy.
Acta. Chir. Scand. 102: 260, 1951.

204. Peters, J. P., Van Slyke, D. D.
Quant. Clin. Chem. Vol. I. Interpretations 2nd Ed., Baltimore, 1946.

205. Phemister, D. B., Aronsohn, H. G., Pepinsky, R.
Variation in cholesterol, bile pigment and calcium salt contents of gallstones formed in gallbladder and bile ducts with the degree of associated obstruction.
Ann. Surg. 109: 161, 1939.

206. Phemister, D. B., Day, L., Hastings, A. B.
Calcium carbonate stones and their experimental production.
Ann. Surg. 96: 595, 1932.

207. Pibram, B. O. C.
The method of dissolution of common duct stones.
Surg. 22: 806, 1947.

208. Pibram, B. O. C.
Post-cholecystectomy syndromes.
J. A. M. A. 142: 1262, 1950.

209. Pibram, E. C.
Zur trage des cholesterinstoffinechels Wahrend der Schwangerschaft und in Wochenbett.
Arch. F. Gynak. 120: 90, 1923.

210. Plater - Quoted by Muleur, G.
Essai historique dur l'affection calculeuse de fois depuis Hippocrate jusqu'a Fourcroy et Pujol.
1801-1802, Paris, 1884.

211. Popper, H., Szanto, P. B.
Intrahepatic cholestasis (cholangiolitis).
G. E. 31: 683, 1956.

212. Potter, A. H.
Cholecystitis in childhood.
S. G. O. 66: 604, 1938.

213. Pulaski, E. J., Fusillo, M. H.
Gallbladder bile concentrations of the major antibiotics following intravenous administration.
S. G. O. 100: 571, 1955.

214. Rabinovitch, I. M.
The incidence of diabetes mellitus in disease of the gallbladder and its ducts.
C. M. A. J. 14: 296, 1923.

215. Radakovich, M., Greenlaw, R. H., Strain, W. H.
Interrelationship of gallbladder and pancreas.
Proc. Soc. Exp. Biol. Med. 6: 77, 1951.

216. Ravdin, I. S., Hagan, W. H.
Diseases of the biliary system.
Med. Clin. N. A. 35: 1679, 1951.

217. Ravdin, I. S., Johnston, C. G.
Gallbladder: Recent advances as applied to treatment.
Penns. M. J. 35: 357, 1932.

218. Rehfuss, M. E., Nelson, G. M.
Bacteriological studies in 2162 cases of gallbladder disease.
M. J. Dig. Dis. 1: 764, 1935.

219. Rehfuss, M. E., Nelson, G. M.
Experimental cholecystitis: Final results of vaccine and filtrate therapy.
S. G. O. 81: 455, 1945.

220. Rehfuss, M. E.
The etiology of cholecystitis.
G. E. 7: 665, 1946.

221. Rehfuss, M. E., Nelson, G. M.
The medical treatment of gallbladder disease.
W. B. Saunders Company, Phila., 1936.

222. Reid, S. E.
Effect of pancreatic juice on the gallbladder.
S. G. O. 89: 160, 1949.

223. Rich, A. R., Duff, G. L.
Experimental and pathological studies on the pathogenesis of acute haemorrhagic pancreatitis.
Bull. Johns Hopkins Hospital. 58: 212, 1936.

224. Richardson, M. W. J.
Boston Soc. M. Sc. 3: 79, 1898.

225. Riedel.
Berl. Klin. Woch. 30: 8-1, 1901.

226. Rienhoff, W. F., Pickrell, K. L.
Pancreatitis: An anatomic study of the pancreatic and extrahepatic biliary systems.
Arch. Surg. 51: 205, 1945.

227. Roberts, J. M.
Cholesterol and cystic duct obstruction in pathogenesis of gallstones.
N. W. Med. 52: 3, pp. 208, 1953.

228. Robertson, H. E.
Silent gallstones.
G. E. 5: 345, 1945.

229. Robertson, H. E.
The preponderance of gallstones in women.
Int. Abst. Surg. 80: 1, 1945.

230. Rokitansky, C.
Handbuch der speciellin pathologischen anatomie, Vol. 2
Wien: Braumueller, Seidel, 1842.

231. Rolleston, McNee,
Diseases of liver and bile ducts. 3rd Edition, 1929.

232. Rosenow, E. C.
J. A. M. A. 63: 1835, 1914.

233. Rous, P., Broun, G. O., McMaster, P.
Relation of carbohydrate to the output of bile pigment.
J. Exp. Med. 37: 421, 1923. (a).

234. Idem.
Studies of the total bile on the bile changes caused by a pressure obstacle to secretion and on hydrohepatosis.
J. Exp. Med. 37: 118, 1923. (b).

235. Idem.
Studies on total bile: The entero-hepatic circulation of bile pigment.
J. Exp. Med. 37: 699, 1923. (c)

236. Rous, P., McMaster, P. D., Drury, D. R.
Observations on some causes of gallstone formation.
J. Exp. Med. 39: 77, 1924.

237. Rous, P., Drury, D. R., McMaster, P. D.
Experimental cholelithiasis in the absence of stasis, infection and gallbladder influences.
J. Exp. Med. 39: 77, 1924.

238. Idem.
On certain special nuclei of deposition in experimental cholelithiasis.
J. Exp. Med. 39: 97, 1924.

239. Rous, P.
Physiological factors in the genesis of gallstones.
Proc. Inst. Med. Chicago, 7: 33, 1928.

240. Rousing, T.
Acta. Chir. Scand. 56: 103,-207, 1923-24.

241. Schade, H.
Ztschr. exper. Path. Ther. 8: 92, 1910.

242. Schaeffer, W.
Arch. Gyn. 150: 696, 1932.

243. Schenken, J. R., Coleman, F. C.
Cholelithiasis.
G. E. 4: 344, 1945.

244. Schoenaheimer-Sperry.
J. Biol. Chem. 187: 97, 1950.

245. Schwegman, C. W., Demuth, W. E.
Acute cholecystitis following operation for unrelated disease.
S. G. O. 97: 167, 1953.

246. Seifert. Quoted by Boysen, J.
Ueber die Struktur und die pathogenese der gallensteine.
Berlin. S. Karger. 1909.

247. Shapira, P. F., Kasabach, Haig, H.
Duodenal ileus, effect of chronic duodenal obstruction on evacuation of the gallbladder.
Arch. Surg. 14: 752, 1927.

248. Singer. See Graham, 1928.

249. Siperstein, M. D., Jayko, M. E., Chaikoff, I. L., Dauben, W. G.
Nature of the metabolic products of $C^{14}$ cholesterol excreted in the feces.
Proc. Soc. Exp. Biol. Med. 81: 720, 1952.

250. Smyth, M. J.
Congenital basence of the gallbladder.
Lancet. 256: 301, 1949.

251. Sobotka, H.
Physiological chemistry of bile.
(Williams and Wilkins Co.), Baltimore, 1937.

252. Sodeman.
Pathologic physiology.
(Gallbladder and pancreas R. Eiman).

253. Sotti, G., Torri, O.
Pathologica 12: 369, 1920.

254. Sparkman, R. S.
Abdominal emergencies following unrelated surgical procedures.
Ann. Surg. 135: 863, 1952.

255. Sperry, W. M.
A study of the relationship of bile to the fecal lipoids with special reference to certain problems of sterol metabolism.
J. Biol. Chem. 71: 351, 1926.

256. Srere, P. A., Chaikoff, I. L., Dauben, W. G.
Sources of cholesterol.
J. Biol. Chem. 176: 829, 1948.

257. Stewart, M. J.
Autopsy report on 6000 cases.
Lancet 2: 617, 1931.

258. Stockinger, W.
Allergic cholecystopathy and secondary cholecystitis following dysentery.
Deut. Med. Wschr. 73: 42, 1948.

259. Strohl, L. E., Diffenbaugh, W. G.
Frequency of gallbladder operations in Elderly patients.
S. G. O. 97: 467, 1953.

260. Sweet, J. E.
The formation of gallstones.
Ann. Surg. 101: 624, 1935.

261. Schwegman, Demuth. See Gleen, Wantz, 1956.

262. Swell, L., Flick, D. F.
The effect of dietary fat and cholesterol on the blood cholesterol level in rats.
Am. J. Phys. 174: 51, 1953.

263. Tennent, D. M., Zanett, M., Atkinson, D. I., Gunther, K., Opdyke, D. F.
The origin of blood cholesterol.
Am. Soc. Study of Arteriosclerosis, Chicago, Nov., 1956.

264. Thenard. Quoted by Thudicum.

265. Thomas, C. G., Womack, N. A.
Acute cholecystitis, its pathogenesis and repair.
A. M. A. Arch. Surg. 64: 590, 1952.

266. Thudicum, J. L. W.
A treatise on gallstones, their chemistry, pathology and treatment.
J. Churchill and Sons, London, 1863. pp. 323.

267. Tonelli. L.
Malaria, relationship to etiology of gallstones.
Policlinico. 38: 241, 1931.

268. Toscano, C.
Fegato e vie Biliari.
Polinclinico. 38: 1597, 1931.

269. Tourinimi.
Beitr. z path. Anat. u. z. Allg. Path. 72: 456, 1924.

270. Twiss, J. R., Carter, R. F., Fishman, B. S.
Infection in chronic cholecystitis.
J. A. M. A. 147: 1221, 1951.

271. Vallejo, E. A.
Acute tuberculous cholecystitis.
G. E. 16: 501, 1950.

272. Venema, T. A.
Berl. Klin. Wschnschr. 54: 815, 1917.

273. Voit, F.
Beitrage zur Frage der Sekretion und Resorption in Dunndarm.
Ztschr. f. Biol. 11: 325, 1892.

274. Vollmer, T. See Long, E.

275. Von Babarczy, M.
Die Bedeudung der cholesterins in der Diatetile der cholecystopathien.
Ztschr. f. Klin. Med. 133: 656, 1938.

276. Walsh, E. L., Ivy, A. C.
Observations on the etiology of gallstones.
Ann. Int. Med. 4: 134, 1930.

277. Walsh, E. L.
Etiology of gallbladder calculi.
Arch. Path. 15: 688, 1933.

278. Walters, W.
Pathology and physiology of stone in the common duct.
Clinical and surgical significance.
S. G. O. 63: 417, 1936.

279. Walters, W., Cleveland, W. H.
Surgical lesions of the pancreas.
Arch. Surg. 42: 819, 1941.

280. Wainwright, C. W.
Intrahepatic obstruction.
New Eng. J. Med. 244: 161, 1951.

281. Weens, H. S.
Cholelithiasis in sickle-cell anemia.
Ann. Int. Med. 22: 182, 1945.

282. Weiser, H. B.
The Rice Institute Pamphlet. 20: 291, 1933.

283. Weitz, G.
Tuberculosis of the gallbladder.
Langenbechs Arch. u. Deut. Zschr. Chir. 280: 318, 1955.

284. Welch, L. H.
Bull. J. Hopkins Hosp. 2: 96, 1891.

285. Wells, H. G.
Chemical pathology. 5th Ed. p. 505, W. B. Saunders & Co., 1925.

286. Weltz, G. H.
Ueber Divertikel der gallenblase.
(Kiel L. Handorff, 1894).

287. Westphal, K.
Muskelfunktion, nervensystem und pathologie der gallenweige.
Ztschr. F. Klin. Med. 96: 22, 1923.

288. Westphal, K., Gleichmann, F.
Ztschr. f. Klin. Med. 115: 329, 1931.

289. Whellon, H.
Symptoms associated with duodenal retention and reverse motility.

290. Whipple, G. H., Hooper, C. W.
Am. J. Phys. 40: 349, 1916.

291. Whipple, G. H.
Arch. Int. Med. 29: 711, 1922. (a).

292. Whipple, G. H.
Physio. Rev. 2: 446, 1922. (b).

293. Whitaker, L. R.
J. A. M. A. 88: 1542, 1927.

294. Wiggers, C. J.
Physiology of health and disease.
(Lea and Febiger), Philadelphia, 1934.

295. Wilder, R. M.
Clinical diabetes mellitus.
W. B. Saunders. Phila., 1940.

296. Wilkie, A. L.
Bacteriology of the gallbladder.
B. J. Surgery. 15: 450, 1928.

297. Wilkie, D. P. D.
Bett, History of some common diseases.
Oxford Univ. Press. London, 1934.

298. Williams, B., McLachlan, D. G. S.
Lancet. 2: 342, 1930.

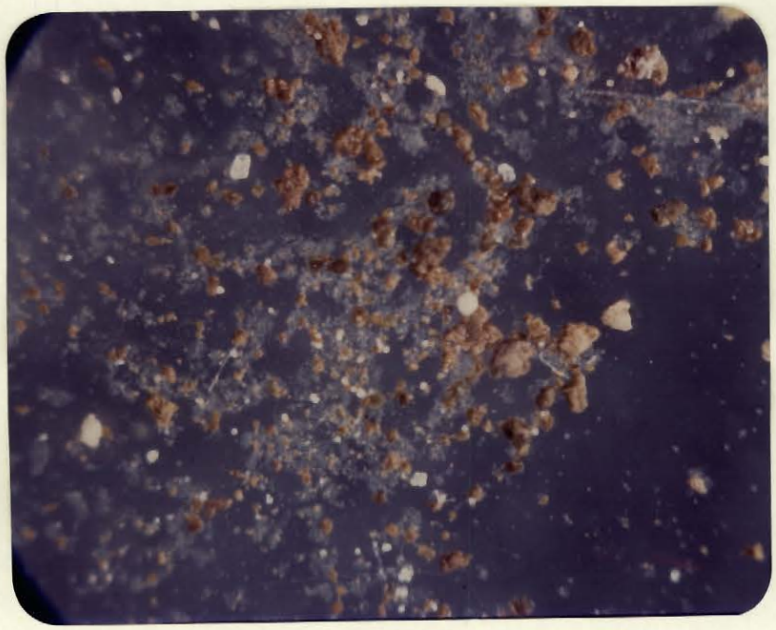
299. Womack, N. A., Haffner, H.
Symposium on abdominal surgery: cholesterolosis, its significance in badly damaged gallbladder.
Ann. Surg. 119: 391, 1944.

300. Wright, S.
Applied physiology. 8th Ed. (Oxford University Press), London.

301. Zaslow, J., Hewlett, T. H., Major, M. C., Lorry, R. W.
The excretion and concentration of aureomycin in the abnormal human biliary tract.
G. E. 16: 475, 1950.

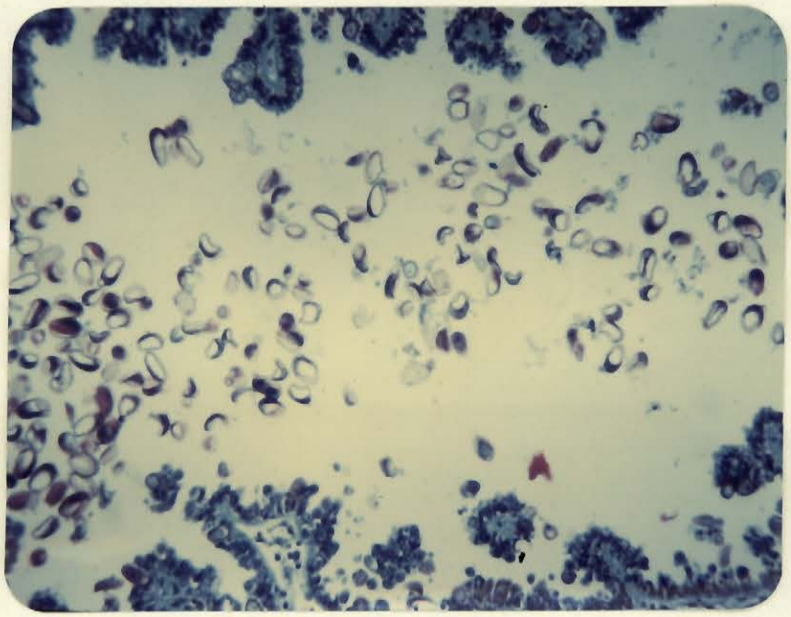
# PHOTOMICROGRAPHS

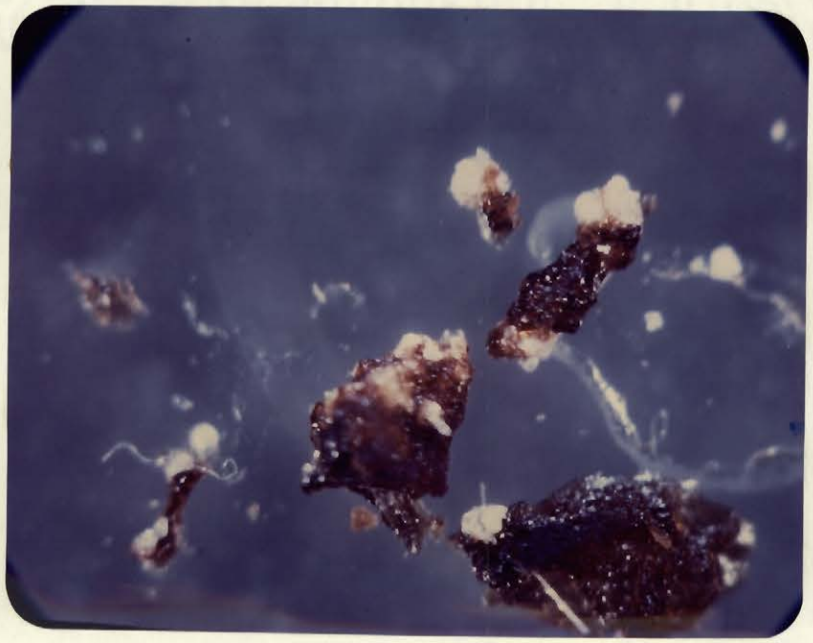
Nos. 1 - 42.



No. 1: Lithogenic diet, Hamster 43A. Aggregation of cholesterol on pigment. L.P.

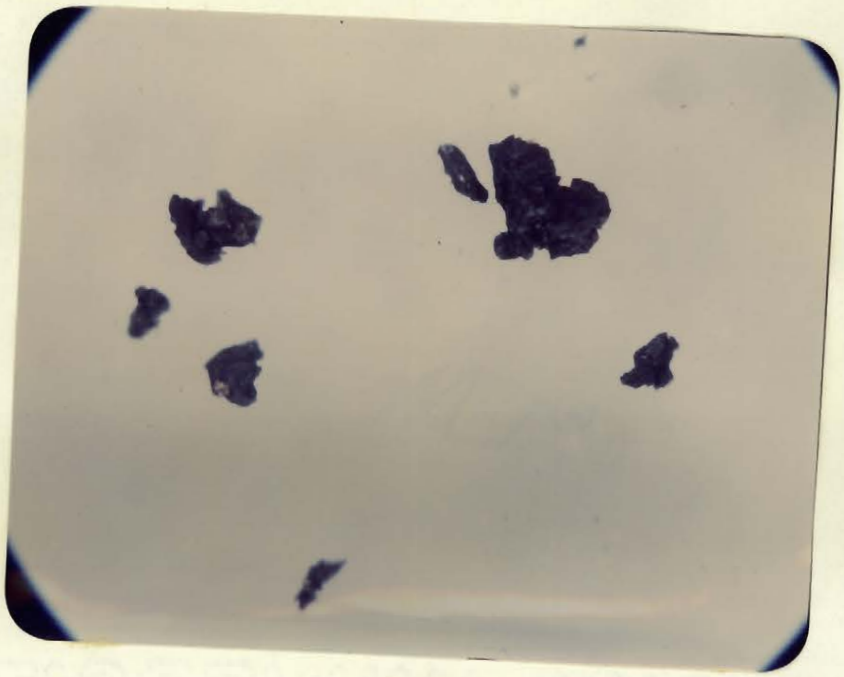
No. 2: Lithogenic diet, Hamster 20A. Cholesterol and pigment calculus. L.P.

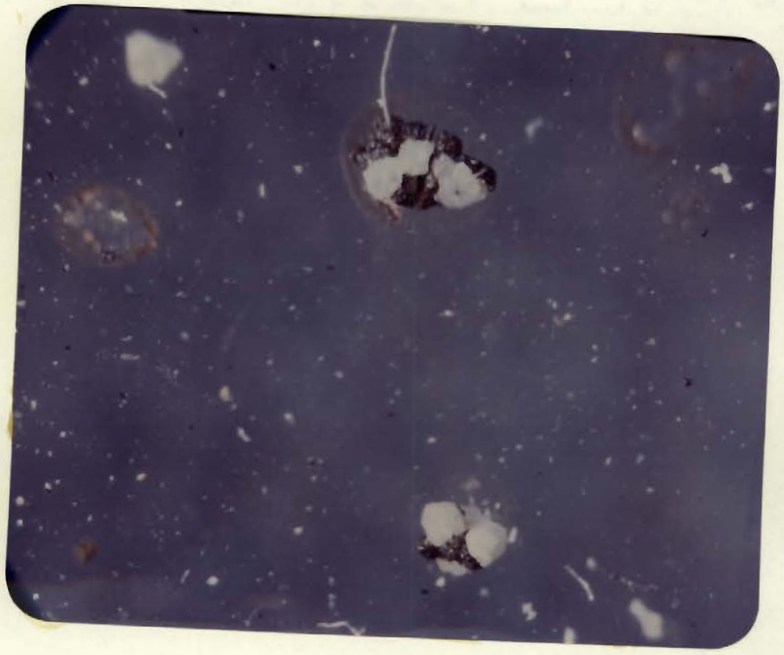




No. 3: Hamster infected with Eimeria Stiedae (coccidia) on "L" diet. (L.P.)

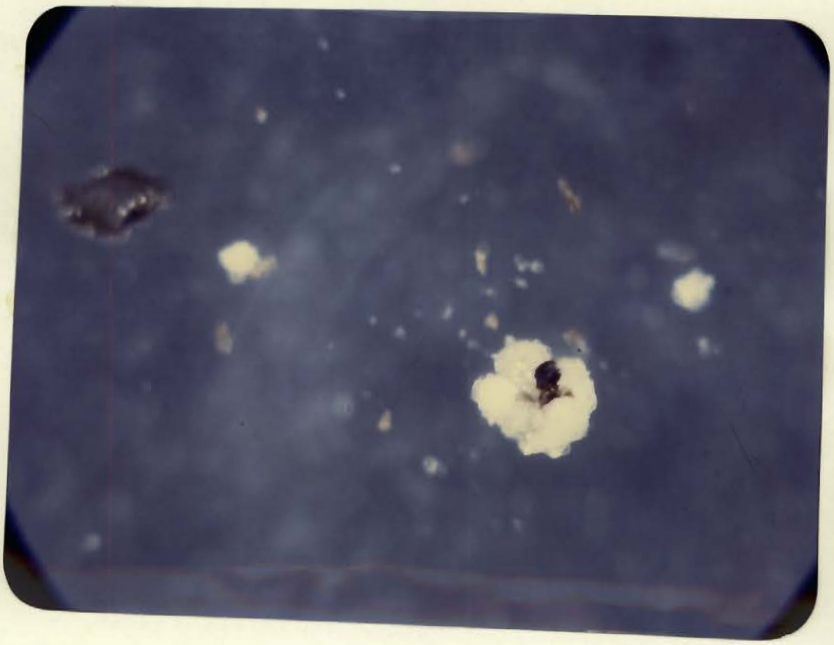
No. 4. Coalescence of blood stained pigment and cholesterol. Hamster on "L" diet. L.P.





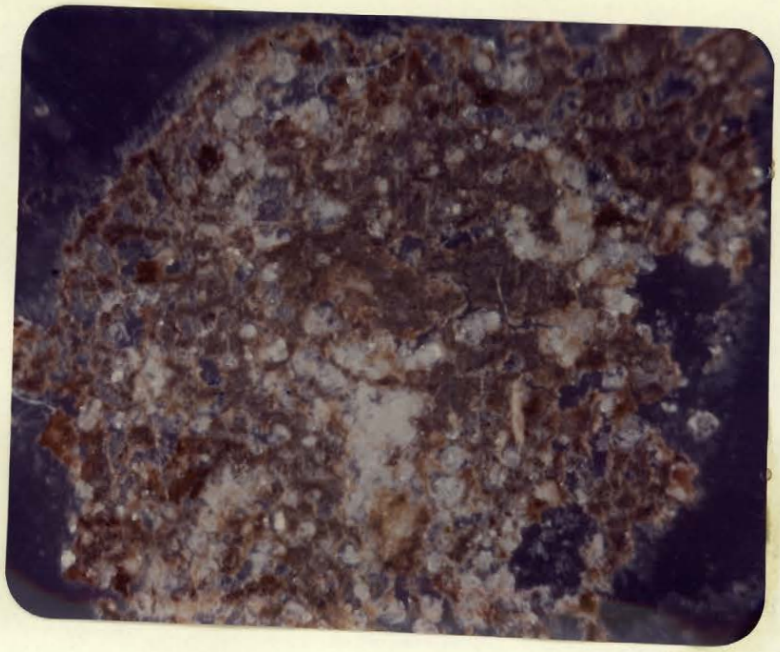
No. 5: Greenish block calculi-like human pigment debris. Hamster on "L" diet. (L.P.)

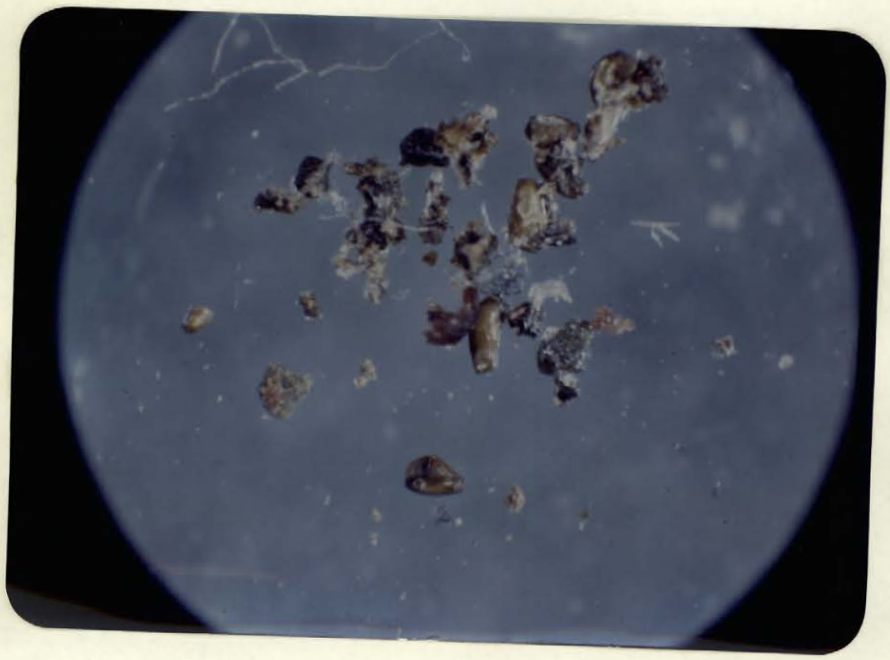
No. 6. Hamster on "L" diet plus oestrogens. Pigment and cholesterol calculi. (L.P.)



No. 7. Hamster on "L" diet plus oestrogens pigment and cholesterol calculi. (L.P.)

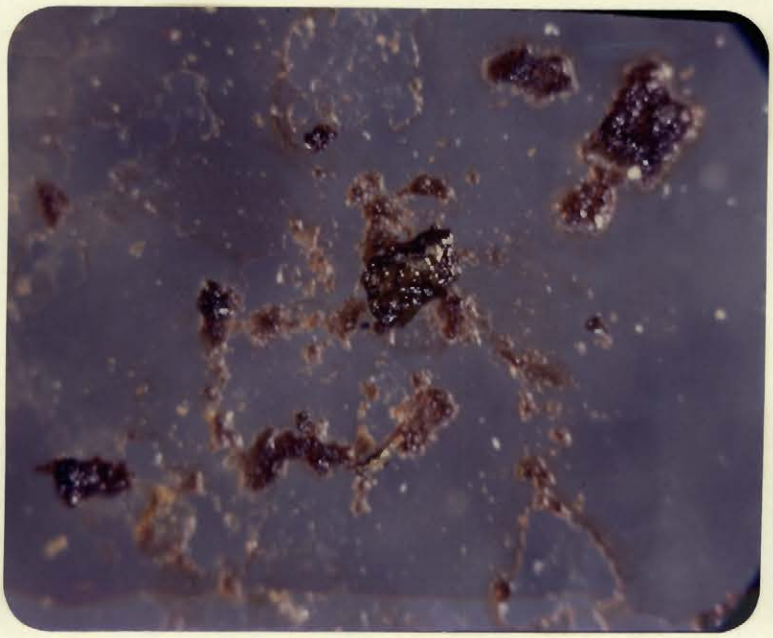
No. 8. Hamster on "L" diet plus progesterone cholesterol and pigment calculi. (L.P).





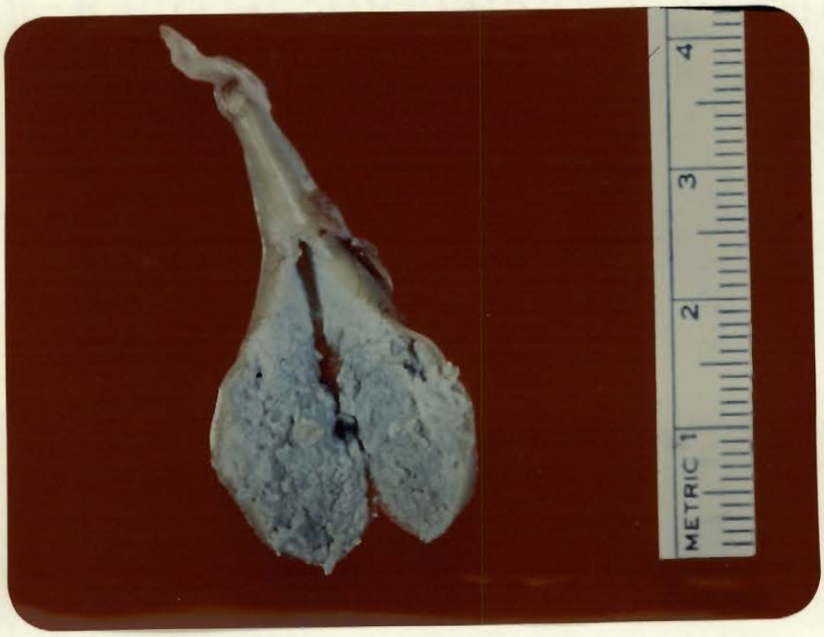
No. 9: Pigment cholesterol calculi. Hamster on "L" diet plus cortisone. (L.P)

No. 9a: Hamster on "L" diet plus chlortetracycline. Pigmented cholesterol calculi. (L.P.)



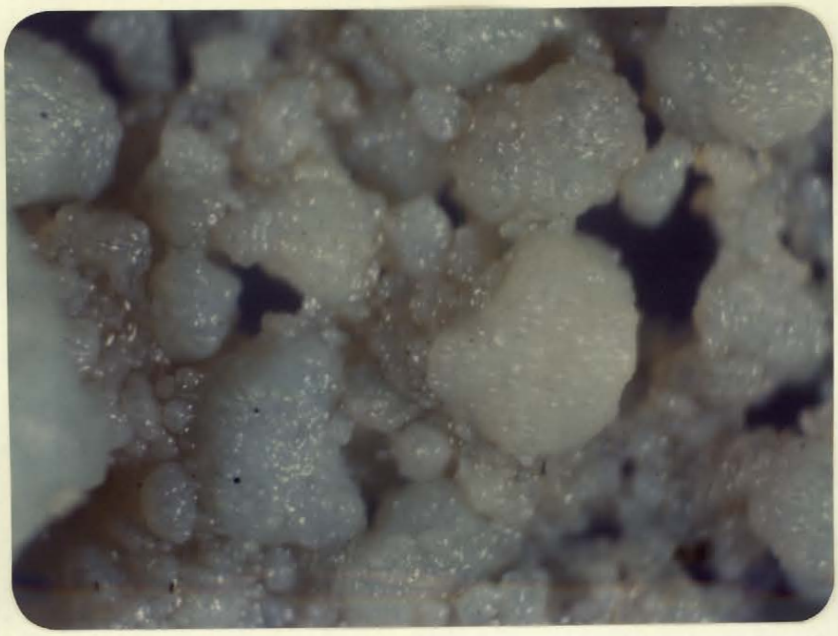
No. 10: Pigment concretions. Hamsters on "L" diet and yeast supplement. (L.P.)

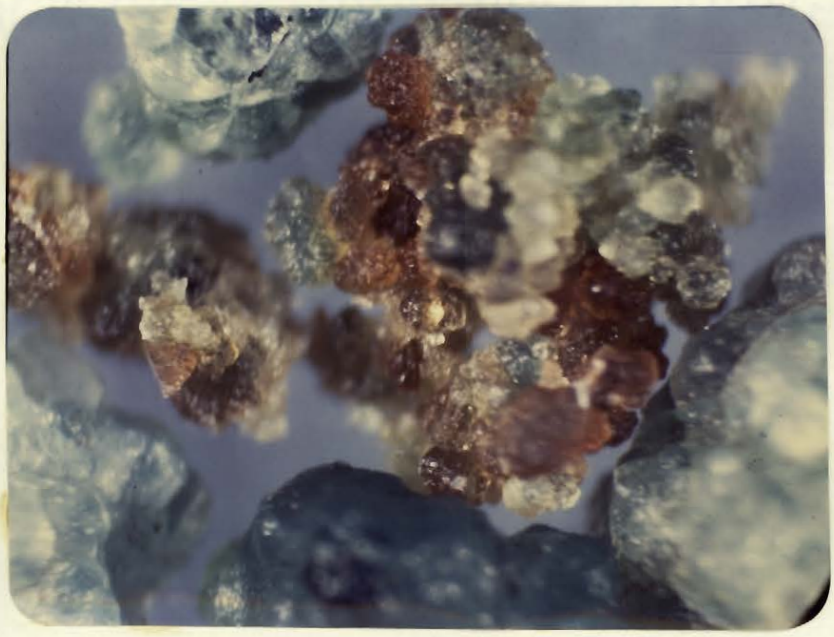
No. 11: Gelatinous deposit. Rabbit on "L" diet. (x100).




No. 11a: Gelatinous white deposit, Rabbit on "L" diet. (L.P.)

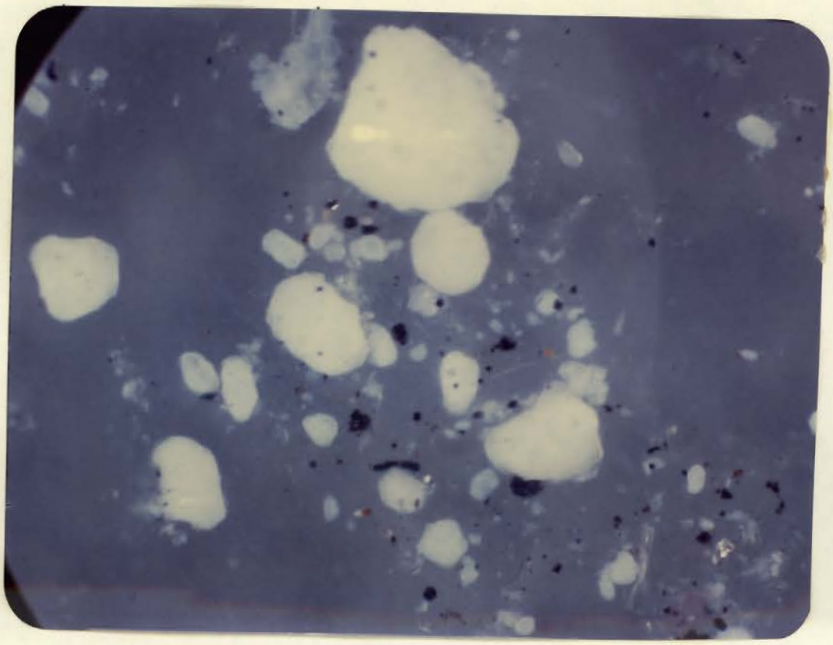
No. 12: Rabbit on 3 beta-cholestanol. G.B. packed solid with gelatinous concretions. (x40).

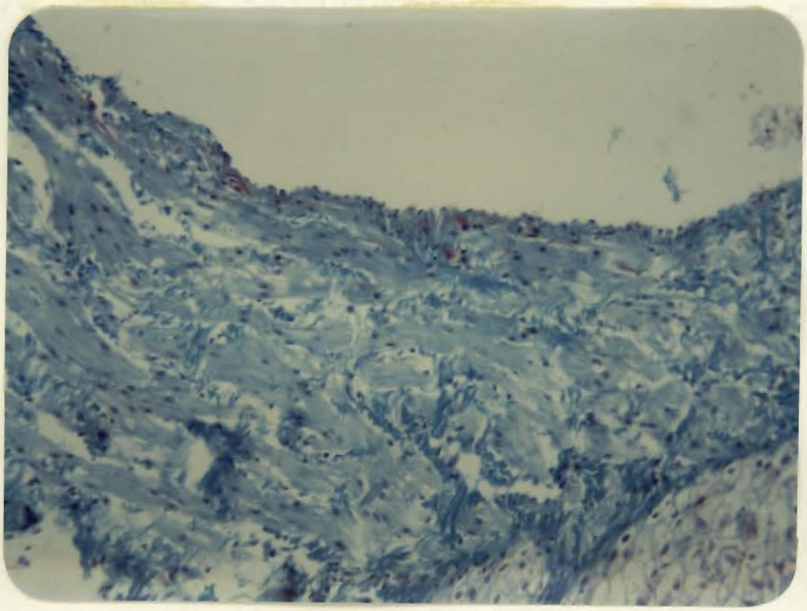




No. 13: Rabbit on 3 beta-cholestanol diet. Gelatinous concretions (x 140) showing aggregation of globules.

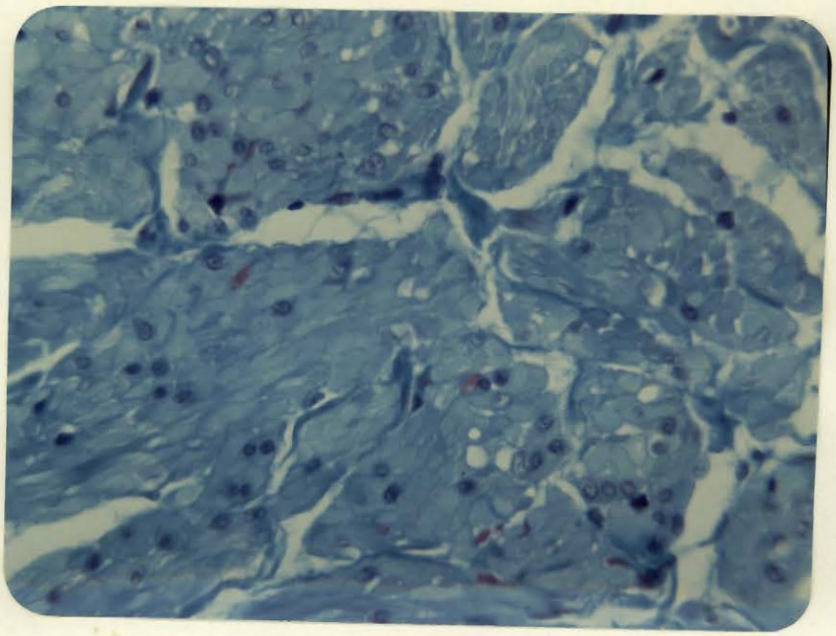
No. 14: Rabbit on 3 beta-cholestanol diet crystalline blood pigment stained calculi. (x140).

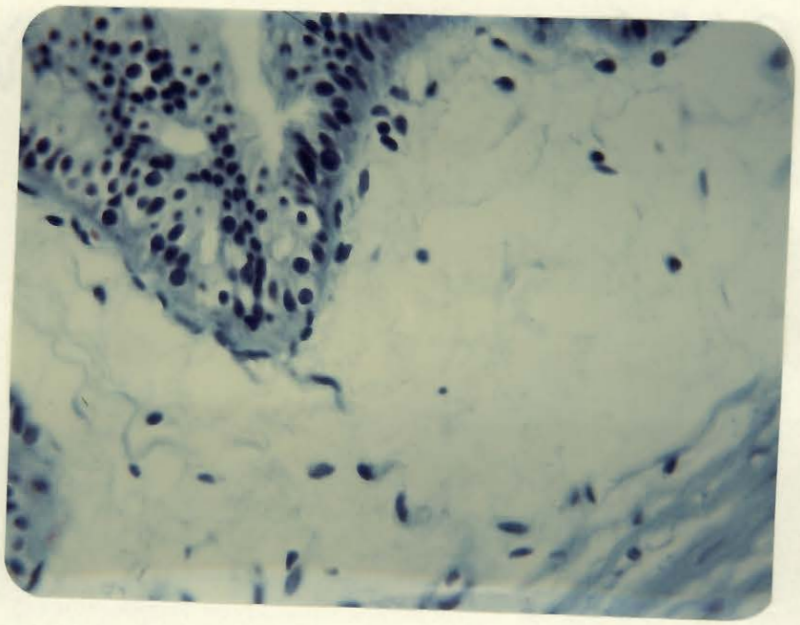




No. 15: Rabbit on 3 beta-cholestanol, pigment concretions and 'gel' forms of concrements. (Bile Acid) (x40).

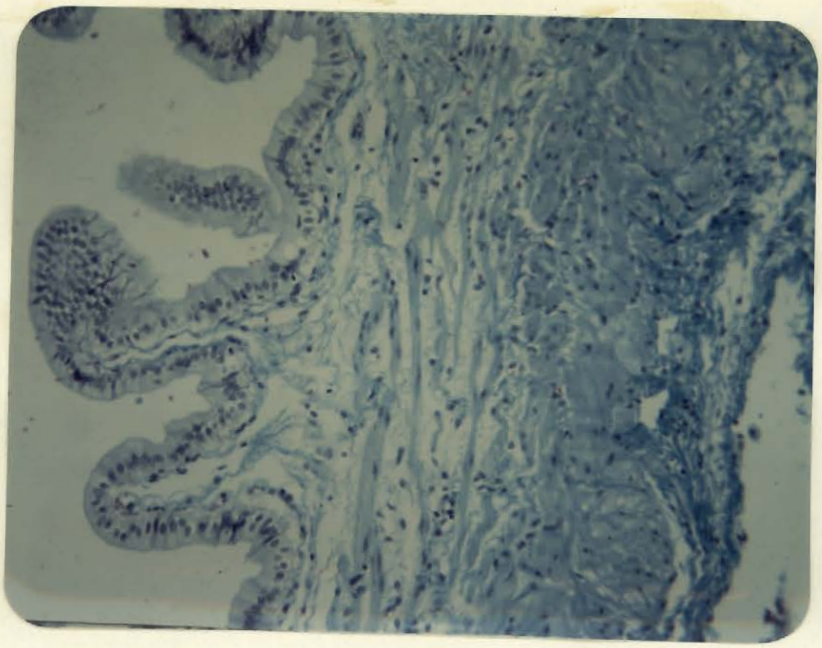
No. 16: Cholecystectomized rabbits on 3 beta-cholestanol diet. C.B.D. inflammatory response. (x100).

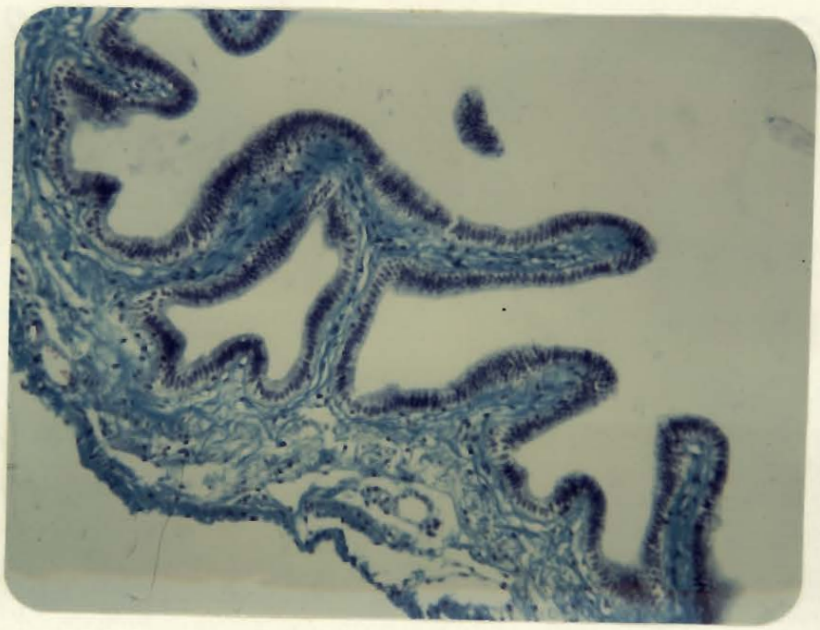




No. 17. Rabbit cholecystectomized on 3 beta-cholestanol diet. C.B.D. (x400).

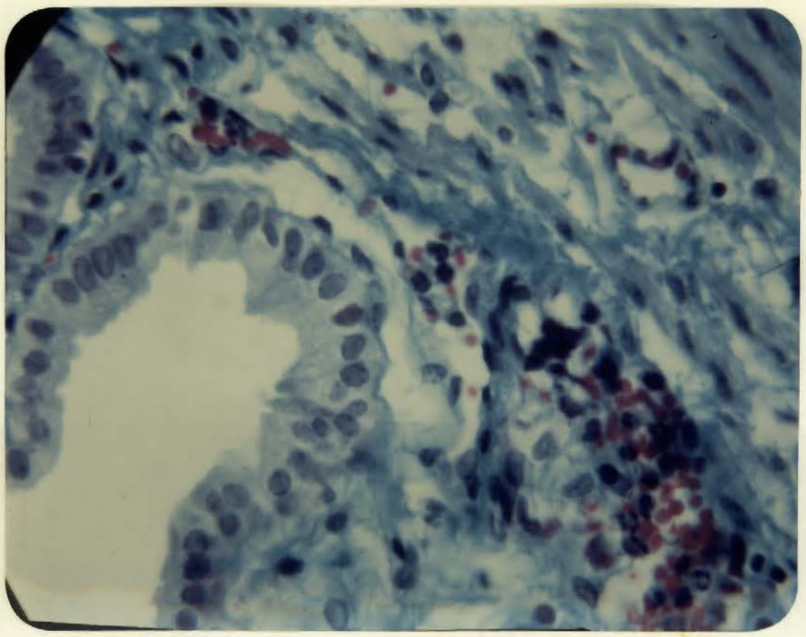
No. 18. Rabbit on 3 beta-cholestanol diet. G.B. (x400).

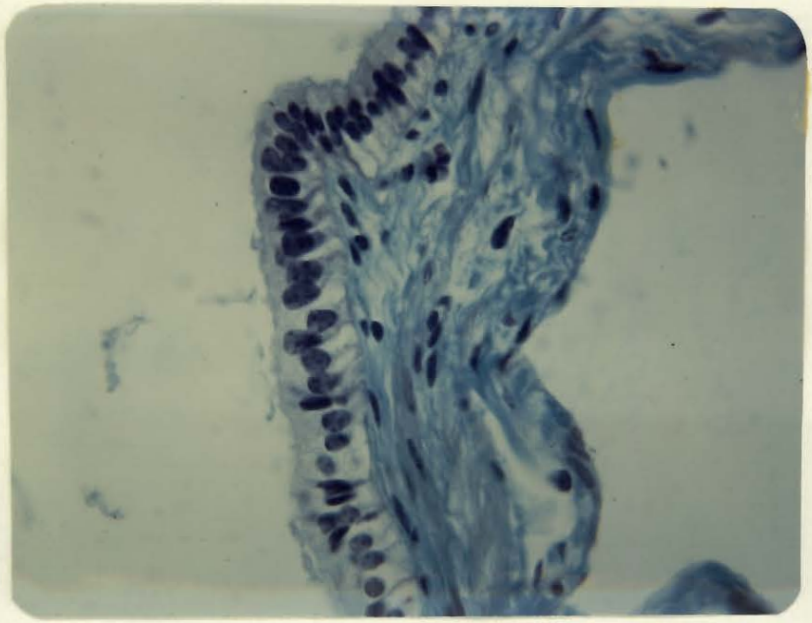




No. 19: Guinea pig 3 beta-cholestanol diet. Gallbladder oedema. (x140).

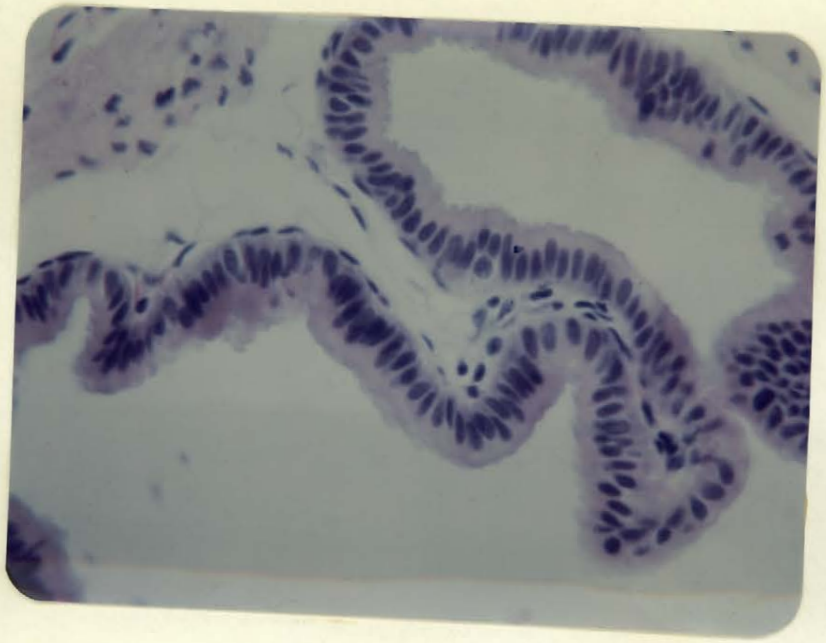
No. 20: Hamster control gallbladder. (140).

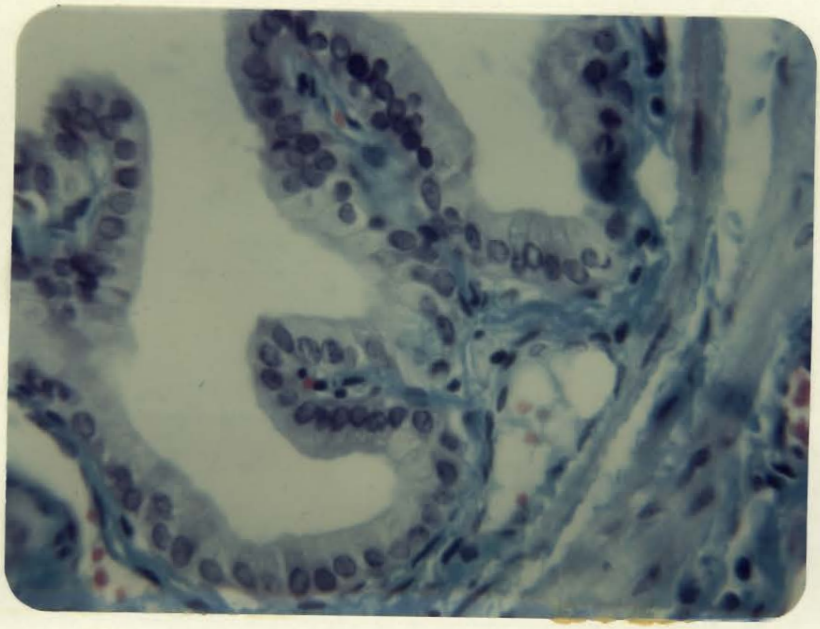




No. 21: Hamster on "L" diet plus cortisone G. B. (x 400).

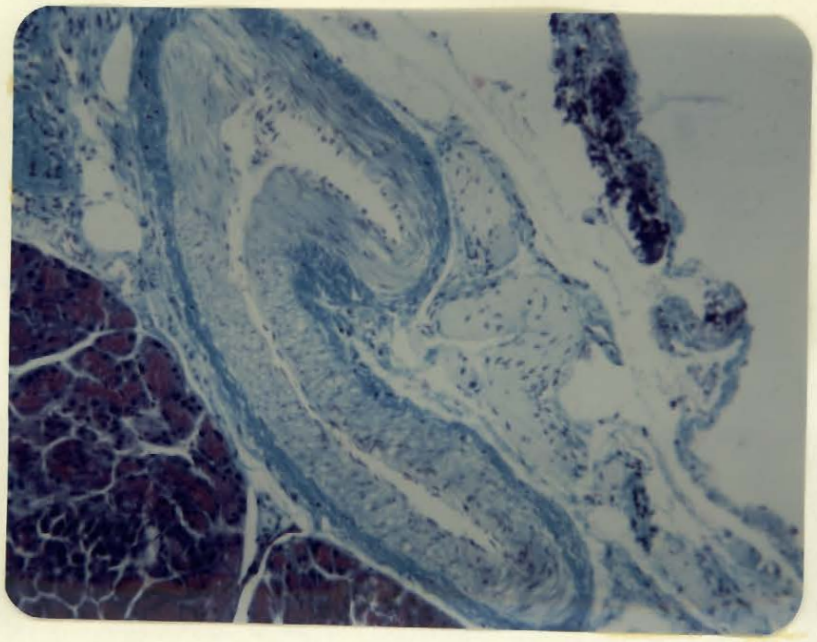
No. 22: Hamster on "L" diet plus oestrogens G. B. (x 400).

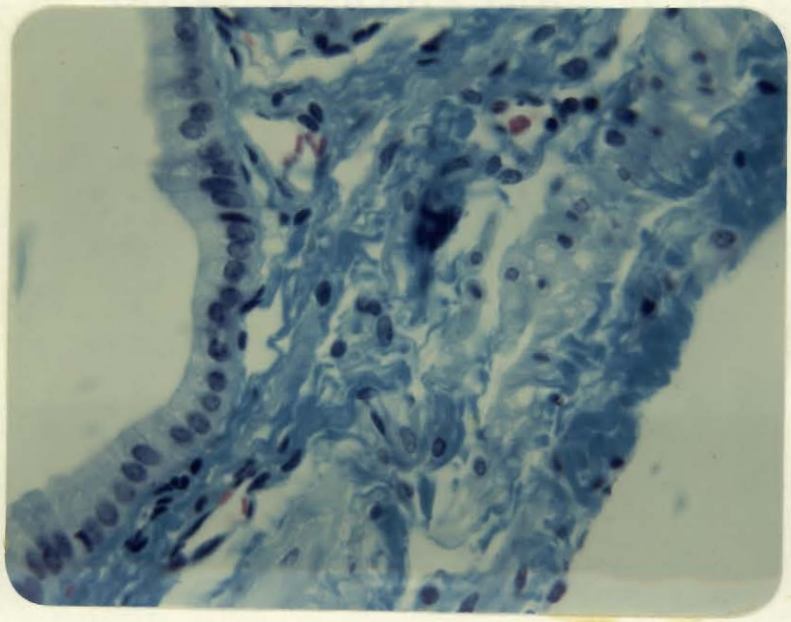




No. 23: Control rabbit gallbladder. (x400).

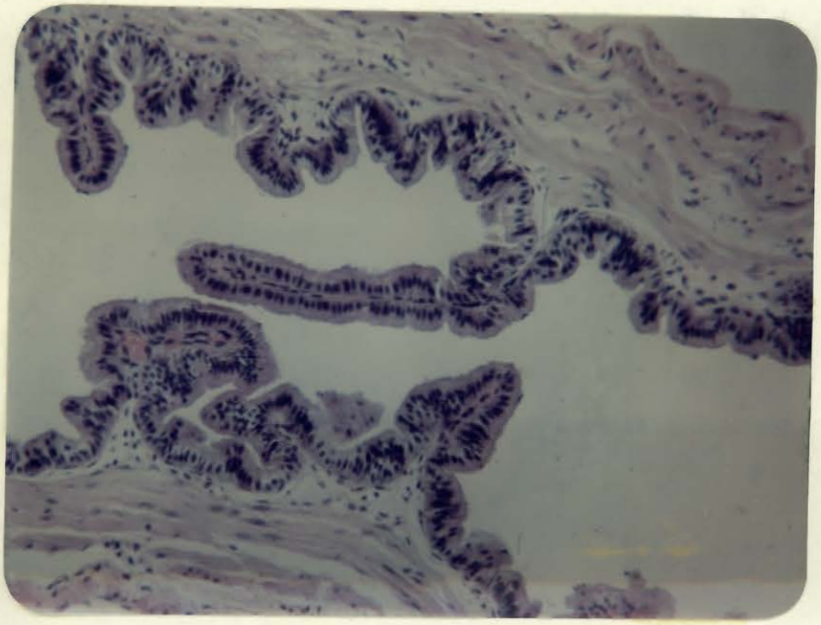
No. 24: Guinea pig normal control gallbladder. (x400).





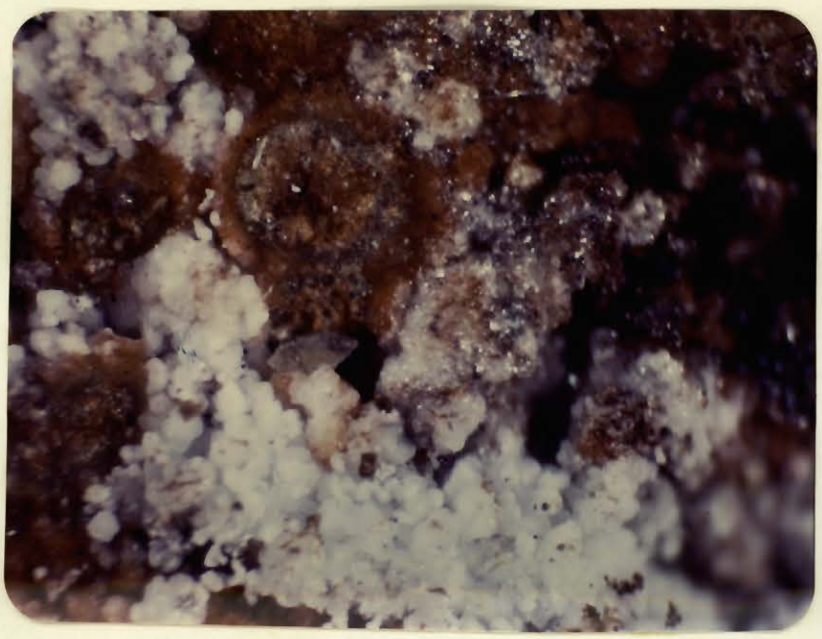
No. 25: Guinea pig on 3 beta diet. (x80).

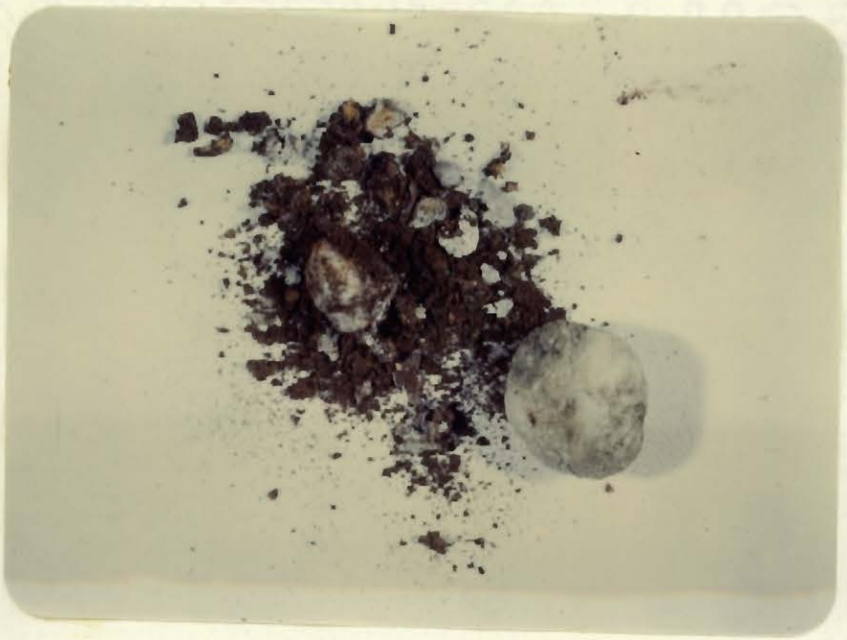
No. 26: Rat on 3 beta diet. C.B.D. (x400).



No. 27: G.P. on 3 beta diet. Gallbladder crystalline and gelatinous concrements.

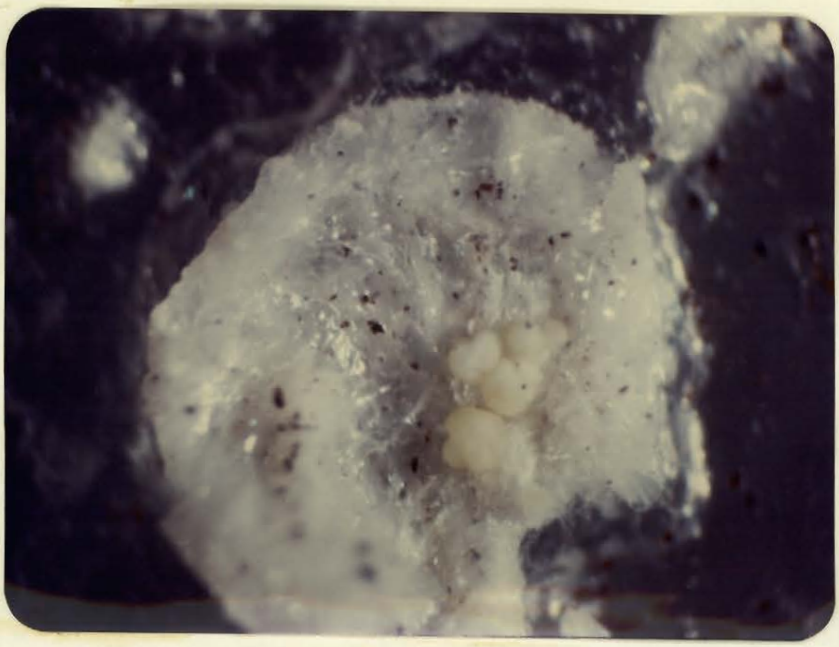
No. 28: Rabbit control gallbladder. (x40).

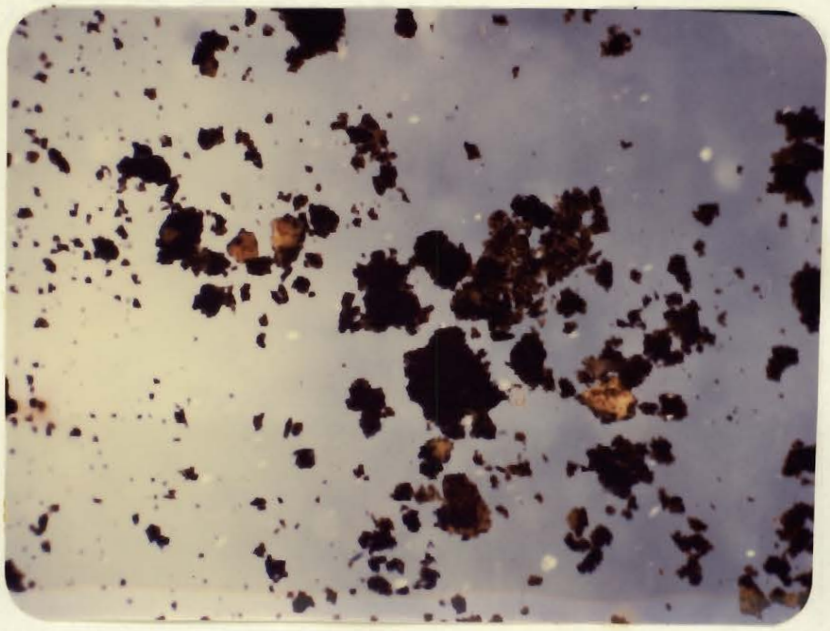




No. 29: Section of large human calculus treated with ether alcohol. Small cholesterol circular aggregates shown. (x20).

No. 30: Twin calculi: 1 partly dissolved after 48 hours in ether. Hard core shown. (x 20).



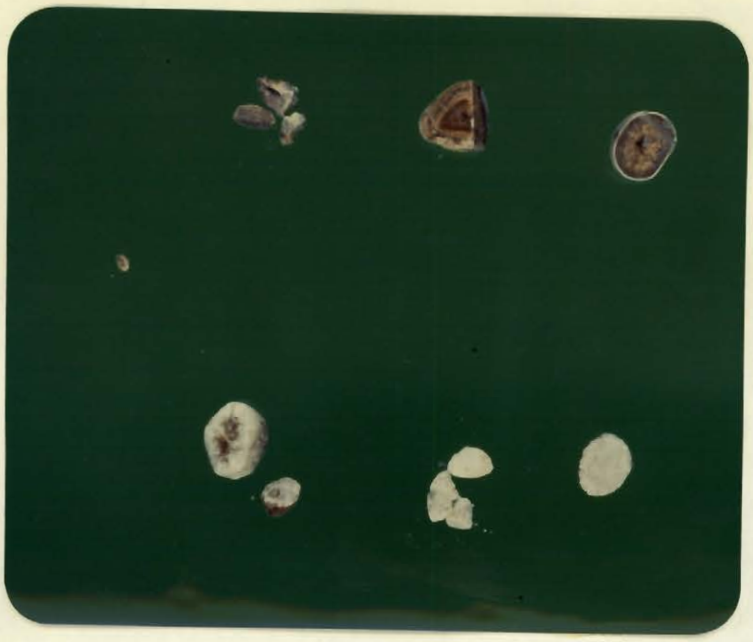


No. 31: Artificially produced small aggregates of 3 beta-cholestanol; crystallization out of xylene on human debris. (x 25).

No. 32: Human calculi insoluble debris. (Ether, alcohol, zylene). (x25).

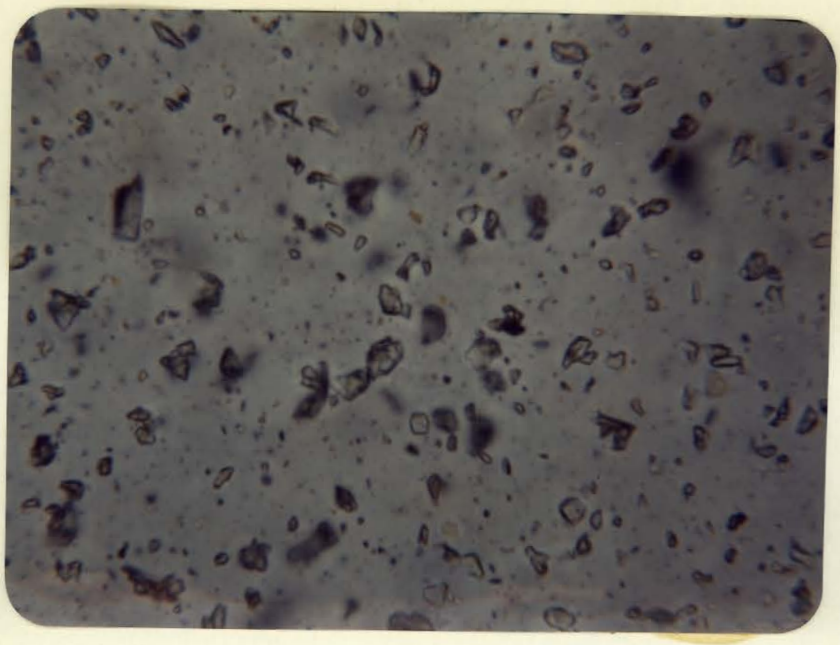

No. 33: X-ray appearance of 6A calculi chosen for petrographic study.

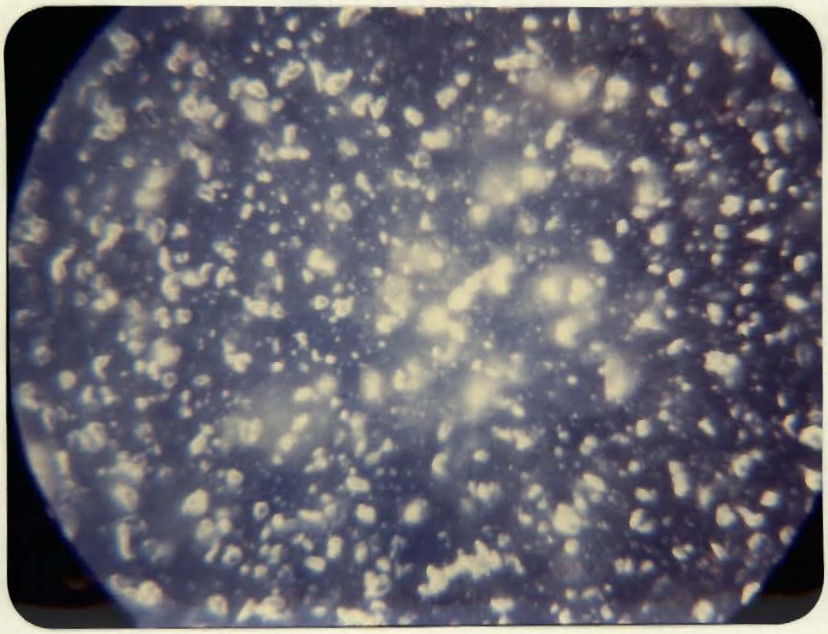
No. 34: Note calculus with adherent sister calculus to outer shell. (Same 6 calculi).



No. 35: 6 calculi used in petrographic series.

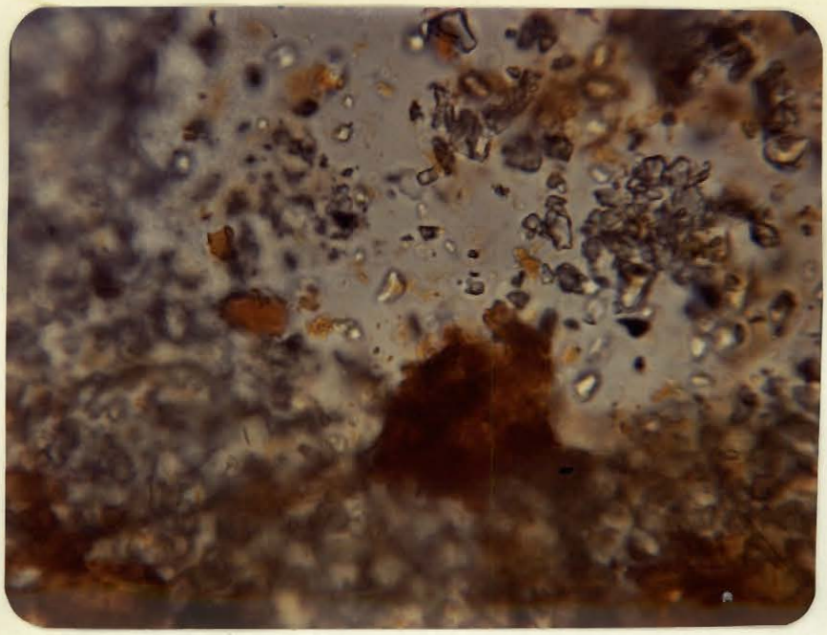
No. 36: Microscopic appearance cholesterol crystals from cholesterol solitaire. (x240).

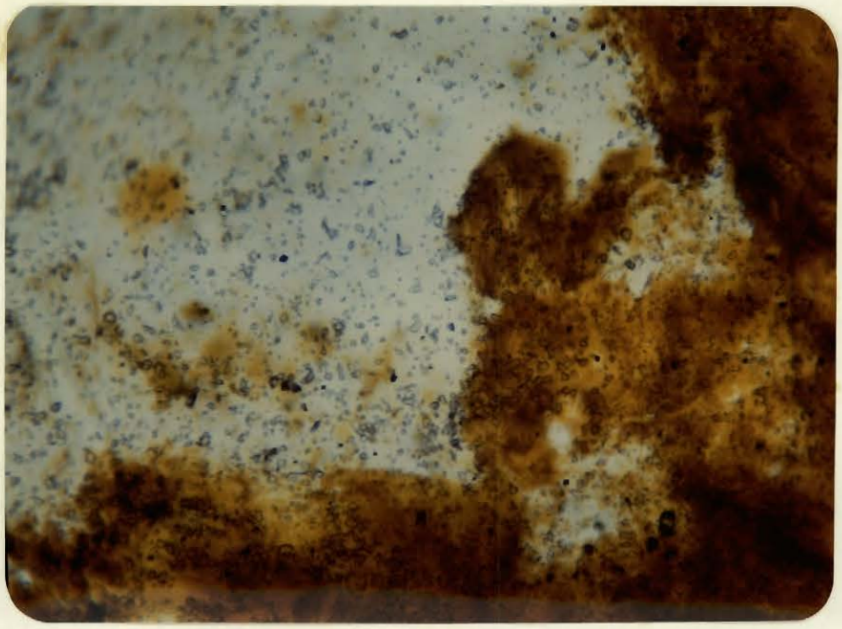




No. 37: Centre of cholesterol calculus (3) showing widely spaced particulate crystals. (x400).

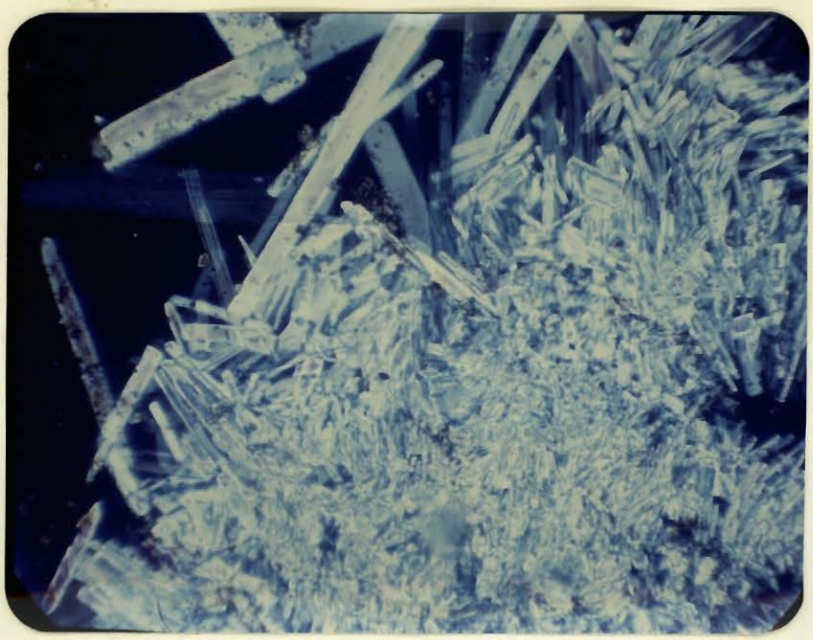
No. 38: Microscopic examination of petrographic section under dark ground illumination. Centre of cholesterol solitaire. (3). (x 240).

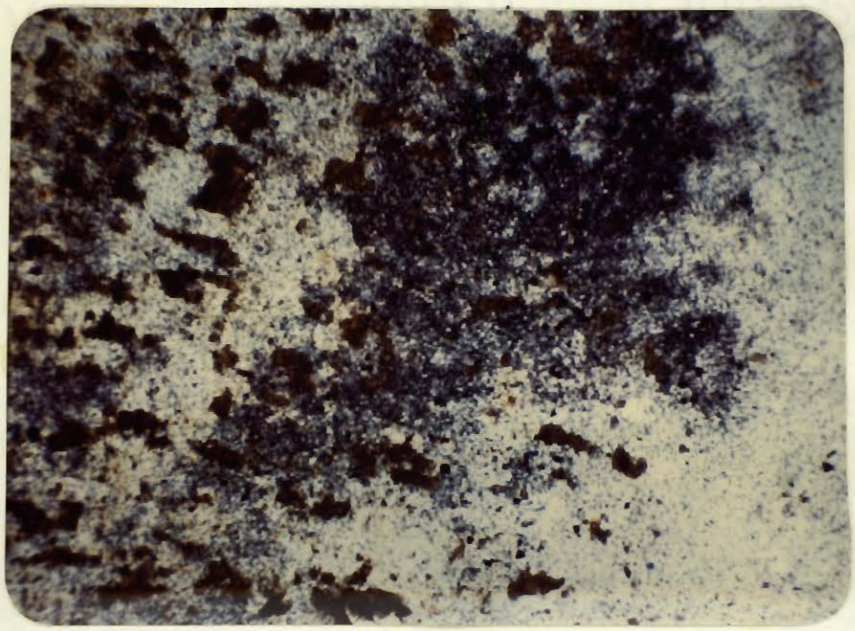




No. 39: Centre of mixed human calculus, (2) petrographic section. Spaces clearly shown. (x400).

No. 40: Centre of mixed calculus showing pigmented Liesegang ring. (x100).





No. 41: Cholesterol calculus, petrographic section examined under polarizing microscope. Cholic acid crystals. (x140).

No. 42: Heavily pigmented calculus. Liesegang rings. Petrographic section. (x20).

TABLE 5

HAMSTERS ON LITHOGENIC DIET (L)

| NO. | SEX | DAYS | MEDICATION | FREE. BLOOD | TOTAL. CHOLESTEROL | BILE CHOLESTEROL | pH | CONCREMENTS |
|---|---|---|---|---|---|---|---|---|
| H.L. 1 | M | 7 | | 62.1 | | Insufficient bile | Alk. | + 1 |
| " 2 | F | 7 | | 30.8 | 98.5 | " | " | + 1 |
| " 3 | M | 12 | | 33.3 | 92 .4 | " | " | + 1 |
| " 4 | F | 19 | | 39.6 | 101.0 | " | " | + 1 |
| " 5 | M | 25 | | 40.7 | 114.0 | 28.6 | " | + 1 |
| " 6 | F | 31 | | 40.7 | 99.0 | 1.B | " | + 1 |
| " 7 | M | 37 | | 42.4 | 191.0 | 24.2 | " | + 1 |
| " 8 | F | 43 | | 44.2 | 151.6 | 32.2 | " | + 1 |
| " 9 | M | 49 | | 61.3 | 145.3 | 1.B | " | + 1 |
| " 10 | F | 55 | | 56.4 | 126.7 | " | " | + 1 |
| " 11 | M | 60 | | 43.2 | 114.9 | " | " | + 1 |
| " 12 | F | 114 | | 48.9 | 137.6 | " | " | + 1 |
| " 13 | M | 114 | | 50.6 | 129.3 | " | " | + 1 |
| " 14 | F | 31 | Yeast | 34.1 | 126.7 | 16.2 | " | + 1 |
| " 15 | M | 37 | " | 29.1 | 112.4 | 8.6 | " | + 1 |
| " 16 | F | 43 | " | 38.6 | 86.5 | " | " | + 1 |
| HC 17 | M | 25 | Cortisone | 76.6 | 191.0 | " | " | + 1 |
| " 18 | F | 31 | " | 81.3 | 212.6 | " | " | + 1 |
| " 19 | M | 43 | " | 75.9 | 198.7 | " | " | + 1 |
| " Controls | | | Average | 39.2 | 117.9 | 26.6 | " | Negative |

TABLE 5

TABLE 6

HAMSTERS ON LITHOGENIC DIET (L)

| NO. | SEX | DAYS | MEDICATION | FREE. BLOOD | TOTAL. CHOLESTEROL | G.B. BILE CHOLESTEROL | pH | CONCREMENTS |
|---|---|---|---|---|---|---|---|---|
| HA 7 | F | 28 | Chlortetra-cyline | 29.0 | 134.9 | | Alk | +1 |
| 8 | M | 28 | " | 31.8 | 120.1 | | " | Neg |
| 9 | F | 28 | " | 34.7 | 155.1 | | " | +1 |
| 10 | M | 28 | " | 45.9 | 114.2 | | " | Neg |
| 11 | F | 28 | " | 43.4 | 121.8 | | " | Neg |
| 12 | M | 28 | " | 42.9 | 126.8 | | " | +1 |
| 13 | F | 28 | " | 38.6 | 118.3 | | " | +1 |
| HO 3 | F | 28 | Oestrogen | 28.6 | 76.0 | | " | Neg |
| 4 | M | 28 | " | 41.7 | 121.0 | | " | Neg |
| 5 | F | 28 | " | 36.8 | 127.0 | 66 | " | +1 |
| 6 | M | 28 | " | 32.3 | 93.9 | | " | +1 |
| 7 | F | 28 | " | 63.2 | 169.0 | 87 | " | Neg |
| 8 | M | 28 | " | 44.4 | 118.0 | | " | +1 |
| 9 | F | 28 | " | 44.3 | 148.8 | 83 | " | Neg |
| 10 | M | 28 | " | 60.8 | 218.9 | 178 | " | +1 |
| 11 | F | 28 | " | 65.7 | 193.5 | 109 | " | +1 |
| 12 | M | 28 | " | 50.9 | 152.5 | 112 | " | Neg |
| HP13 | M | 28 | Progesterone | 35.9 | 109 | 128 | " | Neg |
| 14 | M | 28 | " | 44.8 | 129 | | " | Neg |
| 15 | M | 28 | " | 44.2 | 116 | 163 | " | +1 |
| 16 | F | 28 | " | 39.9 | 133 | 67 | " | +1 |
| 17 | F | 28 | " | 34.7 | 122 | 75 | " | Neg |
| 18 | F | 28 | " | 49.2 | 137 | | " | +1 |
| 19 | F | 28 | " | 46.6 | 136 | 59 | " | +1 |

TABLE 6

TABLE 7

RABBITS ON LITHOGENIC DIET (L)

| NO. | SEX | DAYS | FREE BLOOD CHOLESTEROL | TOTAL CHOLESTEROL | G.B. BILE CHOLESTEROL | pH | CONCREMENTS | REMARKS |
|---|---|---|---|---|---|---|---|---|
| A 9 | F | 12 | 119.0 | 345.0 | 73.4 | 7.2 | Pigt.+ | |
| 10 | F | 12 | 109.0 | 328.0 | 252.0 | 7.4 | Chol.+ | Coccidia |
| 11 | M | 18 | 50.0 | 116.0 | 74.7 | 7.2 | Chol.+ | Coccidia |
| 15 | F | 18 | 111.1 | 387.0 | 64.9 | 7.6 | Pigt.+ | |
| 16 | F | 24 | 63.9 | 210.3 | 141.3 | 7.4 | Neg | |
| 17 | F | 24 | 54.7 | 149.5 | 97.0 | 7.8 | Chol.+ | |
| 18 | M | 30 | 25.8 | 64.6 | 68.8 | 7.2 | Pigt.+ | Coccidia |
| 19 | F | 30 | 35.4 | 130.3 | 36.3 | 7.4 | Pigt.+ | Coccidia |
| 20 | M | 30 | 29.7 | 104.9 | 46.3 | 7.8 | Pigt.+ | Coccidia |
| 21 | F | 30 | 59.9 | 188.4 | 90.0 | 7.4 | Neg. | |
| Controls Average | | | 16.4 | 45.0 | 8.6 | Alk | Neg. | Coccidia |

TABLE 8

RABBITS ON 3-BETA CHOLESTANOL DIET

| NO. | SEX | DAYS | PROCEDURE | FREE. BLOOD CHOLESTEROL | TOTAL. CHOLESTEROL | G.B. BILE CHOLESTEROL | pH | CONCREMENTS & REMARKS |
|---|---|---|---|---|---|---|---|---|
| B 1 | F | 5 | | 67.2 | 164.5 | 36.2 | 7.2 | Nil |
| 2 | M | 9 | | 15.6 | 80.5 | | 7.5 | +2 |
| 5 | F | 13 | | 20.3 | 68.0 | | 7.4 | +3 |
| 6 | M | 13 | | 16.5 | 63.9 | | 7.2 | +3 |
| 7 | F | 17 | | 29.6 | 48.4 | G.B. Absent | 7.1 | +1 CGD |
| 8 | M | 21 | | 3.8 | 7.8 | | 7.6 | +4 |
| 9 | F | 21 | | 19.2 | 73.0 | | 8.0 | +4 |
| 11 | M | 21 | | 34.4 | 119.3 | 81.6 | 7.4 | +3 |
| 12 | F | 21 | | 25.9 | 75.5 | 69.3 | 7.3 | +3 |
| 13 | M | 21 | | 33.0 | 99.5 | | 7.7 | +4 |
| 14 | F | 31 | | 51.3 | 147.0 | 29.6 | 7.2 | +3 |
| 16 | M | 31 | | 11.6 | 37.0 | 48.2 | 7.1 | +2 |
| 18 | F | 41 | | 40.1 | 72.4 | | 7.8 | +4 |
| 20 | M | 41 | | 27.6 | 56.2 | | 7.7 | +2 Fibrosed |
| 22 | F | 21 | Cholecystec-tomy | 19.2 | 73.0 | | | +3 |
| 23 | M | 21 | " | 34.4 | 119.3 | | | +1 |
| 24 | F | 21 | " | 25.9 | 77.5 | | | +1 |
| 25 | M | 21 | " | 33.0 | 99.5 | | | +3 |
| 26 | F | 21 | " | 51.3 | 147.0 | | | +1 |
| 27 | M | 21 | " | 11.6 | 37.0 | | | Nil |

TABLES 7 & 8

TABLE 9

GUINEA PIGS AND RATS ON 3-BETA DIET

| NO. | SEX | DAYS | FREE. BLOOD | TOTAL. CHOLESTEROL | G.B. BILE CHOLESTEROL | pH | CONCREMENTS & REMARKS |
|---|---|---|---|---|---|---|---|
| RATS | | | | | | | |
| E 3 | M | 5 | 13.1 | 38.2 | C.B.D. | 7.4 | Nil |
| 4 | F | 9 | 13.1 | 64.9 | | 7.8 | " |
| 5 | M | 13 | 19.4 | 65.8 | | 7.9 | " |
| 6 | F | 17 | 19.0 | 55.0 | | 8.1 | " |
| 7 | M | 21 | 15.4 | 57.7 | | 7.7 | " |
| 8 | F | 21 | 19.9 | 84.7 | | 7.8 | " |
| 9 | M | 21 | 14.2 | 54.4 | | 7.4 | " |
| 10 | F | 21 | 17.0 | 68.4 | | 7.9 | " |
| 11 | M | 21 | 18.6 | 58.4 | | | |
| 12 | F | 21 | 20.4 | 64.7 | | 7.1 | " |
| Controls | | | 22.6 | 52.0 | | 8.3 | " |
| GUINEA PIGS | | | | | | | |
| 26 | F | 5 | 37.7 | 67.8 | 14.2 | 8.4 | " |
| 27 | M | 9 | 4.1 | 19.3 | 11.9 | 8.0 | " |
| 28 | F | 13 | 4.1 | 12.3 | 10.0 | 7.2 | " |
| 29 | M | 17 | 8.2 | 18.9 | 13.8 | 7.1 | +1 |
| 30 | F | 21 | 4.9 | 23.6 | 22.4 | 7.9 | " |
| 32 | M | 21 | 6.8 | 34.9 | 16.8 | 7.7 | " |
| 34 | F | 31 | 3.9 | 10.8 | 22.6 | 7.1 | " |
| 36 | M | 31 | 16.6 | 57.5 | 14.7 | 7.6 | + 1 |
| 37 | F | 41 | 13.2 | 42.6 | 10.6 | 7.8 | Nil |
| 40 | M | 41 | 10.1 | 16.7 | 15.8 | 7.3 | " |
| Controls | | Average | 11.6 | 32.0 | 13.6 | Alk | " |

TABLE 9