RESEARCHES CONCERNING THE FORMATION OF GALL-STONES*

BY

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"Is there any thing whereof it may be said,
See, this is new?"

—Ecclesiastes i. 10.

One is careful in laying claims to originality in the reporting and measurement of natural phenomena. Research into the literature of the subject will reveal that others have followed similar pathways and you will be reminded of the saying that "there is nothing new under the sun," and that you have merely completed a circle and brought understanding back to where it was some 50—or even 100—years ago. Research is not like that, however; it is a spiral. At each full circle we have achieved an advance in understanding. We are not back to where we were but rather projected forwards. Though gall-stones is an age-old disease of mankind, let us join the spiral and turn on it through the last 200 years. The subject has a certain romance about it, and in giving this account in memory of Alex Simpson Smith I am calling to your minds a surgeon who was imbued with the romance of the art and science of surgery, whose great prominence as an investigator was cut short by the war, never to be resumed.

Two Theories (the results of research by analysis) About the Cause of Gall-stones

In 1769 de la Salle dissolved gall-stones in alcohol and discovered the substance which was named "cholesterin" by Chevreul (1816). Tiedemann and Gmelin (1826-7) identified the bile salts. The early research appears to have come from French, German, and American laboratories. Naunyn's (1892) treatise on the nature of gall-stones and biliary disease is usually taken as a starting-point. He used the classification of the types of stones with which we are all familiar. The common gall-stone is a mixture of cholesterol, bile pigments, and some calcium, and it is found in different shapes and sizes, single or multiple, even unto thousands. Solitary stones composed entirely of cholesterol are rare. Bile-pigment stones may be solitary (about 1 cm. in diameter); multiple, small, irregular, and metallic-looking; and the feathery, almost microscopic, type (Fig. 1). These are not as common as the "mixed" stone.

One of Naunyn's theories about gall-stone formation was that the mixed stones arose mainly as a pultaceous mass which later became invaded by cholesterol crystals,

a process which he called "cholesterinization." It was postulated that stones were caused by inflammation of the gall-bladder resulting in desquamation of cells and the presence of abnormal substances. Certain stones, however, seemed to originate from hard nuclei of calcium bilirubinate. Thudichum's (1863), Boysen's (1900), and Rovsing's (1924) theories are the challenge to Naunyn's theory of inflammation and cholesterinization. They held that most or all stones are primarily pigment calculi formed in the intrahepatic canaliculi as a result of disease of the liver, and that cholesterol is added by layers when the stone reaches the gall-bladder.

In the re-examination of this "pigment" theory and that "cholesterol" theory, we (Rains *et al.*, 1960) decided to carry out a macroscopic examination and qualitative analysis of whole stones and the stone-centres. Stones from 57 patients were examined. Sample stones were broken and the number and the colour of the different layers recorded. The whole stone, the layers, and the centres were analysed for protein, fat, bile pigment, cholesterol, carbonate, calcium, iron, and phosphorus. The results showed that no one substance predominated. Neither cholesterol nor bile pigment was found in every stone or stone-centre. Pigment was identified in only 74% of the centres. However, there is an interesting discrepancy between what the eye sees and the result of the tests for pigment. Eight stone-centres showed macroscopic discoloration of pigment which failed to give a positive chemical result. The reason for the discrepancy may lie in the sensitivity of the method used, but one is reminded by Hammarsten (1914) that Stadeler described a residual bile pigment called "bilihumin." Modern methods of chromatography and pig-

FIG. 1.—Minute feathery pigment calculi found in a normal gall-bladder. This patient had had malaria four years previously.

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ment fractionation should, I think, reveal the nature of this "bilihumin." Also from our analysis protein and carbonate were found to be equally as common, except in the analysis of stone-centres. Modern methods of electrophoresis will be of value in determining the nature of the proteins present. Carnot and Gruzewska (1928) claimed to have found an abnormal protein, "cholenuclein," supposedly derived from the inflamed mucous membrane of the gall-bladder. Proteinous substances appear to have a specific significance in gall-stone formation, and this is discussed below.

Bacteria and Infection as the Cause

Coli-typhoid Infection

Welch (1890) demonstrated the presence of micro-organisms of the coli-typhoid group in the centre of gall-stones. Blachstein (1891) grew typhoid bacilli from the bile of an animal after inoculation. In one case the organism grew 128 days after injection, though it had disappeared from every other part of the body. Cushing (1898) also detected typhoid and coliform organisms in stones and reported that Flexner had found *Bacillus typhosus* (*Salmonella typhi*) in 50% of cases with cholecystitis. Richardson (1898) described cases of clumping of typhoid bacilli in gall-bladder bile and suggested that these were evidence of an immune reaction and were therefore the forerunners of stones.

In experimental work Gilbert and Fournier (1897) demonstrated that stones can be formed after the intravenous injection of attenuated cultures of typhoid bacilli. Richardson confirmed this. Mignot (1898) produced calculi in a guinea-pig as a result of the injection of *Bacillus coli* (*Escherichia coli*) into the gall-bladder. Mignot also showed experimentally that aseptic foreign bodies do not give rise to the formation of stones, but if, for example, cotton-wool was impregnated with colon bacilli and introduced into the gall-bladder calculi would be formed. The results of these clinical observations and such experiments suggested that gall-stones are caused by some infection of the wall of the gall-bladder as a result of a generalized infection: that exfoliation of inflammatory cells and the production of abnormal protein formed the chemical *milieu* for the birth of a gall-stone, thus giving force to Naunyn's theory. We can also understand why Moynihan referred to the gall-stone as being a "monument in memory of dead bacteria." Dubois (1901) had in fact described the pearl as being "the brilliant sarcophagus of a worm." Parasites and foreign bodies are rarely found in the centre of gall-stones, but pins, pieces of hydatid membrane, the ova of bilharzia and roundworms are always being recorded, especially in stones occurring in the

Japanese (Fig. 2). Homans (1897) found seven calculi crystallized on sutures 20 months after cholecystotomy.

The Streptococcus

The next full circle on the research spiral was reached when Rosenow (1914, 1916) reported the isolation of streptococci in 25 out of 30 cases of gall-stones. *E. coli*, *Cl. welchii*, and typhoid organisms were also isolated in six samples. Cultures of the wall of the gall-bladder, the bile, and the cystic gland gave similar results. Rosenow believed there was a selective affinity of a type of streptococcus for the gall-bladder. He injected streptococci intravenously in rabbits and found that it would develop lesions in the gall-bladder provided the virulence of the organism was not so great as to kill the animal. However, if the virulence was diminished by repeated cultivation it acquired an affinity for the stomach and appendix. On heavy passage and increased virulence the pancreas was most likely to be affected. In nine instances he demonstrated the presence of this organism in the small black stones that formed as a result of such experimentally produced cholecystitis. Brown (1919) confirmed Rosenow's findings and went further by showing that streptococci from the tonsils of patients will produce cholecystitis and stones. These findings suggested that cholecystitis and stone formation is a result of blood-borne infection from a focal source.

Streptococci were also found to be the commonest type of bacteria by Wilkie (1927) and Illingworth (1927), but were more likely to be isolated from the deeper layers of the wall of the gall-bladder while the mucous membrane and bile were often sterile. When Wilkie gave intravenous injections of streptococci to rabbits small calculi of cholesterol and calcium were formed. This occurred within the inflamed gall-bladder whether or not the cystic duct was ligated. Illingworth found that 7 out of 23 stones were infected: two with streptococci, three with *E. coli*, and two with staphylococci. Stones were not always present in cases of cholecystitis in which the gall-bladder wall was infected.

Mårtensson's Bacillus. Specific Antibodies

When Mårtensson (1941) studied this problem he dissolved out the organic calcium, the cholesterol, and the pigment from the stones. A fine network of organic, entirely combustible material was left in which epithelial cells could be made out. From this stroma he isolated what he called the "typical" bacilli—Gram-positive rod-shaped structures joined in long threads or bundles having rounded ends or endospores. These were reminiscent of, though serologically distinct from, the subtilis group. By injection into rabbits he was also able to demonstrate the occurrence of gall-bladder lesions and the formation of stones, and he embarked on tests to show that some patients with stones possessed antibodies to this organism. Such a study of antibodies reminds us that Sterling (1939) showed that agglutinins appeared in the bile of dogs in response to a specific infection such as typhoid. Bacterial agglutinins (*E. coli* and *Salm. typhi*) were present in patients who had cholelithiasis but absent in those who were free from biliary disease.

Actinomyces

In research into this aspect we (Rains *et al.*, 1960) examined sample stones from 57 patients. After being sterilized on the outside by heat, the stones were broken, crushed in a mill, and cultures and microscopical

FIG. 2.—Gall-stones, one large and barrel-shaped and the other small and like a ring doughnut, have formed around a nylon suture. (Mr. Fauset Welsh's case.) Reproduced, by permission, from the *Annals of the Royal College of Surgeons of England*.

specimens were prepared. On aerobic culture *E. coli* was found in four stones, streptococci in two, and staphylococci in eight. However, the results of anaerobic culture were startling, for a type of *Actinomyces* was cultured in 30 out of the 57 cases. This organism grew only very slowly in primary culture under strict anaerobic conditions, requiring 10 to 87 days' incubation before it appeared. It was also identified in 5 out of 57 preparations made direct on to a glass slide. Staining showed Gram-positive rods or coccobacilli in branching, cluster, or beading forms. In order to identify and compare the mycetes found in each stone agglutination suspensions were made. Agglutination tests showed them to be homogenous in their serological characters when tested against the experimental immune rabbit sera. Absorption tests show that they differ distinctly in serological character from the *Actinomyces bovis* of Wolff and Israel.

The finding of actinomyces or similar organisms in calculi is not entirely new. Pearce (1901) found threads of a leptothrix in the gall-stones of a patient with cholecystitis. Mayo Robson (1905) successfully operated on a case of actinomycosis of the gall-bladder. One of the very first bacteriological investigations of stones was by Galippe (1886), who examined dental tartar and renal calculi and found some kind of a mycete. Söderland (1913) and Naeslund (1925) confirmed this in a study of dental tartar and salivary stones and held that the organisms were responsible for the precipitation of calcium into a stony mass. In fact, Citron (1945) demonstrated an alkaline phosphatase activity of the *Actinomyces israeli*, showing that calcium precipitated in a solution containing organic phosphate.

While previous investigators have spotlighted the part that aerobic organisms may play in stone formation, this report of the prevalence of an anaerobic organism revives interest in the theory that the disease is the result of a blood-borne spread from a focus of infection. A mycete exists in the mouth, but extensive serological testing is necessary to confirm that the organism found in the dental tartar is the same as that found in gall-stones.

Comment on Infection as a Cause

The overall evidence is that infection of the gall-bladder plays a part in the formation of some stones, either as a primary cause with clumps of dead bacteria and desquamated cells or as a secondary factor which dictates that additional layers are added to a small stone already present—a small stone which might have arisen as a result of a chemical and an aseptic phenomenon. Neither Naunyn's (infection) nor Boysen's (pigment) theory can be firmly upheld or refuted by these results. It is interesting to note the change of bacterial flora found in gall-stones since investigations were started in 1890. One can only suppose that this is in some way related to the general prevalence of a particular infection at a certain time. Thus as typhoid fever became less common so did the finding of bacilli in the stones. Prior to the antibiotic era, diseases due to streptococci were prevalent: thus streptococci were found. In the antibiotic era we might expect the prevalence of staphylococci and fungi.

Some Metabolic Aspects

The division of opinion at the beginning of this century regarding the causal theories resulted in an

apposite classification of gall-stones. Aschoff and Bacmeister (1909) divided them into "infective," "metabolic," and "static" stones (or causes). Let us consider some metabolic aspects.

Race and Diet

It is well known that gall-stones are rare in certain races, especially those of the Orient. In the Japanese, stones containing cholesterol are infrequent but pigment stones are well known. Maki (1961) discloses that calcium bilirubinate stones, including those of parasitic origin (10%), are 60 to 80% of all gall-stones. Most gall-stones in Western people are found in the gall-bladder, but in the Japanese half or more are found in the bile-ducts, including the intrahepatic ducts. In relating occupation to the type of gall-stone, Maki found that the professional group had a ratio of 32 cholesterol to 29 pigment stones, and the labouring group only 8 cholesterol to 61 pigment stones. The food of the high-income Japanese has something in common with that of the West, but the food of the Japanese farmers is deficient in fat and protein. There is no difference in the incidence or type of stones in those Chinese or Japanese who are resident in the U.S.A.

Thus the important thought that arises from these findings (and similar findings by others) is that the level of cholesterol in the serum does affect gall-stone formation in that it is more likely to be deposited on an already formed stone nucleus—such as a small pigment stone. This is in fact what Thudichum's and Boysen's theories were—that minute pigment stones appear first and that cholesterol is deposited on them in the gall-bladder as a secondary phenomenon. According to Piper and Orrild's (1956) study of 12 families (400 people) suffering from hypercholesterolaemia there was no increased incidence of cholelithiasis, though secondary arterial and skin deposits were very common. Patey (1934) showed in rabbits with hypercholesterolaemia that stones did not form, but cholesterol was deposited in areas wherever damage to tissues occurred.

Thus these researches and observations support the theory that cholesterol is present in gall-stones as a secondary addition and support the "pigment" theory, which also held that the gall-bladder is not necessary for the primary formation of a calculus.

Hyperbilirubinaemia

If the kernel of truth about gall-stones is to be found in an abnormality of bile-pigment excretion by the liver much experimental work is required in the future, for information is sadly lacking. We are all familiar with the formation of multiple pigment stones in haemolytic diseases (such as congenital acholuric jaundice) where an increased amount of circulating blood pigment is excreted by the liver in the bile. The relationship between pigment or mixed stones and other haemolytic states, such as malaria (Fig. 1), and recurrent concealed haemorrhage (gastro-intestinal, ovarian, and uterine) would be an interesting study. The effect of toxins on the liver mechanism of pigment excretion also requires fresh examination. Hammarsten (1914), for example, recalled that Minkowski and Naunyn found that if the livers of healthy geese were damaged by arsenurated hydrogen sulphide a liberal formation of pigments occurred. Advances in such research on bile pigment are now possible through the new understanding of pigment fractions and chemical properties (Billing *et al.*, 1957).

Experimental Dietary Deficiencies

Experimental studies in animals on the effect of diet have also produced interesting results. In 1926 Fujimaki noted that rats would develop stones in their bile-ducts (and in the urinary tract) after a long period of vitamin A deficiency. Dam and Christensen (1952), and Christensen and Dam (1954) were investigating the effects of vitamin deficiencies in hamsters and quite by chance noticed the formation of gall-stones in those animals fed on a generally deficient diet. These stones were said to be mainly composed of cholesterol. The animals also developed changes in fur, skin, and teeth. It was then found that when yeast, polished rice, linseed oil, or copper were added to this "lithogenic" diet, gall-stone formation was inhibited in greater or less degree.

Age, Sex, and Endocrines

It is firmly established that the frequency of gall-stones increases with age beyond the age of 70, when over 20% suffer from the condition. In the middle years of life gall-stones are certainly more frequent in women than in men. It is after the female climacteric that the sex difference diminishes (Horn, 1956). Research into hormone influence on cholesterol metabolism and excretion in the bile is suggested by this sex-and-age distribution and by the fact that the frequency of stones is increased with child-bearing. This aspect is still a mystery, though, according to those who favour a bile-pigment upset as the primary cause of stones and who hold that cholesterol deposition is a secondary phenomenon, no specific hormone need be involved. Menstruation, pregnancy, and labour are recurring physiological phenomena in which the red blood cell is specially connected. Pigment particles appear in the bile, and it is on to these that cholesterol—in a hypercholesterolaemic state—is deposited. An equally important factor is impairment of biliary flow (stasis), for with normal flow and proper emptying of the gall-bladder the fine pigment particles are passed down the bile-ducts into the duodenum. Certainly there is altered function of smooth muscle in the gastro-intestinal and genito-urinary tracts (hydroureter) during pregnancy. We do not yet know whether there is increased tone of the sphincter of Oddi and impaired biliary flow at such a time.

Physico-Chemical Research

So far I have sketched in the "bacterial" theory and the "pigment" theory, brought to mind some interesting metabolic possibilities, and reintroduced the other classical factor, stasis of bile. But rather than dwell too long on the physiological possibilities regarding impaired flow of bile I wish to indicate how stasis may be associated with the actual mechanism of stone formation. This section is concerned with the chemical and physical phenomena whereby cholesterol is held in solution in the bile and the manner and *milieu* in which crystallization occurs.

Solubility of Cholesterol in Bile Salts—Their Physico-chemical Properties

Since cholesterol does not dissolve in water it is natural to inquire into the properties of those constituents of the bile (bile salts and lipids) which may be responsible for its solution, and then to consider the mechanism of precipitation. The salts of the bile acids have a formula which is similar to that of cholesterol,

and one is reminded of the old axiom that "like dissolves like."

Bile acids have another interesting similarity, that of the end-chains between the bile acids and the paraffin chain compounds (which give us soaps and detergents). The molecules of both contain water-attracting and water-indifferent parts (amphipathic), and it would seem that the bile salts, like these paraffin chain salts, have foaming detergent and emulsifying properties consequent upon their power to reduce surface tension. If we measure these properties in solutions of increasing concentration of a paraffin chain salt it is found that the power of reducing surface tension and obtaining

FIG. 3.—Composite graph showing the change of phase in a solution of a long-chain paraffin salt at increasing concentration. The shaded area indicates the critical concentration at which micelles begin to form as shown by alteration of the curves of the physico-chemical characteristics. μ =Equivalent conductivity. D =Detergency. ST =Surface tension, recorded as absolute surface tension. g =Osmotic coefficient, being the ratio of the value of the observed lowering of freezing-point to that which would be expected for an "ideal" fully dissociated electrolyte (such as potassium chloride).

detergency changes at a low and critical point, often around 0.2 molar strength. Such change is also measured by plotting the equivalent conductivity and the osmotic coefficient (Fig. 3). Such a change is a change of phase—a change in ionization. McBain (1913) first showed that this phenomenon is due to an aggregation of ions, called a "micelle." It is often represented schematically as a sphere or laminated structure in which the water-repellent parts of the ions face the interior (Fig. 4). Detergency is effected by dirt particles and insoluble substances being brought

FIG. 4.—A schematic representation of a micelle. (After Hartley.)

into solution by being adsorbed or engulfed on to or into the ionic micelle (which is described as becoming swollen in the process).

While Hartley (1936) suggested that solutions of bile salts might behave in the same way as the detergent salts, Bashour and Bauman (1937), Roepke and Mason (1940), and Johnston and McBain (1942) did not find any striking evidence that this is so. However, these workers were examining the bile salt solutions at 0° C., 20° C., and 25° C. We (Rains and Crawford, 1953) decided to re-examine the phenomenon at 37° C. using sodium cholate, desoxycholate, glycocholate, and taurocholate in physiological as well as dilute concentrations. By studying the equivalent conductivity, osmotic coefficient, and surface-tension effects and turbidity we demonstrated a change of phase at concentrations between M/160 and M/80 in each of the bile salts studied. This indicates ionic aggregation (micelle) formation, which persists in a range of concentrations normally found in human bile. Ekwall, Fontell, and Norman (1957) have confirmed this phenomenon, for small-angle x-ray scattering patterns show that an association of ions to colloidal aggregate or micelles occurs in bile-salt solutions. It also confirms the finding that conjugation of the bile salts does not greatly influence the phenomenon.

The next question to settle was whether cholesterol is held in solution by the ionic micelle of bile salt in the same way as the detergent micelles adsorb dirt in laundering processes. The technique of the experiments which were then performed consisted in shaking up an excess of cholesterol in different concentrations of bile-salt solutions for 48 hours at 18 and 37° C., filtering off the excess cholesterol, and determining the amount held in solution by the Liebermann-Burchardt test. There was little affinity for cholesterol at 18° C., but in accordance with the micellar theory there was a steady uptake at 37° C. At the critical concentration for the beginning of the formation of micelles a definite peak in the affinity for cholesterol occurs (Rains, 1955).

Besides this physico-chemical method of carrying cholesterol in the bile it is now believed that lecithin and any fatty acids also present in the bile act with the bile-salt micelles to increase cholesterol-carrying capacity (the L.B.S.-lecithin-bile-salt system). But according to Hoffmann (1961) it is suggested that the lipids, rather than assisting in cholesterol carriage, are themselves carried by the same micellar mechanism.

Precipitation and Crystallization of Cholesterol

The bile salts have a buffering capacity and will resist mild acidification. Once the buffering action is overcome, however, the pH will fall rapidly. If cholesterol is being carried by the bile salt it precipitates (Fig. 5). Such a precipitation phenomenon favours the infection theory of gall-stones because the bile becomes more acid in the inflamed gall-bladder. However, cholesterol precipitated by acidification from the bile-salt micelles does not crystallize into stones. Similarly, if the bile salts are diluted so that the micelle cannot hold the cholesterol the sterol precipitates but does not crystallize.

Stasis and Influence of Colloids on Crystalline Form

In the course of our experiments with the cholesterol/bile-salt solution, observations were made on the effect of allowing such a mixture to stand undisturbed for about 14 days. In the cholesterol/sodium-desoxycholate

solutions small white bodies appeared and moved slowly to the sides of the glass tubes, and in those of a higher molar strength a soft white moulded mass was formed. This was cholesterol not in crystalline form but as a mass of interlacing tube-like refractile processes, the alternative or myelin form with which some investigators of 100 years ago were very familiar (Ord, 1879). Ord had reproduced the experiments of Montgomery (1866) in showing the development of a non-crystalline form of cholesterol in egg albumen. These were small rounded bodies or spherules which in time tended to coalesce to form larger spheres.

The pioneer work concerning the formation of spherules by otherwise crystalline substances is generally attributed to Rainey (1858), an original contribution to this aspect of mineralogy and colloid chemistry which has only been spasmodically applauded down the years. Rainey's simple experiment was to use two filtered solutions of gum arabic of different specific gravity, one of which was saturated with carbonate of potash. The heavier solution was put into a glass phial, and the lighter solution carefully poured on top, so that the solutions were mixed as little as possible. The phial was kept for three to six weeks, and a glass slide which had been laid in the direction of the long axis of the

FIG. 5.—Buffer curve for 4% sodium desoxycholate and desoxycholate/cholesterol complex. Reading graph from right to left the resistance to acidification is demonstrated. When this is overcome, as shown by rapid change of the pH reading from 6.5 to 2.5, cholesterol appears to precipitate. —, = 5 ml. 4% sodium desoxycholate. ---, = 4% sodium desoxycholate + cholesterol filtrate.

FIG. 6.—Spherulith formation of calcium carbonate by layering of gum arabia showing the growth of spheruliths by aggregation. ($\times 110$). (Rainey's experiment reproduced, 1961).

phial was examined under the microscope, and drawings of the spheruliths of calcium carbonate were made (Fig. 6). However, these spherules were later observed to aggregate and change into a radiating crystalline structure. Ord, as I have said, confirmed the work of

FIG. 7. — Gall-stones being formed by aggregation. Reproduced, by permission, from the *Annals of the Royal College of Surgeons of England*.

Rainey and of Montgomery and showed how cholesterol formed stones after the same manner. This phenomenon of spherule formation and aggregation is often seen in gall-stone structure (Fig. 7).

Similar ideas regarding the early formation of stones were voiced by Meckel von Hemsbach (1856), for he too thought that stones were primarily formed from minute stone embryos. And while the early observations of those interested in gall-stones concerned the phenomenon of crystallization of cholesterol out of solution a somewhat later though similar observation was made regarding bile pigments by Steinmann (1889), who studied the separation of bile pigments in egg albumen. And, comparatively more recently, Peyton Rouse, McMaster, and

Drury (1924) studied the appearances of bile in the biliary fistulae of dogs and found that such microspheruliths of calcium carbonate and calcium bilirubinate occurred on the sides of the glass connexions of the rubber drainage-tubes. In studies on gall-stones and bile they observed similar nuclei within mature stones as well as in the free state.

Effect of Stasis and Layering of Bile

It thus becomes evident that this influence of colloids on crystalline form, the formation and aggregation of spheruliths, is related to stasis, and, as Rainey demonstrated, to the occurrence of what is called an interface between two solutions containing proteinous substances of different specific gravity. Within the gall-bladder the bile tends to stratify in layers (Tera, 1960), so that conditions similar to those described above will exist when emptying of the viscus is impaired, for layers of bile of different specific gravity (fresh hepatic bile/old concentrated gall-bladder bile) come into contact (Fig. 8). It was with this impaired physiology of gall-bladder emptying in mind that Fitz-James and Burton (1949) studied the precipitation of cholesterol *in vitro* due to a difference in rate of diffusion of cholesterol and bile salts across the interface (mimicking the interface between concentrated gall-bladder bile and a fresh influx of hepatic bile). This diffusion led to a local fall of the critical ratio for cholesterol solvency and the formation of what they called a "pseudo" stone.

To summarize what is known about the physico-chemical effect of stasis we may say: (a) that it favours the formation of a stone embryo—either in a proteinous milieu or as a result of an interface between layers of bile of differing specific gravity; (b) that it favours the aggregation of spherules and the addition to minute stones by layering and crystallization of cholesterol in

the radiating crystalline form; and (c) that without stasis small stone embryos or bile grit would pass down the bile ducts and would not remain in the gall-bladder. Aggregation and the addition of layers would not be possible.

FIG. 8.—Cholecystogram showing layering phenomenon in the gall-bladder and gall-stones lying at the interface between bile with different specific gravities. Reproduced, by permission, from the *Annals of the Royal College of Surgeons of England*.

Conclusions

Claude Bernard said, "You must not assume that you have the explanation for any phenomenon of nature until every alternate hypothesis has been exhausted." Where gall-stones were concerned the various hypotheses have not been so exhausted. Research into the nature of their causes may be of greatest interest to those with the detective mind, for there is a vast amount of circumstantial evidence. Much is incomplete and some aspects of this lecture have, of necessity, been sketched in very lightly here. One does, however, move towards certain conclusions favouring stone formation and growth.

The evidence acquired from a continuous spiral of research and knowledge of over 100 years shows:

(1) That the primary cause may be found by studies in bile-pigment metabolism and its physico-chemical properties.

(2) The phenomenon of stone growth by aggregation of microspheruliths and layering by cholesterol can be understood through a study of the physico-chemical characteristics of bile salts, cholesterol, and lecithin.

(3) Stasis of bile is of importance as it favours the aggregation and subsequent additional layering of stones in a milieu containing proteinous material or layers of bile of different specific gravity. Stasis also prevents the natural voidance of early minute stones into the duodenum. Hence minute stones can form without stasis, yet without stasis no gall-stones of clinical importance can form.

(4) Infection plays its part as a cause of stasis, and it provides the proteinous milieu in which stones will grow.

(5) Other causes of stasis within the biliary tree have yet to be determined with accuracy, but they seem to be those which impair the function of the sphincter of Oddi and may well be related to other conditions in which there is an alteration in the physiology of smooth muscle—pregnancy, gastro-oesophageal reflux, and diverticulosis.

Summary

This lecture concerns the theories on gall-stone formation, and includes the products of personal research and interest over the past 10 years.

The types of gall-stones are described. Classical observations by pathologists such as Naunyn favour infection as the primary cause of the common gall-stone, to which pigment calculi bear no relation. Contrary to this theory is one (Rovsing) which states that all stones are formed from the pigment centre. Macroscopic appearance suggests that this may be so, but analysis by standard methods has not so far confirmed this.

Since 1886 bacteria have been cultured from gall-stones. Colityphoid organisms, streptococci, staphylococci, and now actinomyces have been isolated. Stones can be produced experimentally by intravenous injections of attenuated organisms. Infection may thus be held to be a primary cause, and that a stone forms from a nucleus of clumps of bacteria or desquamated cells, or as a secondary factor which dictates that layers are added to a small stone (? pigment) already present in the bile.

The character of gall-stones appears to change with diet and the standard of living, and "Westernization" of the Eastern races has shown a significant change from a preponderance of pigment to the cholesterol mixed stones. Dietary deficiencies in experimental animals produce stones.

The frequency of gall-stones increases with advancing years. The predominance in females in the middle years falls away after the climacteric.

Much of the interesting and maybe rewarding research into stone formation lies in the realm of physico-chemical research. This includes the physical mechanism by which cholesterol is held in solution, and the way in which stones form from minute spherulites in a proteinous or colloid milieu—growing by aggregation and changing from an amorphous to a crystalline form. Imperfections in the flow of bile (forms of stasis) will allow the growth of such stones, particularly when layers of bile of different specific gravity come into contact through imperfect gall-bladder function. Without impairment of biliary flow, stones would be voided while small and not become a clinical problem.

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"Raoul Island, most hospitable of the isolated Kermadec group perched on a submarine Pacific Ocean ridge some 670 miles north-east of Auckland, New Zealand, houses nine residents, the men of a permanent meteorological station that is part of a New Zealand-administered chain of Pacific weather observation and reporting posts. . . . The expedition leader is trained beyond first-aid standard and can measure a dose, give an injection, and put in a stitch. Thus he can deal with cuts, injuries, fractures, and minor infections, should the need arise. In the event of a suspected serious illness he can be linked by radio-telephone, at any hour of the day or night, to the Air Department's Director of Medical Services in Wellington, to receive detailed instructions on the treatment of the patient. Should atmospheric conditions interfere with the efficiency of the radio-telephone link, the leader refers to a typewritten list of treatments for the diseases or emergencies most likely to arise, and the medical officer then advises, 'Use treatment "A,"' or 'Use treatment "B,"' as the case may be, repeating this instruction until the leader of the expedition signifies that the message has been received. He, in turn, repeats the message back to the M.O., who thus assures himself that the correct treatment will be given. Contact is maintained with the island and confirmatory instructions and reports on the patient's progress are exchanged when radio communication conditions improve." (*Forefront*, August, 1962.)