OBSERVATIONS ON SOME CAUSES OF GALL STONE FORMATION.

I. EXPERIMENTAL CHOLELITHIASIS IN THE ABSENCE OF STASIS, INFECTION, AND GALL BLADDER INFLUENCES.

BY PEYTON ROUS, M.D., PHILIP D. McMASTER, M.D., AND DOUGLAS R. DRURY, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

PLATES 12 TO 15.

(Received for publication, August 6, 1923.)

In the present paper and others to succeed it we shall report experiments on the causation of gall stones. Whatever the significance of our findings, or lack thereof, there can be no doubt that the problem of cholelithiasis waits upon the development of an experimental material. The literature of the subject, like that of many another medical theme, exhibits the difficulties which sometimes effectually thwart attempts to solve a riddle in etiology by the study of clinical, which is to say uncontrolled, instances. It has been from early times a literature top-heavy with hypothesis and dismal with uncorrelated observations. The admirable studies of Naunyn, Aschoff and Bacmeister, and others which have so greatly furthered knowledge on the classification and incidence of stones have not explained their causation,—witness, for example, the prevailing uncertainty as to whether infection plays an essential rôle therein. Gall stones have frequently been encountered and produced in animals, but only under conditions of infection and biliary disturbance which render the circumstances of their occurrence not a whit more easy to understand than in man.

The lithiasis with which we have had to do developed in dogs permanently intubated for the collection of sterile bile.¹

Most of the successful attempts to induce gall stones in animals have been carried out with the herbivora (rabbits, guinea pigs). This is not for lack of work with species such as the dog which approach man more nearly in their physiological processes; but in them calculi have proved difficult to engender, for reasons as yet uncomprehended. Stadelmann\textsuperscript{2} mentions stone formation upon the surface of a broken cannula left in a dog with a bile fistula. Gilbert,\textsuperscript{3} Gilbert and Fournier,\textsuperscript{4} and Mignot\textsuperscript{5} all caused cholelithiasis in dogs by infecting the gall bladder with organisms of low virulence and hindering the evacuation of bile. They were interested only in the relationship of infection to the lithiasis and give no detailed description of the stones,—nor for a like reason does Rosenow\textsuperscript{6} who has reported stones as a consequence of the elective localization of streptococci in the canine gall bladder. Harley and Barratt,\textsuperscript{7} and Hansemann,\textsuperscript{8} following Naunyn, introduced cholesterol stones from man into dogs and saw that instead of increasing in size they gradually dissolved away, a fact explained by the low content in cholesterol of dog bile.

Gall stones developed in fourteen out of twenty-two intubated dogs which we have studied with reference to the phenomenon. Of the eight animals free from them, one had been intubated for only 7 days.

The make-up of the tube system has already been described.\textsuperscript{1} It consisted of a glass cannula (Fig. 1, A) 1 to 2 cm. long with a bore of 1½ to 3 mm., joined to a U of rubber tubing measuring 7 to 10 cm. on a side. The sides of the U were connected by a short, curved segment of glass (Fig. 1, B), and that nearer the duct consisted of soft, black rubber while the further one was of more resistant red material. All this portion of the apparatus lay within the peritoneal cavity, but the red tubing was prolonged to pass out through a stab wound in the abdominal wall and connect with a rubber balloon into which it delivered the bile. Close to the balloon, as a provision to empty it, a side tube was incorporated in the system by means of a T of glass (Fig. 1, C). The total length from cannula mouth to balloon ranged between 25 and 30 cm. Small bulbs had been blown in the glass connections used in some cases, to facilitate deposition out of the bile. Prior to assembling the apparatus, the rubber segments were boiled in a sodium bicarbonate solution, soaked in weak hydrochloric acid, and repeatedly

\textsuperscript{2} Stadelmann, E., Der Icterus und seiner verschiedenen Formen, Stuttgart, 1891.
\textsuperscript{3} Gilbert, A., Arch. gén. méd., 1898, x, series 8, 257.
\textsuperscript{5} Mignot, R., Arch. gén. méd., 1898, x, series 8, 129.
\textsuperscript{7} Harley, V., and Barratt, W., J. Physiol., 1903, xxix, 341.
\textsuperscript{8} von Hansemann, Virchows Arch. path. Anat., 1913, ccxii, 139.
flushed with distilled water. The glass connections were cleaned with aqua regia and well rinsed. And finally the apparatus was autoclaved twice in a large flask of distilled water.

**Incidence of the Stones.**

The bile out of which the stones formed was liver bile. For the neck of the gall bladder has been severed between ligatures at the initial operation or tied off at several points, effectively isolating it from the duct system, as proven at autopsy. Stasis also can be ruled out as a factor in the lithiasis. It is true that temporary obstruction to the flow from the liver was designedly produced in certain of the animals by clamping the collecting tube, and that on other occasions brief obstruction occurred through accidental twists in the tube; but an analysis of the incidence of calculi has failed to bring out any influence thereon of the stasis, which lasted at most only 48 hours. In many of the dogs developing stones, the flow of bile was never interrupted.

Thirteen of the animals were kept with special reference to maintenance of health, procedures likely to disorder them being avoided. Stones developed in seven of these, and in four of the number the bile was consistently sterile on day-to-day culture, while staining of the sediment thrown down with the centrifuge failed to disclose organisms. The secretion had become infected in four of the six animals which did not show stones at autopsy. Nine intubated dogs were submitted to procedures that may have, and often undoubtedly did, produce pathological changes in the secretion, as for example, the intravenous injection of hemoglobin or calcium chloride, prolonged chloroform anesthesia, and toluylenediamine poisoning. In all save two such animals, one intubated for but a few days as already mentioned, stones occurred. The bile was infected in two of the seven instances of lithiasis and in one of the two dogs that were free from stones.

The diet of the animals was so varied that the question of its influence must remain an open one.

The organisms recovered from the infected animals were always found in pure culture. They differed greatly in character. Some

---

strains may well have acted to prevent or determine the occurrence of calculi, though they produced no obvious disorder of the biliary tract. In the lack of precise information on the point, attention will be concentrated on the lithiasis developing under sterile conditions.

Stones were found as early as 14 days after intubation. The longer the period of intubation, the larger in general they tended to be. In three cases in which the bile was sterile they led to total biliary obstruction after 21, 24, and 50 days intubation, respectively, through gradual closure of the lumen of the cannulas wherein they were situate.

**Location.**

Always the calculi were restricted to the system of glass and rubber tubes that had been connected with the common duct (Figs. 1 and 2). They were never found in the biliary system proper. This was true even when they gave rise to obstruction and the ducts were in consequence dilated, and also when, as not infrequently happened, there was a circumscribed irritation of the duct mucosa where the lip of the cannula had rubbed upon it. Even the cul-de-sac resulting from ligation of the gall bladder neck was regularly free from stones. They were sessile upon the foreign tubing; multiple; discrete in inception though often secondarily merged; and larger and more numerous the nearer their position to the bile source.

When the calculi were small they were sometimes present only in the cannula. Usually, though, other and smaller ones were to be found on the adjoining rubber tube. When those in the cannula were large, the incrustation was usually to be noted in diminishing amount for some centimeters further down, occasionally as far as the glass T-tube (Fig. 1), that is to say more than 20 cm. from the common duct. The alternation of glass and rubber surfaces was without obvious influence on the number or character of the stones; but wherever there was an eddy in the bile current or a shallow dead space, as just below the constriction in the cannula and at the junctions of rubber and glass, they were relatively numerous and large (Fig. 2). They were never found, significantly enough, on the outside of that portion of the cannula which was included within the duct by the ligature, nor, save once, were any present at the immediate entrance to the cannula or on the first few millimeters of its wall, in contrast to the state of affairs existing further down (Fig. 1). In the instance referred to, an occluding calculus in the cannula projected upwards as a jagged mass into the duct. Inspection showed that it had had its inception at a point several millimeters below the cannula mouth.
The stones were always relatively large and abundant on the side of the tubing that was lowest during the life of the dog (Figs. 1 and 2), and frequently were found only there. The materials of which they were principally composed caused them to be much heavier than the bile. Those near any one point tended to be remarkably uniform in size and constitution, as if of the same "crop" or "generation" (Figs. 3 to 5). In exceptional instances a second and even a third "crop" could be discriminated, scattered amidst the first (Fig. 1). The deposition was punctate to begin with, the stones enlarging as separate entities until they touched each other when they often became secondarily united in a matrix. Ultimately the cannula became lined in some cases with a rigid tube of calculous matter, the merged entities of which could still be recognized here and there.

A considerable length of the tube system lay outside of the body, and the bile coursing through it must have been cooled somewhat. An influence of the factor on stone formation and localization could not be discerned, but that is scarcely surprising since deposition out of the bile had spent itself close to the duct. Furthermore the outside tubing was handled so often as almost of necessity to dislodge incrustations upon it.

Structure.

The calculi were found to be unaffected by water, so the tube system was gently rinsed out prior to scrutiny of them. The early stages in their development and association could be studied by direct inspection with the microscope through the walls of the glass connections. Those on the rubber were scraped off into water for examination. In general the stones exhibited little variety of structure and composition as compared with human stones. Those situate nearest the bile source were regularly the most pigmented, being usually of an opaque brownish or greenish black to the unaided eye, like small, angular pepper grains (Fig. 1). In an unique instance a few calculi having place on the side of the cannula which was uppermost during life resembled small, dark brown stalactites with prolonged points, in contrast to others, on the lower side, which had the usual angular contours and were but lightly pigmented (Fig. 6). Not infrequently calculi situate far down in the tube system were almost colorless, and in one instance this was true of those in the cannula itself. They appeared mustard yellow, like Lycopodium seeds in the gross.
The slightly pigmented stones showed evidence of crystalline structure (Figs. 6 and 7) and even when relatively large sometimes retained the crystal shape, appearing like flattened rhombs or plates (Figs. 8 and 9). Arrow-head forms and forms like angular pears were frequent. The admixture of pigment evidently interfered with the crystallization; and many of the heavily colored stones, as for example the stalactites above mentioned, appeared to consist of a nearly amorphous material. Concentric layers of varying degrees of pigmentation could frequently be made out. The center of many of the stones was more pigmented than the periphery, often pronouncedly so (Figs. 6 and 10). On the other hand a deposition of colored layers on a non-colored core was never encountered. Whatever the composition of the stones, they were nearly always hard and brittle, grating under the knife, and shattering into many fine splinters when forcibly pressed. Only occasionally were small concentric yellow-brown forms noted (Fig. 11) that appeared less rigid, separating on pressure along radial and concentric lines into pieces with rounded corners.

A specimen example of the cholelithiasis will be described:

Dog 30, a male terrier of 18½ kilos, was killed with chloroform on the 38th day of bile collection. The secretion had remained sterile throughout. At autopsy the ducts were somewhat more distended than is normal, though the walls were not stretched. On an attempt to run saline through the tube system, from above downwards, a slight resistance was met which was relieved by the expulsion of a plug of soft mucus.

Stones.—The upper portion of the cannula was clean, save for a thin, transparent layer of mucus; but the lower part was thinly coated with organic débris and in its midst was a tongue-shaped group of calculi that averaged about ¼ mm. in diameter. They were roughly spherical, with an almost black, well defined, rounded nucleus and a well-nigh colorless, narrow, surface layer. Those nearest the duct were separate, but others a little further down on the glass were secondarily united in a pale green, translucent crystalline matrix. The wall of the rubber tubing next the cannula was encrusted all around with a similar deposit, lying in a layer of organic matter; but lower, and in the curved glass connection between the two limbs of the U, stone formation was limited to the dependent side of the tube, as also was the organic débris. The stones had the form of pale green, translucent, crystalline masses, some with relatively small, dark centers. When crushed, they separated into numerous jagged splinters and gave no evidence of concentric or radial structure. On the dependent side of the red rubber near where it was attached to the glass connection a bright brown skim was to be seen.
which when scraped into water was found to consist of minute, spherical or ovoid, red-brown bodies caught in organic débris. The smallest were of about the size of an erythrocyte. The larger showed concentric layers of deposition and radiating striae (Fig. 11). They broke readily on pressure along both the concentric and radial lines, and the fracture angles appeared somewhat rounded as if the calculi were soft. Further down were no stones but only a little organic débris. The plug of mucus flushed from the tube at autopsy showed a few calculi that had evidently been caught and dislodged in its passage.

Composition.

Calculi from different levels in the tube system of the first six animals with lithiasis were scraped into water, washed by centrifugation, and tested for bilirubin, phosphates, cholesterol, carbonates, calcium, and calcium bilirubinate. Less extensive analyses were carried out with the later material.

The method of analysis follows:

The washed material was dried on a watch-glass, powdered in an agate mortar, twice extracted in an excess of boiling water, and separated therefrom by sedimentation. Often some floccular débris failed to come down, and this was collected on a filter and tested for cholesterol (Liebermann-Burchard reaction). The sediment after drying was twice extracted with an excess of boiling chloroform (Merck’s reagent), which was in turn filtered off and examined for the presence of bile pigment and cholesterol. After the extracted material had again been dried, separate portions were tested for calcium (oxalate reaction), for phosphates (with ammonium molybdate), for carbonates (barium hydroxide), and for calcium bilirubinate. The reactions of this last substance find scant mention in the literature on gall stones. According to the original description of Städelter, it differs from bilirubin among other respects in being insoluble in chloroform, but is readily broken up by the action of weak acids, the bilirubin liberated going into solution when chloroform is present.

Carbonates were present in some of the stones from every animal as shown by the fact that they effervesced with weak hydrochloric acid and that the gas evolved caused clouding of barium hydroxide. None of those examined for phosphates contained them, and cholesterol was also absent, save in one instance in which the trace found was evidently derived from pus about the exit wound some of which had been inadvertently drawn into the tube system at autopsy. A trace of green pigment came away not infrequently in the watery extract of the stones, but the bulk of the pigment of those that were dark, and amorphous failed to go into solution in either water or chloroform. After treatment of them with weak acid, which evoked slight or no effervescence, almost all of the coloring

---

mattered came away into chloroform, conferring on the solvent an intense yellow-brown hue which changed gradually to green on exposure to the air. The pigment gave the Gmelin reaction brilliantly. In the residue after the extraction much calcium was regularly demonstrable. The inference seems justified that the original pigment complex was calcium bilirubinate. There was often present, in addition, some brown coloring matter which could not be identified (bilihumin?). There exists, of course, the possibility, indeed the probability, that the stones contained a little uncombined bilirubin which had come down incidentally in the precipitation of calcium carbonate, and was protected from solution in chloroform by this latter. The amount, though, was certainly negligible as compared with that of other pigment in the dark calculi.

Types of Stones.

Three general types of stones could be discriminated, one consisting almost wholly of calcium bilirubinate, another of calcium carbonate, and a mixed type in which both compounds were present. The composition and form of the stones varied, ordinarily, with the distance from the bile source. The relatively large, opaque, and deeply pigmented amorphous stones sometimes found in the cannula and upper tubing contained much bilirubinate; those lower down in the same animal showed usually a nucleus or core in which the substance predominated, enclosed in a more or less translucent matrix of concentric layers of tinted carbonate which gave some indications of the crystal form; while the lowest ones in the collecting system were frequently of almost pure carbonate, markedly crystalline in character, and scarcely pigmented at all. In one instance already mentioned, carbonate stones were alone found throughout the tubing, irregular crystalline masses, mustard yellow in the gross, of greenish tint under the microscope, and yielding on analysis a negligible amount of pigment. In another the minute calculi present side by side in the cannula were of two markedly different sorts, some of them being heavily pigmented, dark and amorphous, while others were translucent, nearly colorless, and obviously crystalline (Fig. 7). These latter dissolved in weak hydrochloric acid with a marked effervescence of carbon dioxide, and the solution yielded much calcium; whereas the pigmented elements scarcely effervesced at all, altered little in appearance, but were softened as shown by the fact that they now spread out on pressure instead of splintering. The stalactites situate on the upper side of the cannula in an instance previously referred to
consisted almost wholly of bilirubinate (Fig. 6, c), whereas the roughly crystalline elements on its lower surface contained, some of them, well-nigh pure carbonate, others a pigmented center and a carbonate envelope (Fig. 6, a, b, and d). No stones of a structure definitely radial were ever observed though scattered radial striae were sometimes to be seen (Fig. 11). The varied forms of the calculi can be explained on the basis of the relative amounts of their two main constituents, the amorphous, calcium bilirubinate, and the crystalline carbonate. The organic débris in which some of them originated may sometimes have played a part in determining their shape (Fig. 6, d). Every stone, no matter how little colored, and how crystalline in appearance, possessed an organic scaffolding or shadow as could be seen by dissolving out its major constituents, which was readily accomplished with acid alcohol (Fig. 5). This shadow possessed the exact form of the original stone. On pressure it flattened out like mucus, which it resembled in appearance, returning to approximately its original shape when the tension was released. Not infrequently a glassy skim of mucus could be seen in a half light overlying the surface of calculi (Fig. 6, c, d),—the outermost, freshest layer of the scaffolding.

The Stones Not a Result of Bile Deprivation.

Total loss of the bile over long periods of time, such as occurred in our animals, may well be productive of grave changes in the general metabolism. Certainly there are radical alterations in the bile itself, practically from the first. The amount of it is lessened owing to absence of the normal stimulus to secretion from cholates delivered into the intestine and reabsorbed therefrom; and the total pigment content is cut down through the prevention of a like enterohepatic circulation of it. If the stones we have described are the result of these and other biliary abnormalities, then they possess merely a curious interest. To determine the fact, recourse was had to experiment. Short segments of the common and hepatic ducts of dogs were excised and replaced with sections of glass tubing in which small bulbs had been blown. This was done with as little trauma to the duct system as possible and with no disturbance of its intes-

---

CAUSES OF GALL STONE FORMATION. I

tinal connection. The gall bladder was not interfered with. The operations were carried out by an aseptic technique, under ether.

Seven animals were thus treated. The abdominal wound healed by first intention in all. One dog died of an acute pneumonia after 27 days. At autopsy a few hours later, stones of the familiar, pepper grain type and consisting of mixed carbonate and bilirubinate were found in the single glass connection that had been inserted into the duct. Unfortunately no cultures of the bile were taken. There was no obstruction or inflammation of the duct system and no definite liver change. The glass connection had been placed in the large main duct from the liver just above the point where by entrance of the tributary from the lateral hepatic mass (made up of the right lateral and caudate lobes) it becomes the common duct.

In two dogs biliary obstruction with jaundice developed after some weeks as the result of complications resulting from the presence of the glass tubes. One animal, killed 29 days after operation, and presumably after a week or more of obstruction, showed no calculi. Tubes had been inserted into the common duct and that from the left lateral and central liver lobes. The obstruction had occurred in the tube first mentioned, through clotting within it of blood from the abraded duct wall. The tarry bile which filled to distension all of the ducts and the gall bladder proved sterile on culture. The other animal was killed when heavily jaundiced, 30 days after operation. It had by contrast a heavily infected bile and a liver which showed scattered small necroses. The lower end of a glass tube originally placed in the common duct was found to have worked through the wall and now lay in an occluding blind sac of scar tissue. Both in this tube and in another lying in the course of the duct from the lateral mass of the liver there were present lightly pigmented carbonate concretions of characteristic crystalline appearance, scattered here and there in the midst of a heavy coat of cellular débris.

Three animals, sacrificed after 29, 31, and 66 days, respectively, yielded no calculi. The one kept for the longest period had a bile heavily clouded with a pure culture of micrococci. The gall bladders of the others yielded a normal-looking secretion that proved to be sterile. In a final instance, typical carbonate stones were found associated with organic débris in a tube placed in the common duct of an animal killed while in excellent condition 35 days after operation (Figs. 8 and 9). The bile proved sterile on agar and bouillon culture, as, furthermore, on microscopic examination of the slight sediment obtained by centrifugation.

These experiments suffice to prove that the stones developing in our intubated animals were not essentially consequent on the alterations in the bile involved by loss of this secretion from the body.
Relation of Organic Débris to Stone Formation.

In twelve out of the fourteen intubated dogs in which lithiasis was encountered organic débris was found closely associated with the stones. Only twice did they develop when it was absent, which was the case in eight animals. The débris took the form of a thin coating on the tube surface of translucent or transparent, slightly granular and pigmented material containing occasionally a few degenerated cells. In some instances an obvious source for the material was discovered in a local irritation of the common duct where the lip of the cannula had rubbed. Sometimes the mucosa was raised here into a reddened papilla. In other cases in which the liver had been damaged with chloroform or toluylenediamine, the centrifuged bile of the day-to-day specimens showed desquamated cells in addition to much mucus, and the organic coating on the tubes at autopsy was thicker than usual, often extending far below the region in which the stones were located.

The nature of the association between the organic débris and the calculi became clear on a microscopic inspection of the latter in situ, on the walls of the cannula and the glass connections. In an instance in which small calculi of two sorts existed in the cannula, carbonate concretions and others predominantly of bilirubinate, it was noted that only those of the carbonate had an association with débris. At the center of small individual masses of this latter lay one or several stones (Fig. 7). One might suppose either that the calculi had been secondarily enclosed in the organic material or that it had to do with their inception. But if they were enclosed, why had not this happened to the bilirubinate calculi intermixed with them? In another case, already several times mentioned, there existed on the dependent side of a cannula an organic layer deep within which layered carbonate stones were present. On the upper side of the same cannula were relatively large stalactites of bilirubinate, by contrast practically free from organic débris (Fig. 6). A search through all the instances of lithiasis has disclosed the fact that calculi formed primarily of carbonate were only once observed in the absence of the débris, whereas those of bilirubinate were often
completely free from it, and in other instances only seated or lodged upon it (Fig. 7) as if their original nuclei of deposition had been caught and retained by it. In one unusual case, already mentioned, all of the calculi in a tube system consisted of carbonate. There was present in this instance an unusual amount of organic débris. Altogether the facts indicate that the slight amount of organic material settling in the tube system played an important rôle in determining the formation of carbonate calculi. These never developed on the surface of the material but always within it as if determined by some special condition there. Occasionally carbonate crystals were found scattered diffusely through the layer of organic débris. Frequently this latter extended far below the zone of stone formation,—a fact which sufficiently proves that the calcium salts were derived not from it but from the bile.

The Rôle of Nuclei in the Stone Formation.

By treatment with acid alcohol the stones could be rid both of pigment and of calcium salts (Fig. 5) and their scaffolding subjected to direct inspection for special centers of deposition. In a number of instances staining was done to disclose bacteria but none were found when the bile itself had been sterile; and when it had become secondarily infected,—for it was never so in the first days of bile collection,—the central portions of the scaffolding proved free from the organisms that were abundant in the peripheral regions. The association of the carbonate stones with organic material has already been stressed. In but a single instance were calculi of this composition, devoid of special centers of deposition, found upon clean tubing. Occasional stones were observed which had particles of talc from the rubber at their center. But most interesting were the many which contained minute, rounded, pigmented bodies, so different in appearance from the general substance of the calculus as to suggest that they had a special origin, and so located within it that they might well have acted as centers of deposition. These were found in stones of all sorts but especially in such as contained much bilirubinate. Their presence could be brought out by treatment of the calculus with acid alcohol, for although much of their substance dissolved, they left
P. ROUS, P. D. McMASTER, AND D. R. DRURY

discrete, heavily pigmented shadows deep within the general scaffolding of the stone. In one case, that of Dog 30, already given in detail, we were fortunate in finding elements identical in appearance with these "nuclei" free on the wall of the lower portion of the tube system as well as within stones there and higher up.

It may be recalled that in this animal, Dog 30, minute red-brown bodies (Fig. 11) were present on the surface of the rubber below the glass connection in the U-tube. The smallest of these were the "nuclei" referred to. They were rounded, rather soft, and highly refractile. On treatment with a dilute watery solution of hydrochloric acid, they effervesced mildly and lost in refractivity but otherwise did not alter greatly in appearance, showing that they contained but little carbonate. About many of them a concentric deposition of mixed bilirubinate and carbonate had taken place (Fig. 11). When subjected to acid alcohol, they left discrete dark shadows. Similar shadows were recognizable after the alcohol treatment, deep within certain of the large stones from close to the bile source, stones which had bilirubinate cores and carbonate peripheries of varying thickness. Other of the stones, translucent, pale green, crystalline masses of almost pure carbonate, found in the usual association with cellular débris, did not contain them.

In the instance described, some of the stones were so small that the nuclei could be directly perceived within concentric layers of deposit. That they had served as centers of the deposition was evident. The most that could be said of those within the larger calculi of this and those in other animals was that they had place deep within the more or less homogeneous scaffolding left after the treatment with acid alcohol. Such treatment tends to destroy even well marked lines of concentric deposition, as we have noted repeatedly in the case of bilirubinate calculi overlain with carbonate.

The character and rôle of the nuclei will be more closely scrutinized in a succeeding paper. We wish to emphasize here merely the fact of their existence and of their evident relationship to the development of some stones.

DISCUSSION.

The findings make plain the fact that gall stones can on occasion be formed in the absence of influences to which many authors have accorded a primary significance in their etiology. We refer to infection, stasis, and gall bladder activity.
Gilbert, Mignot, Miyake, Cushing, and others, working in the nineties of the last century, were able to induce cholelithiasis in animals by combining injury to the gall bladder with infection. Their demonstration in rabbits, guinea pigs, and dogs was so conclusive as perhaps to have overshot the mark. It has led to a general belief that without infection stones fail to occur. But recently Kehr has observed stone formation upon the free ends of threads left at operation within the human gall bladder under conditions of surgical asepsis; and Kretz has recorded a like happening in rabbits and dogs. Aschoff and Bacmeister report the occurrence of stones of special type in uninflamed human gall bladders which they suppose never to have been infected. These authors maintain that one type of human lithiasis is due to disturbances in the general metabolism, a view that has been actively combated, notably by Lichtwitz and Naunyn.

No one attempts to deny that infection of the biliary system is a frequent cause for gall stones; and intermittent biliary stasis is conceded by all to have an important influence thereon. Whether stasis acts merely by facilitating infection or by favoring deposition out of the bile has been a moot point. The remarkable concentrating activity of the gall bladder, which finds special scope when the bile is retained long, may be recalled in this connection. Naunyn showed that concentrating human bile as such does not result in increased deposition from it; but on the other hand, he furnishes instances of the rapid growth of stones lodging in the common duct which tend to encourage a belief that the concentrating ability of the gall bladder plays, on occasion, a major rôle in lithiasis. He and others have emphasized the importance for stone formation of a pathologic bladder mucosa. Yet the frequency of intrahepatic stones in the human liver shows that the rôle of the organ is no indispensible one.

Riedel has remarked that wherever bile passes, gall stones may form; and he has produced striking illustrations in point. Yet it is none the less surprising to find, as we have in the present experiments, that there exists a marked tendency to precipitation out of
normal liver bile. The point immediately comes up, why did not stones form within the bile passages of the intubated animals? The ducts possess a musculature and are far from being fixedly distended like our tubing. They possess the ability to rid themselves of organic débris, such as, collecting on glass and rubber, favors a formation of calculi. Furthermore, they elaborate a secretion of their own, which, if it does nothing else, must act to keep their lining cleansed. That it may perhaps exert a direct influence to prevent lithiasis is suggested by the absence of stones from the immediate mouths of the canulas of our animals (Fig. 1), in exception to the general rule that stone formation on the tubing was especially great at situations close to the bile source.

The solubility of calcium carbonate is affected by slight changes in the reaction of the fluid in which it is contained. A priori, one might suppose the deposition on our tubing to have been determined by a diffusion of alkali from the glass or rubber. The precautions taken to remove soluble matter from the tubing have been described, yet some may still have come away gradually into the bile. Perhaps the best evidence against such a cause for the lithiasis lies in the failure of carbonate calculi to form when the tube surface was free from organic débris. A further indication that the character of the tubing was of little consequence is to be found in the fact that the calculi from adjoining surfaces of glass and rubber were alike in character and size.

Stones were absent not only from the ducts of all of the animals, but from the tube system as well, of some that had been long intubated. This was true of two instances yielding sterile bile for 49 and 85 days, respectively, and of a third, intubated for 86 days, the secretion of which had become infected after a time. The shortest of the periods mentioned was more than sufficient for the development of good sized stones in other animals, and sometimes even of occluding ones. It is true that the tubing was free from organic débris in the three exceptional cases, but so, too, was that of a dog already referred to in which discrete carbonate stones, lacking evident centers of depo-

---

tion, were found after 46 days of bile collection. The conclusion seems justified that individual differences in the character of the bile must sometimes have determined the occurrence of calculi.

Human gall stones are of far more varied character than those here described for the dog, as would follow from the diverse pathological states in which they have been noted to develop. It seems wholly likely from the established relation between peculiar local conditions and human stones of special character that, if said conditions were as narrowly controlled in man as in the experimental animals of the present work, the resulting lithiasis would be quite as uniform in type.

Gilbert and Mignot seem to have assumed that the stones developing in their dogs under the joint influence of local infection and tissue injury consisted of cholesterol; but they present no data on the point. Dog bile contains relatively little cholesterol. The substance never, so far as we have been able to discover, precipitates from it on standing, as frequently happens in the case of human bile. And cholesterol stones of human origin introduced into the canine gall bladder are dissolved away. In view of these facts the complete lack of cholesterol in our calculous material is scarcely surprising. Surprising it is, though, to find calcium carbonate the major constituent of dog stones. For though stones formed of the salt are the commonest variety in herbivora, they are seen in man only under very special circumstances; and one might suppose from the dietary habits of the dog that the same would be true of this species.

There exists much difference of opinion as to the source of the calcium in the carbonate stones of human beings. Not a few instances are on record of carbonate casts of inflamed ducts, resembling those which occur rather often in cattle. The general association of carbonate stones with marked inflammation of the passages has led to the view that the calcium has its source in inflammatory exudate. But within the tubing from some of our animals, and more especially within the cannulas taken, similar casts were present. They had formed by the union in a carbonate matrix of calculi primarily punctate (Fig. 10). Though a thin layer of organic débris was present with them, the calcium cannot in such instances have been the product of duct inflammation. For usually the ducts were

\[ \text{Goodman, E. H., } \textbf{Beitr. chem. Physiol. u. Path.}, 1907, \text{ ix, 91.} \]
normal and the débris represented merely the accumulated products of normal desquamation from their surface. The fact has already been emphasized that the formation of carbonate stones went on, not upon but within, organic débris, as if induced by some secondary change or condition there. The question seems worth asking whether the active inflammation found in association with human carbonate stones may not tend to their formation by providing in organic débris a chemical nidus for deposition, while so interfering with the ducts that this débris is not voided as it would be under more ordinary conditions but retained to undergo changes preliminary to carbonate deposition. We shall present data which support such a view in a succeeding paper. Lichtwitz\textsuperscript{7} has already given reasons for doubting the rôle of exudate in the production of carbonate stones, and Bacmeister\textsuperscript{24} has shown that small amounts of calcium carbonate fall out of sterile human bile incubated \textit{in vitro}, though not enough, he believes, to account for carbonate stones. A well marked precipitation of the substance usually takes place in sterile dog bile allowed to stand a few hours, as we have many times noted. Infection is in some way responsible for the carbonate stones met with at the postmortem table. But it may be so merely through its rôle as the principal agent whereby long standing inflammation of the biliary system is maintained in clinical instances.

Little is known of the conditions which lead to a deposition of calcium bilirubinate out of human bile. Bacmeister\textsuperscript{25} has found bilirubinate stones in uninflamed and sterile gall bladders, but they are more frequent in the infected and damaged organ. In our dogs, calculi consisting predominantly of the substance were much less common than those of calcium carbonate, and they were usually restricted to the cannula and always to the upper reaches of the tubing. During the development of the calculi, carbonate was frequently deposited on bilirubinate stones—almost invariably, in fact, when the period of accretion was long—whereas the reverse process, bilirubinate on carbonate, was never observed despite the not infrequent secretion of a bile more concentrated than that put out in the days

\textsuperscript{24} Bacmeister, A., \textit{Beitr. path. Anat. u. allg. Path.}, 1908, xlv, 528.

\textsuperscript{25} Bacmeister, A., \textit{Ergebn. inn. Med.}, 1913, xi, 1.
immediately after intubation, when the bilirubinate core of the stones must have been laid down. There can be no doubt then that the conditions in the bile were, relatively speaking, unfavorable to bilirubinate deposition. It was noteworthy how often, just as in man, a central pigmented core of bilirubinate was present within calculi for the rest of another constitution. And as in the human creature, whole crops of such stones were sometimes present side by side. It was as if at some critical period in bile collection bilirubinate had come out of solution, providing here and there centers favorable to subsequent layering with another material,—serving in this respect just as foreign bodies (talc particles) were sometimes observed to serve, and as many sorts of foreign bodies have been noted to serve in man. The pronounced similarity in character of the stones of each crop or generation—for sometimes there was more than one—indicates that they were begun at one time and submitted to like growth conditions.

The punctate inception and development of the stones were to have been expected in the absence of preformed centers or nuclei, since deposition from slightly supersaturated solutions ordinarily takes the punctate form. But there existed special reasons therefor in many of the animals in the shape of the minute pigmented bodies about which stones tended to form. The source and composition of these bodies will be taken up in a paper to follow.

The presence in each calculus of an organic shadow or scaffold is in line with what is known of stone formation in general. Moritz, Pfeifer, and Kleinschmidt have noted that crystals falling out of dilute urine possess such shadows. The organic material of which they are composed comes down as an incidental to the deposition. Yet sometimes, as we have observed of carbonate stones forming in organic débris (Fig. 6, a, b, d, Fig. 7), the scaffold precedes the calculus, which latter is, so to speak, the result of petrifaction. Even in the case of bilirubinate calculi, the scaffolding sometimes grew faster than the stone because of the successive thin coatings with mucus that this latter received. The presence on the tube wall nearby of

26 Moritz, cited by Bacmeister.
27 Pfeiper, cited by Bacmeister.
28 Kleinschmidt, cited by Bacmeister.
a similar mucous glaze rules out the possibility of a special influence of the stone to induce its deposition.

**SUMMARY.**

Gall stones frequently form in dogs intubated for the collection of bile under sterile conditions, in the absence of stasis and of gall bladder influence. The stones consist almost entirely of two substances—calcium carbonate and calcium bilirubinate—and they are remarkably uniform in character, as would follow from the limiting conditions of their development. They are not the result of bile loss, for similar ones may be recovered from the wall of glass tubes interpolated in ducts with intestinal connection undisturbed. The study of them has brought out evidence on the general problem of cholelithiasis. Some factors in their causation and that of gall stones as a class will be considered in succeeding papers.

**EXPLANATION OF PLATES.**

**PLATE 12.**

**FIG. 1.** Stones on the walls of the glass connections in a tube system,—from a dog sacrificed 24 days after intubation. The bile had been sterile. A, cannula containing calculi, some of which had become secondarily united and led to biliary obstruction. The absence of stones from about the entrance to the cannula should be noted. B, the curved tube that connected the arms of the rubber U. Here two “crops” of discrete calculi are to be seen. C, the T-tube from just above the collecting balloon. It contains small calculi only. × about 4½.

**FIG. 2.** Calculous deposit on the rubber tubing immediately below the glass connection in a U,—to show the discrete character of the stones and their greater frequency and larger size on the dependent side of the tubing and in the angle between rubber and glass. From the dog furnishing the material for Fig. 1. The tube has been laid open by a cut through the side that was uppermost during life of the animal. The area completely free from stones had been covered by the glass connection. × about 4½.

**PLATE 13.**

**FIGS. 3 to 5.** Calculi scraped from tubing through which sterile bile had been collected for 23 days. Figs. 3 and 4 show the stones by transmitted and reflected light respectively × 20; and Fig. 5 the shadows of them left after treatment with acid alcohol. × 36.
PLATE 14.

Fig. 6. A drawing of individual calculi from here and there on the wall of the cannula depicted in Fig. 1. a, b, d—carbonate calculi developing in the organic débris which had accumulated on the lower side of the tubing. a gives indications of the crystal form; and d has a pigmented center. c, stalactite forms of calcium bilirubinate from the upper side of the tubing. They are covered with a transparent skim of mucus. × 54.

Fig. 7. Drawing of stones of two distinct types as they existed side by side on the walls of a cannula. The dark ones consisted of calcium bilirubinate, and those that were pale, and crystalline, of calcium carbonate. The latter were situate within small masses of organic débris. The dog had been intubated for 67 days, and the bile was infected after the 8th day. × 54.

PLATE 15.

Figs. 8 and 9. Carbonate concretions from the wall of a glass tube that had been interpolated in the common duct of a healthy dog 35 days previously. The development of the stones by deposition in successive layers with retention of the crystalline form is well shown. × 77.

Fig. 10. Fragments of a stony cast of the inside of a cannula which had been connected with the common duct for 36 days during which time the bile remained sterile. An almost colorless deposit of calcium carbonate is to be seen on the large dark calculi. It had united these latter in a matrix. × 58.

Fig. 11. "Nuclei," some of them showing secondary deposition, from far down in the tube system of the same animal. All were intensely pigmented, but this feature is not evident in the photograph, owing to the use of a color filter which acted to minimize it. × 209.
(Rous, McMaster, and Drury: Causes of gall stone formation. I.)
(Rous, McMaster, and Drury: Causes of gall stone formation. 1.)
(Rous, McMaster, and Drury: Causes of gall stone formation. 1.)