OBSERVATIONS ON SOME CAUSES OF GALL STONE FORMATION.

III. The Relation of the Reaction of the Bile to Experimental Cholelithiasis.

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In previous papers it has been shown that there exists a well defined tendency for calculi to form out of the sterile liver bile of dogs, irrespective of whether or not these animals are losing the secretion;[1] and that after hepatic injury of various sorts potential centers for lithiasis are frequently abundant therein.[2] Why, then, are gall stones almost never encountered in autopsies on dogs dying from ordinary causes? We have suggested an answer to this question in so far as it concerns the ducts. There are several evident safeguards against stone formation within them,—their motility, the cleansing and possibly antagonistic action of the secretion they themselves elaborate, and the flushing out effected by an intermittently quickened bile stream. But how is one to explain the absence of calculi from the gall bladder, a viscus which, according to the latest evidence, empties itself but poorly, and in which the bile often undergoes great concentration[3] and must frequently be held for many hours together. Nearly half of the score or more of normal canine gall bladders that we have examined contained an abundant débris consisting of shreddy mucous particles and flakes of long desquamated epithelium not infrequently stained black as result of secondary changes in pigment during the course of their sojourn in the organ. Once actual quartz sand was present,—small granules resisting aqua regia, like those not infrequently recovered

[1] Rous, P., McMaster, P. D., and Drury, D. R., *J. Exp. Med.*, 1924, xxxix, 77.

[2] Rous, P., Drury, D. R., and McMaster, P. D., *J. Exp. Med.*, 1924, xxxix, 97.

[3] Rous, P., and McMaster, P. D., *J. Exp. Med.*, 1921, xxxiv, 47.

from the biliary system of other domestic animals,[4] into which they were presumably forced from the intestine by the muscular contractions of the gut. Yet never have gall stones been present. In view of the marked tendency for calcium carbonate to come down out of liver bile on standing, and for it to deposit within old organic débris,[1] it seems certain that the normal gall bladder must either effect some change in the secretion in the direction of safety, or else add some preventative against deposition. Both possibilities are within reason, since the *vesica fellea* is known to alter markedly the fluid coming to it.

Influence of the Gall Bladder on the Bile Reaction.

The solubility of calcium carbonate, the substance most abundant in the gall stones that we have induced experimentally in dogs, is greatly affected by the reaction of the fluid in which it is contained. Lichtwitz[5] has touched upon the possible influence of the bile reaction on gall stone formation in his discussion of the puzzling absence of calcium bilirubinate from normal human bile. Naunyn[6] before him had tentatively advanced the view that a special reaction may be necessary for the formation of the substance. Following the line of thought suggested by our own work, and by such speculative reasonings, we have tested the reaction of bladder bile of the dog and have compared it with that of the secretion as derived from the hepatic tissue. To our surprise bladder bile proved frankly acid to litmus in a large proportion of instances, whereas liver bile was nearly always somewhat alkaline, and usually markedly so. The literature showed that the difference had already been noticed by Okada[7] for the dog, and by Neilson and Meyer[8] for the rabbit. Curiously enough, it has failed to attract attention in its possible bearing on gall stone formation.

Okada used the capillary electrometer. He ascertained that the pH of the gall bladder bile of dogs ranged from 5.43 to 6.97. His animals almost without exception had been fasting; but the data he obtained in other species supported

[4] Kitt, T., *Lehrbuch path. Anat. Haustiere*, Stuttgart, 3rd edition, 1906, ii.

[5] Lichtwitz, L., *Ergebn. inn. Med.*, 1914, xiii, 1.

[6] Naunyn, B., cited by Lichtwitz.[5] See also Naunyn, B., *Mitt. Grenzgeb. Med. u. Chir.*, 1921, xxxiii, 1.

[7] Okada, S., *J. Physiol.*, 1915–16, l, 114.

[8] Neilson, N. M., and Meyer, K. F., *J. Infect. Dis.*, 1921, xxviii, 511.

the view that bladder specimens may be alkaline, acid, or neutral, with a tendency for them to be relatively acid after fasting. Liver bile, procured through a fistula of Schwann, was, by contrast, always alkaline, with a range in pH of 7.54 to 8.15. Fasting was without an evident influence upon such specimens. Those exposed to air rapidly became more alkaline.

Neilson and Meyer made use of a colorimetric method and studied rabbit bile especially, but some other biles also. They corroborated Okada's findings and traced the change in pH on exposure to air to a loss of CO_2. They showed further that base-forming diets notably increase the average titrable alkalinity of rabbit bile, whence they inferred that this alkalinity is due to the presence of carbonates.

We have employed the potentiometer. The readings were made in duplicate at 37°C. Exposure of the specimens to air was avoided by withdrawing them from the collecting balloons, or from the gall bladder, directly into Luer syringes cleansed of alkali by long previous use and boiled in water for present purposes. The determinations were made as soon as possible after the bile had been procured, though tests showed that even several hours in the syringe did not notably alter the pH.

Day-to-day pH determinations were made on the liver bile of twelve dogs studied with special reference to the bile sediment. The observations in some instances extended over many weeks. In addition, the total calcium content of the bile was determined as routine by ashing, precipitation with sodium oxalate, and titration with potassium permanganate. The results of the analyses will form the topic of a separate communication. They will be considered here only in so far as they bear directly on the problem under discussion.

The liver bile regularly proved alkaline, often more strongly so than Okada found. The pH ranged from 7.07 to 8.55 and tended to approximate 8.20 ordinarily. But Okada's specimens, unlike ours, had been subjected to gall bladder influence, owing to the circumstance that the organ formed a link in the fistula system. On the other hand some carbonate precipitation had occurred in our specimens during their period of sojourn in the collecting balloons. This should have rendered the bile less alkaline, if anything, owing to the fact that calcium carbonate represents the union of a strong base with a weak acid. The precipitate was present in proportionate amount in the samples tested.

Fasting is attended by a great diminution in the amount of bile secreted, and in a marked and in some instances closely reciprocal increase in the concentration of certain of its constituents.[9] According to Okada, the reaction undergoes no change. But we find to

[9] McMaster, P. D., Broun, G. O., and Rous, P., *J. Exp. Med.*, 1923, xxxvii, 395.

TEXT-FIG. 1. Influence of fasting on the amount and pH of liver bile.
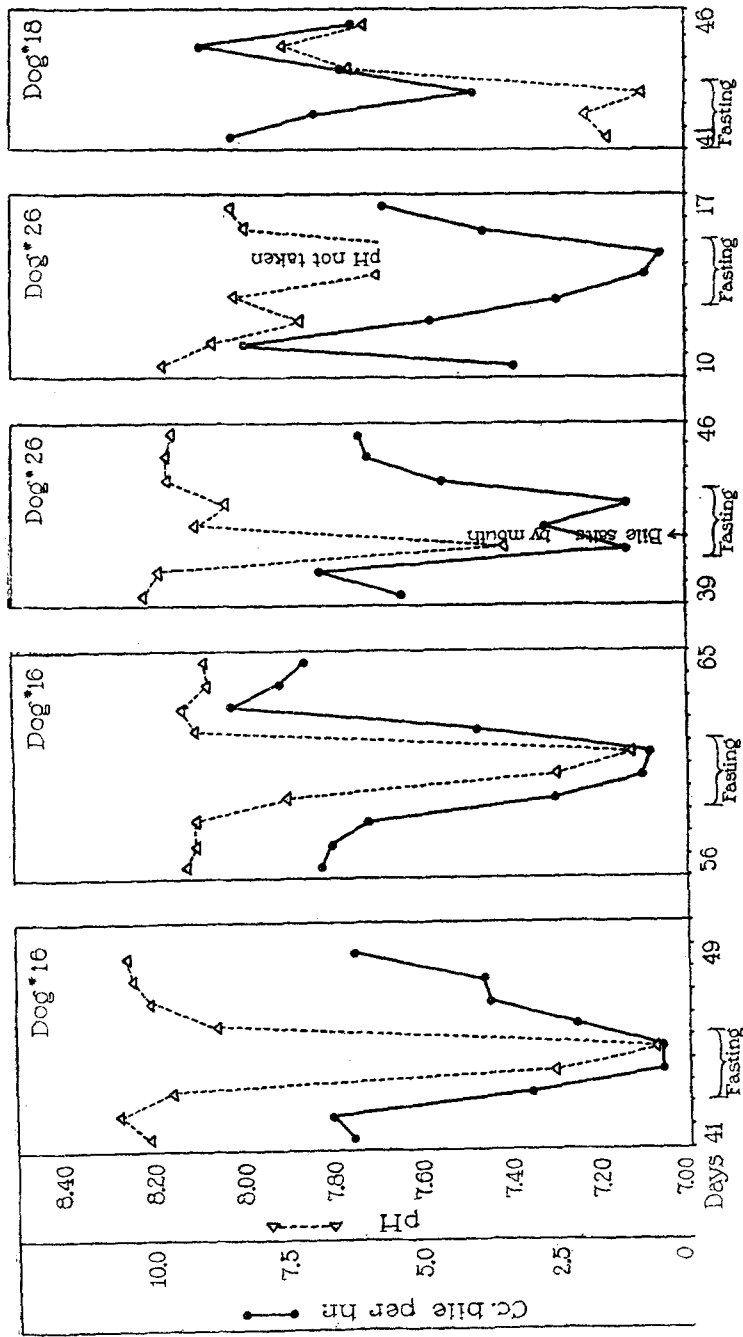
All biles sterile except that of Dog 18.

the contrary that the bile regularly becomes less alkaline during a fast, and, as this progresses, may become approximately neutral to litmus, though the pH has never in our experience quite reached 7.00 (Text-fig. 1).

The gall bladder bile of a number of normal dogs was procured by operation under ether. As in the case of liver biles, the individual differences in reaction were greater than those noted by Okada,— which would naturally follow from the larger and more various material at our disposal. The gall bladder specimens obtained a few hours after a meal were either neutral to litmus or alkaline—not infrequently as alkaline as some liver biles,—whereas those procured after 48 hours of fasting, were as a rule markedly acid and reddened litmus promptly. Specimens of this latter sort were heavily pigmented and syrupy, appearing greatly concentrated. It has long been supposed on good evidence [10,11] that during intervals between meals bile accumulates in the gall baldder and is concentrated and stored there, to be more or less gradually voided and replaced with new when the digestion of a meal takes place. Recent observations which indicate that the viscus empties itself imperfectly do not invalidate this view. Fresh support for it is to be found in our observations. In normal dogs recently fed, the reaction of the gall bladder contents approximated that of liver bile, whereas after fasting it was found acid, and concentrated and thickened, all to a degree never encountered in liver bile, as procured from fistulas. That the prevailing alkalinity of liver bile is not due to the loss of secretion entailed by the fistula may be safely inferred from the alkaline character of the gall bladder contents of full fed normal animals (Table I).

The alteration that takes place in the reaction of bile within the gall bladder may conceivably be the result of chemical changes occurring in a labile secretion as result of its prolonged retention at body heat, or on the other hand, of some influence exerted by the organ of retention. That the latter is the true explanation may be inferred from the fact that liver bile collected into a balloon and kept warm remains highly alkaline, as further from the demonstrated activity of the gall bladder

[10] Naunyn, B., *Arch. Anat., Physiol., u. wissensch. Med.*, 1869, 579.

[11] Babkin, B. P., Die äussere Sekretion der Verdauungsdrüsen, Berlin, 1914, 344.

to influence the bile in other ways, and from Neilson and Meyer's[8] observation that in gall bladders so injured by infection as to lose the function just referred to, the pH of the bile remains that of the secretion as derived from the liver.

Influence of the Bile Reaction on Carbonate Precipitates.

Granting, then, that the gall bladder acts to render the bile acid, it has remained to demonstrate an influence of changes in the reaction of dog bile upon carbonate precipitation. This has been sufficiently accomplished by a series of simple experiments with bile that contained such a precipitate. They need be but briefly described.

TABLE I.

The pH of Gall Bladder Bile and Its Relation to Fasting.

Dog No.	pH	Remarks.
1	5.18	
2	5.67	No food for 24 hrs. and but little at last feeding.
3	5.41	
4	6.20	
5	5.31	No food for nearly 24 hrs. and long ether anesthesia.
6	5.57	
7	7.05	
8	6.82	No food for nearly 24 hrs.
9	6.27	
10	7.06	
11	7.43	
12	7.33	
13	7.61	Food within a few hrs.
14	7.03	
15	7.00	
16	7.55	

Several like portions of a single specimen of dog bile, each with its share of crystalline carbonate, as obtained from the collecting balloon, were distributed in 50 cc. Pyrex centrifuge tubes previously rinsed with distilled water. Some were rendered neutral to litmus, and others made faintly acid thereto, while still others were kept as controls. The acids employed for the purpose were N/10 hydrochloric acid, concentrated lactic acid, and 3 per cent acetic acid. The

specimens treated with hydrochloric acid became opalescent owing to the development of a finely divided mucous precipitate, but the others appeared unchanged. All were now brought to the same bulk by the addition of distilled water, and either centrifuged at once or allowed to stand overnight at room temperature before so doing. The experiment was several times repeated. The controls regularly yielded the sediment of carbonate originally present, whereas in the portions made neutral or acid it was completely or partially dissolved. Some of the original specimens contained pigmented nuclei, potential centers for lithiasis, of the sort heretofore described,[2] as well as carbonate crystals. These nuclei dissolved, but somewhat less rapidly than the associated carbonate crystals.

Several unsuccessful attempts were made to determine a precipitation of calcium carbonate out of liver bile by exhausting it of free carbonic acid. For the purpose, the secretion was taken from the collecting balloon into large centrifuge tubes filled with CO_2 gas, deprived by centrifugation of the sediment previously formed, separated into portions under CO_2, and some of these portions placed *in vacuo* for several hours, with the container tilted so that a large surface of fluid was exposed. No carbonate came out, nor did it in the controls. Evidently the precipitation of the salt occurring in the balloon had reduced the bile content of it far below the saturation point.

Efforts to dissolve a carbonate sediment by bubbling CO_2 through the bile were likewise unsuccessful, probably because the period of a few hours allotted to the tests was too short. The greatest change accomplished was a dubious rounding of the crystal edges. The results have an interest in connection with the not infrequent presence of carbonate calculi within glass bulb connections interpolated in the course of the common duct of animals otherwise normal, which connections must have been flushed at intervals with more or less acid bile from the gall bladder. The persistence of the calculi may be laid to the transitory nature of this flushing. The bulbs when removed from the animals always contained alkaline bile.

It would seem from these experiments, crude though they are, that no other explanation is needed for the lack of carbonate deposition in the normal gall bladder than the changes in reaction of the bile effected by this viscus. Further observations upon the changes occurring in the liver bile during a fast, and on those in the bile that assembles and distends the gall bladder after obstruction of the common duct clearly indicate that the reaction of the secretion undergoes an alteration in the direction of safety when there might otherwise be danger of carbonate precipitation.

The Calcium Concentration and Reaction of the Bile as Affected by Fasting.

On the withdrawal of food from the intubated dog for 2 or 3 days, the bile output usually lessens greatly, and may diminish to less than one-tenth of the ordinary amount.[9]   Such alterations are accompanied

(Bile sterile)
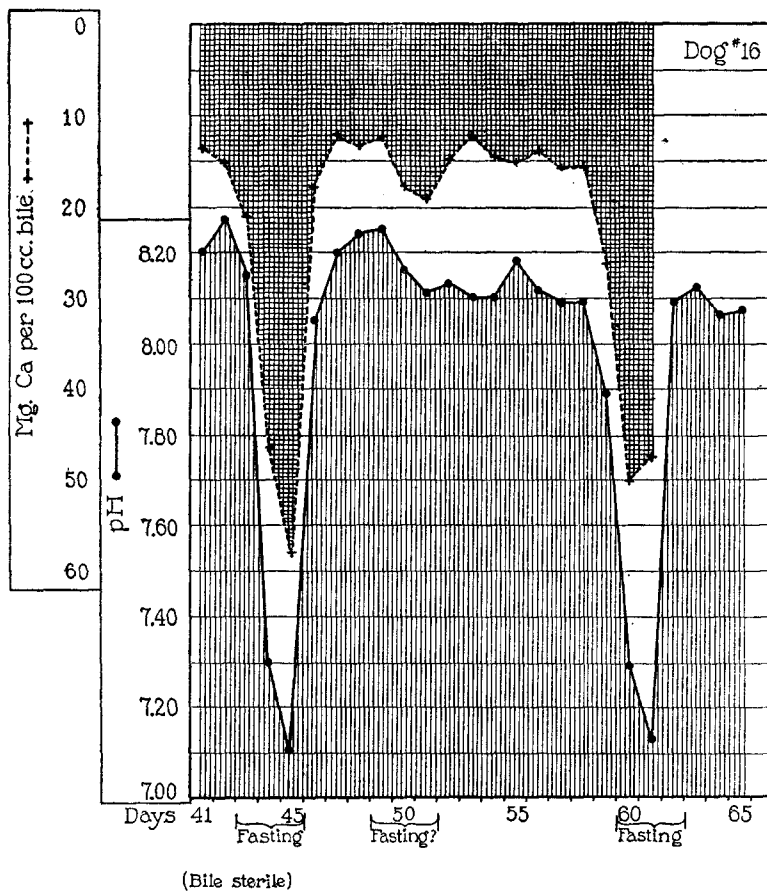
TEXT-FIG. 2. Calcium concentration and pH of liver bile.

by a reciprocal increase in the concentration of bilirubin, and the total output from day to day remains the same.   There is a marked but less considerable increase in the calcium per cc. (Text-fig. 2).   And *pari passu* with this increase and the pronounced slowing of the bile stream,

changes both that, other things being equal, would favor precipitation within the duct system, there occurs a change in the reaction of the bile such as would tend to rule out this possibility. The pH falls from an average of approximately 8.20 to close to 7.00, that is to say to approximately the neutral point for litmus (Text-figs. 1 and 2). The precipitate found in balloon specimens collected under these circumstances is often far less than the ordinary, and never greater despite the high concentration of the bile.

The Calcium Concentration and Reaction of Stasis Bile.

When the common duct of a previously normal animal is obstructed a very great concentration of the bile pent in the bladder may come about through the action of this viscus.[12] Yet, as is well known, stones fail to form out of the dark, thick fluid.

We have, in seven instances of experimentally induced stasis, ascertained the reaction of the bladder bile and determined its bilirubin and calcium content.

Obstruction was produced by tying and cutting the common duct in every instance save one in which it followed the clotting of blood within a glass tube which had been interpolated in the duct. Asepsis was maintained during the operations and the eventual stasis fluid regularly proved sterile on culture. It was taken into syringes under the precautions already described. For comparison with it the gall bladder content was removed at the initial laparotomy through a rubber catheter run up into the viscus by way of a slit made in the common duct below the point at which this was to be ligated. The dogs had purposely been fed full a few hours before operation. Thereafter they as a rule ate fitfully, if sometimes well.

From the table (Table II) it will be seen that the period of obstruction ranged from 2 to 6 days. The eventual bladder contents showed, in comparison with that originally procured, a pigment concentration which was sometimes nothing less than startling. In the case of Dog 1, there was 18 times as much pigment per cc. in the stasis specimen as in the normal sample originally had, and in Dog 6, 53 times as much. Part of this increase is attributable to the influence of operation on the character of the bile[9] but most of it is undoubtedly due to the con-

[12] Rous, P., and McMaster, P. D., *J. Exp. Med.*, 1921, xxxiv, 75.

centrating activity of the *vesica fellea*.[3]   The change in calcium con-
centration was, relatively speaking, negligible, though in four out of
five instances there was a well defined increase, once to double the
initial amount.   No calcium precipitate was ever found.   The pH
of the initial specimens has been either alkaline or neutral, whereas
that of the stasis bile was acid, sometimes notably so.

TABLE II.

The Reaction and Calcium Content of Stasis Bile.

Dog No.	Stasis.	Bile character.	Bilirubin per cc.	pH	Total calcium per 100 cc.	Remarks.
	days					
1	0	Watery, clear, yellow-brown.	0.47	7.61	22.8	Fed on day of operation.
	3	Greenish black.	8.42	6.79	59.0	
2	0	Pale amber, clear.	0.56	7.55		Fed on day of operation.
	5	Brownish black.	4.66	5.82	53.3	
3	0	Amber, clear.	0.46	7.0	38.0	Fed on day of operation.
	6	Greenish black.	2.2	6.1	44.6	
4	0	Dark brown, flaky.	1.03	7.03	28.5	Fed but ate little on day
	4	Dark brown.	1.78	6.85	21.8	of operation.
5	0	Pale yellow.	0.3	7.43	30.7	Fed on day of operation.
	2	Greenish black.	4.26	6.15	47.4	
6	0	Pale yellow.	0.18	7.06	30.7	Fed on day of operation.
	2	Greenish black.	9.61	6.14	53.0	
7	?	Greenish black.	0.78	5.18	39.7	Tissue icterus, so obstruction had probably lasted at least 1 week.

All the bile specimens proved sterile.

The changes in reaction which occur in normal bladder bile, in the
liver bile of fasting animals, and in stasis bile take place at times when
the calcium content of the bile is increased.   That they are not an
indirect result of such increases is sufficiently shown by the lack of
correspondence between the two under conditions of stasis (Table II).

Gall Bladder Shortcomings and Stone Formation.

If changes in bile reaction effected by the gall bladder act as the normal preventative of carbonate lithiasis, when these changes are suppressed stones should form. We have been spared the labor of experiments on the point through the existence in the literature of observations which suffice to illustrate it and which are the more telling because recorded in another connection.

Meyer, Neilson, and Feusier[13] have noted that in rabbits with a typhoid infection of the gall bladder, stones consisting of calcium carbonate regularly develop when the animal survives for a considerable period (100 days). They attribute the calculi, as have others in the past, to the inflammation set up by the infection, and they believe that all carbonate stones are traceable to infection, a view rendered untenable by our work.[1] In a separate paper, Neilson and Meyer have dealt exhaustively with the reaction of normal and pathological bile but in no reference to stone formation. They state that the liver bile of the rabbit is regularly alkaline, having a pH of 7.6 to 7.9, whereas that from normal gall bladders may be acid, neutral, or alkaline, the pH ranging between 5.7 and 7.7, much as in the dog. The bladder biles of typhoid carrier rabbits were, by contrast, alkaline with the pH never less than 7.00 and on the average approximating that of normal liver bile. They conclude that "the gall bladder of a 'carrier' animal has apparently lost its concentrating function and therefore fails to change the reaction of its content." They mention that some of the carrier biles they tested contained carbonate sediment.

In brief, these observations clearly show that gall bladders which have been damaged by the typhoid bacillus fail to alter the character, and in special the pH, of the bile coming to them from the liver. When this abnormal state of affairs endures for some time a carbonate lithiasis inevitably develops. That there is a relationship of cause and effect between the bladder shortcomings and the development of stones cannot be definitely affirmed. Yet the findings bring support by the method of difference to the conception,—based initially on evidence through the method of agreement,—that the formation of carbonate stones in the gall bladder of the dog and rabbit is prevented under normal conditions by changes in the reaction of the bile that the organ effects.

[13] Meyer, K. F., Neilson, N. M., and Feusier, M. L., *J. Infect. Dis.*, 1921, xxviii, 456.

The Reaction of the Bile in Its Relation to Human Cholelithiasis.

Stones formed predominantly of calcium carbonate are rare in man. Human calculi are of such varied character that a single factor can scarcely be the prime agent in the genesis of all. Yet the question is worth asking whether the reaction of the bile, or to speak more concretely, the failure of a pathologically damaged gall bladder to alter the reaction of the bile, may not have a share in determining human lithiasis.

In a preceding paper[2] we have described spheroliths of calcium carbonate occurring in the sterile bile from human gall bladders containing calculi, and serving as centers for a formation of secondary stones. It has been interesting to compare the reaction of biles containing such spheroliths with that of specimens in which they were not present. Nine samples of bile in all have been tested, which were removed from the gall bladder at operation, and also subsequent fistula specimens from four of the cases. The derivation and general characters of the material have been described in the paper just referred to. We are indebted for the specimens to Doctor Allen Whipple of the College of Physicians and Surgeons of Columbia University.

The bladder biles were aspirated direct from the organ into Luer syringes previously submitted to boiling water; and the drainage specimens were collected, under oil occasionally, in Pyrex centrifuge tubes. The patients had received no food on the day of operation. While care was exercised to prevent exposure of the bladder contents to air, in most instances this was not wholly prevented. Dog or rabbit bile becomes more alkaline on contact with air, and some alteration in the same direction may be expected in the human instance.

When the amount of bile was sufficient its reaction was determined in duplicate with the potentiometer; but frequently reliance had to be placed on litmus.

In two out of four instances of cholelithiasis with mild chronic cholecystitis the bladder bile had a pH of 7.00 and of 6.98, respectively; and in one of the others,—the sole one of them with an infected bile, —it was neutral to litmus, and in the fourth alkaline. The bile from a peculiarly shaped but otherwise normal gall bladder had a pH of 6.72; that from a case with dubious cholecystitis in the absence of infection a pH of 7.28; while in an instance of mild chronic inflammation with infection, the pH was 7.06. The sterile white bile associated with a carcinoma of the head of the pancreas that had metastasized to the

gall bladder wall was markedly acid, having a pH of 6.29; whereas in another instance of a growth localized as yet to the pancreas but producing intermittent biliary obstruction the tarry specimen proved alkaline to litmus, possibly as result of the presence of blood intermixed with it when procured. There must have been little, if any, impairment of the concentrating activity of the bladder in this last case. All of the fistula specimens were highly alkaline, their pH averaging 8.00. But they were collected during the adjustment period after obstruction, while the patient was still jaundiced, and hence must be regarded as abnormal.[9] Also there had been a relatively great exposure-of them to air.

The reaction of human bile has received much notice in the past, though usually of a perfunctory character. The secretion is stated to be either alkaline or neutral to litmus. Little distinction has been made between gall bladder and duct specimens, and some authors do not mention the indicator that they employed. Neilson and Meyer have cited the literature. They alone made pH determinations, on fistula specimens from a single patient. They found that the pH from day to day approximated 8.00. They do not mention precautions to prevent exposure of the material to air.

An immediate conclusion which follows upon the inspection of our scanty data together with the prior ones of Neilson and Meyer is that many more cases must be studied, and under conditions better controlled, before certainty will be reached as concerns the effect of the gall bladder upon the reaction of the bile. But the available data show at least that the gall bladder bile of man is in some instances neutral or slightly acid to litmus, whereas liver bile tends to be alkaline. And the findings as a whole support the supposition that the secretion is alkaline as it comes from the liver, but that it undergoes an alteration toward the acid side during its sojourn in the *vesica fellea*, just as in the rabbit and the dog.

Changes in Reaction Determining Precipitation from Human Bile.

Is the solubility of the substances out of which human stones form affected by slight changes in the bile reaction? We have made some simple experiments on the point.

Experiment 1.—Into each of five small test-tubes, there was put 1 cc. of a medium brown bile with pH of 7.00 from a gall bladder containing "old" cholesterol stones. The fluid was sterile but slightly cloudy, because of the distribution through it of fine, myelin-like bodies, as the microscope showed. No sediment came down from it on centrifugation. To four of the tubes were added respectively 1, 2, 3, and 4 drops of N/10 potassium hydrate in water. All were now allowed to stand overnight at room temperature and again centrifuged. From the tube to which nothing had been added and from that containing 1 drop of potassium hydrate there was recovered a copious, light brown sediment of myelin-like material which dissolved almost completely in acetone and proved on test to be mainly cholesterol. The supernatant bile was alkaline to litmus in both instances. Acidification of it with HCl failed to cause the sediment to go into solution again. In the tubes containing 2 to 4 drops of hydroxide, no precipitation had occurred.

Experiment 2.—A bile, dark brown, thin, and faintly cloudy, from a case of chronic cholecystitis, was rid of a slight mucous sediment by centrifugation and distributed in ¾ cc. portions in each of four small test-tubes. The fluid had been sterile, with a pH of 7.06. To one portion a drop of 1 per cent lactic acid was added, to others, 1 and 2 drops of potassium hydrate, respectively, while a fourth was kept as such. All were allowed to stand at room temperature for 3½ hours and then centrifuged. The acid specimen yielded no sediment, whereas that kept as such gave a slight one of mucus and myelin bodies (cholesterol), while those containing potassium hydrate yielded a well marked one of the same materials.

Experiment 3.—A sterile, medium brown, clear, and slightly syrupy bile from a case of dubious cholecystitis was centrifuged to rid it of a few cells which it contained; and 1 cc. specimens were placed in each of three test-tubes. The initial pH of the fluid was 7.28. To one tube a drop of 1 per cent lactic acid was added, to another a drop of N/10 potassium hydrate, while the third was left as such. After 1½ hours at room temperature all were centrifuged, sedimentation noted, and they were let stand overnight and centrifuged again. No further sediment had come down during the interval. In the tube receiving alkali there had been present, after the initial hour and a half, a considerable deposit of cholesterol in myelin form, and in the tube let be as such, a slight sediment of the same material, whereas in that containing acid no deposition whatever occurred.

Another experiment similar to that just described, carried out with a ropy, black bile, failed of result in the way of sediment, as did one with a thin, lightly pigmented, and presumably highly dilute, fistula specimen secreted during the readjustment period after relief from obstruction.

Our finding, that small quantities of base or acid will determine or prevent a precipitation of cholesterol from human bile, might perhaps have been predicted from the known complexity of the secretion and,

in special, from that of the relationships whereby cholesterol is maintained in its distribution through the fluid. Yet the results do not lose in signficance for this reason.

Human bile is a well buffered fluid; and the quantity of hydrate or acid added in our tests cannot have greatly altered the reaction. Neilson and Meyer neutralized some rabbit biles with concentrated lactic acid or N/10 HCl and found that there occurred only a temporary change in the hydrogen ion concentration which in some instances returned to the initial figure within 24 hours. Others before us have noted that cholesterol frequently falls out of human specimens that have stood for a few hours *in vitro*. An explanation for the phenomenon may be found in alkali derived from the glassware; and another in a presumptive loss of carbon dioxide on exposure of the bile to air. Whatever the cause for the deposition, it has, in our experience, proved preventable through the addition to the bile of a trace of acid. On the other hand, bile rendered acid to litmus may have no effect to dissolve a cholesterol deposit that has once formed. Certainly it had none in the single test we made.

In three of our instances of cholelithiasis there was present in the gall bladder a quantity of bile sand in addition to large stones. Fragments of the older stones together with cholesterol plates made up the bulk of this sand in two of the cases; but there were present as well, in all, minute concretions, spheroliths which consisted of calcium carbonate in impure form. These have been described in detail in a previous communication.[2] They were beautifully concentric in structure, and on the surface of some of them a deposition of cholesterol, or of pigmented amorphous material, or of both had taken place, while still others were situated deep within larger stones. Their interest in the present connection lies in their association with a heavily pigmented, thick bile, one that is to say which had undergone alterations within the gall bladder. For it may be recalled that in the case of the dog and rabbit carbonate precipitation tends to take place from the unchanged liver bile on standing; and we have referred the absence of precipitation within the gall bladder to the activities of this viscus to prevent it by altering the bile. There can be no doubt that in the human instances now under discussion one of these activities, namely that through which concentration of the bile is effected, had still

been retained in considerable part by the pathologically altered bladder; while that the ability to alter the reaction of the secretion had not been wholly lost seems probable from the fact that one specimen containing spheroliths was neutral to litmus and another had a pH of 6.98. The third bile was faintly alkaline to litmus.

The circumstances under which the spheroliths originally formed are of course unknown. We have already drawn attention to the fact that carbonate concretions may not readily redissolve in bile,—this while discussing their presence upon the walls of glass tubes interpolated in the common duct of dogs and intermittently flushed with normal bladder bile. It is possible as accounting both for their origin and survival in our human cases that there was a pathological increase in the bile content of calcium; or that the concentration of the secretion effected by the gall bladder was of an abnormal sort, preventing solution of the spheroliths despite favorable changes in the bile reaction, or that these changes themselves were never sufficiently great to accomplish this. Speculation upon the point is idle. But the findings themselves serve to forecast a difficulty which may be expected in attempts to correlate the reaction of human bile with the presence of stones.

DISCUSSION.

The problem of cholelithiasis presents two major facets to the pathologist, why stones begin, and why they grow. In a previous paper we have considered some aspects of that first mentioned and have shown that in the dog, at least, potential opportunities for stones to begin are offered in many sorts of liver disturbance. Out of the bile elaborated under such pathological conditions there come down "showers" of little concretions, nuclei which can serve as centers of stone formation, and which frequently did so under the abnormal circumstances of our experiments. Potential nuclei of other sorts, organic débris in special, must pass along the biliary channels at some time or other in the life of many individuals.

In the present communication we have been concerned with why stones grow, or, to put the problem in a form more accessible to attack, why, given the recurring opportunities just mentioned for stones to arise they remain rare, relatively speaking. The question is an old one,

if stressed anew by our observations. For the statement has often been made, with a query appended, that gall stones seldom form on the sediment so frequently contained in the gall bladder; and the fact has repeatedly been noticed that although foreign bodies frequently serve as the nuclei of gall stones, their presence in the biliary tract is not of itself sufficient to cause lithiasis.[14]

The experiments and observations here recounted afford an explanation for the occurrence of calcium carbonate stones in laboratory animals which is more than merely rational being probably the real one. A study along similar lines of the reaction of the bile in its possible relation to the development of calculi consisting of calcium bilirubinate would appear to be highly desirable, because of the important rôle conceded to the substance in human stones. But the indications are that this may prove difficult. For the physical chemistry of bilirubinate precipitation is a complicated matter; while furthermore the methods at present available do not always enable one to identify the substance with certainty in the test-tube. It is deposited from the bile of the dog only on special occasions and then in small amount.[1] The existing literature on stones of the guinea pig and rabbit suggests that in these species as well it is seldom formed in quantity.

According to the thesis supported by our work, carbonate stones develop in intubated dogs and in typhoid carrier rabbits as the result of a tendency for the salt to be deposited out of the normal bile of both species. In healthy animals of the sorts mentioned this tendency is counteracted in the gall bladder by a change that the organ effects in the reaction of the bile, as result of which carbonate remains in solution despite the great diminution its fluid vehicle undergoes. When the gall bladder function is so impaired that it fails to effect this change and the reaction of the bile remains what it was when first derived from the hepatic tissue, carbonate lithiasis may automatically ensue.

Does this explanation of the occurrence of a certain type of calculi under controlled conditions in some laboratory animals bring with it any implications as regards the causes for human cholelithiasis? Needless to say, a single factor can scarcely be expected to account for

[14] Mignot, R., *Arch. gén. méd.*, 1898, x, series 8, 129.

all the diverse categories of human stones. Yet the fact is worth recalling that calcium carbonate is no uncommon constituent of these stones, if seldom a principal one. As already mentioned, we have recovered calcium spheroliths from human gall bladders in which they were serving as centers for a secondary formation of gall stones. The suggestion that calcium bilirubinate may be precipitated from human bile at a certain reaction only was made years ago.[5] And the test-tube findings of the present work sufficiently prove that a precipitation of cholesterol out of the bladder bile of man can be determined or prevented *in vitro* by slight additions of base or acid which would tend to effect alterations in the bile reaction such as there is reason to suppose may be decisive within the organism. These are suggestive facts. Our purpose is not to emphasize them but to outline, irrespective of them, an attitude toward the problem of human cholelithiasis which may perhaps have advantages besides novelty. It has become old habit to ask, why do stones form? But might not the question better be, why are they ever absent? May not a gall bladder or duct functionally impaired through infection or by other means (recurring stasis?) become *particeps criminis* to stone formation as result of its sins of omission? Faulty evacuation of the bile channels has long been deemed a contributary element in lithiasis. But may not a failure on the part of the gall bladder to modify the bile committed to it be sometimes the decisive factor?

To test the hypothesis in its bearing on the human case will require a large material. Quite possibly the normal gall bladder effects other changes in the bile besides those of reaction, that are inimical to stone formation, serving in this way to offset the favoring influences of concentration and retention. But much remains to be learnt in relation to the influences of the bile reaction. It is needful to ascertain first of all whether, as our findings seem to indicate, the normal gall bladder of man effects changes in the reaction of its content comparable with the changes occurring in the dog and rabbit. In these days of "prophylactic" ablation, data on the point will not be difficult to procure. But to determine whether stones form when the gall bladder fails to alter the reaction of the bile may prove an arduous task. A few of the difficulties have already been touched upon in connection with the figures on the pH of human biles containing carbonate spero-

liths. The problem was simpler in the case of typhoid carrier rabbits, because of the limiting conditions and the type of calculus encountered. But in patients coming to operation, the manifestations of physiological principles are only too often overlaid by phenomena irrelevant to them, for example those of infection. That the human gall bladder, though containing stones, may retain part at least of its ability to concentrate the bile is certain. Our findings suggest that the ability to alter the bile reaction may likewise not be totally lost under these circumstances. Fitz[15] has concluded as the result of a series of observations upon operative material, that bladder bile from gall stone patients tends to be less pigmented, of lower specific gravity, and less rich in nitrogen than that from cases of cholecystitis only. The finding might perhaps be taken to suggest, in line with our hypothesis, that a failure of the bile reservoir to modify the secretion coming to it from the liver is concerned in the development of stones. But the need of the moment is for data.

Conjectures only can be made as to the means whereby alterations in the reaction of the bile come about in the *vesica fellea* of the dog and rabbit. It is reasonable to suppose that the same processes of osmosis and diffusion may be responsible which bring about a reduction in the bulk of the secretion. Were this the case, one would expect to find an impairment of the concentrating ability constantly associated with a greater or less failure to alter the bile reaction,—unless indeed the permeability of the bladder wall for some substances may be altered without affecting that for others.

SUMMARY.

As previous papers from our laboratory have shown, there exists a well defined tendency for calcium carbonate to come out of solution in the normal liver bile of the dog, and for it to be deposited on certain nuclei not infrequent in the secretion under pathological circumstances. Gall stones that had arisen in this fashion were a frequent occurrence in the intubated animals we studied. The present paper is concerned with the reasons for the absence of such stones from dogs with an intact biliary tract.

[15] Fitz, R., and Aldrich, M., *J. Am. Med. Assn.*, 1922, lxxix, 2129.

The solubility of calcium carbonate is known to be markedly affected by the reaction of the fluid in which it is contained. The normal liver bile, out of which it tends to precipitate, is alkaline, with an average pH of 8.20 but in the gall bladder where conditions might otherwise seem especially favorable to precipitation, the secretion undergoes a change toward the acid side, becoming on long sojourn there, strongly acid to litmus (pH 5.18 to 6.00). From bile as thus altered, no carbonate precipitation takes place, even when it becomes greatly concentrated as in fasting animals or after obstruction of the common duct. Furthermore, carbonate which has precipitated out of liver bile on standing dissolves again in it when the fluid is rendered slightly acid *in vitro*, or, in some cases merely neutral to litmus.

There are several obvious reasons for the absence of carbonate stones from the normal ducts under ordinary conditions,—notably the motility of these latter, the flushing that they undergo from an intermittently quickened bile stream, and the cleansing and possibly antagonistic action of the secretion elaborated by the duct mucosa. In the fasting animal, one at least of these influences is almost done away with, the rate of bile flow is so greatly cut down; while furthermore the calcium concentration of the secretion undergoes a considerable increase. But *pari passu* with these changes there occurs one in the bile reaction, a diminution in alkalinity so great that the pH often approximates that of the neutral point for litmus. That this change is not a direct consequence of the increase in calcium, may be inferred from the findings with stasis bile, the calcium content and reaction of which were observed to vary independently, if in general in the same direction.

These adjustments within the organism, some of which may be thought to exhibit an element of the purposeful, when considered with the test-tube experiments, strongly suggest that the reaction of the bile plays a critical part in determining the occurrence of carbonate stones, as furthermore that their absence from the normal gall bladder is a consequence of the changes in the bile reaction there occurring. The changes come about through a functional activity of the bladder. This being the case, one might suppose that the failure to act would be followed by a formation of carbonate stones. There is sufficient evidence available in the literature to indicate that this happens, in rabbits at least.

It is important to know whether changes in the bile reaction play any part in determining the cholelithiasis of man. To determine the matter will require a large material. But this much we have shown, that carbonate spheroliths not infrequently serve in human beings as centers in a formation of secondary stones of carbonate and cholesterol, as further that cholesterol precipitation out of human bladder bile can be induced or prevented by slightly altering the reaction of the fluid toward the alkaline and acid sides, respectively.

The possibility that cholelithiasis may be a consequence of sins of omission on the part of the biliary channels and reservoir deserves to be considered.