The rôle of calcium salts is admittedly an important one in the formation of gall stones. There is no need to detail in this connection the large literature on calcium carbonate stones, which are the common calculi of the herbivora and of the dog, or to recount the hypotheses that have been advanced to explain the occasional occurrence of carbonate stones in human beings and the more frequent calcium bilirubinate concretions. The precise sources of the calcium, the influences modifying its amount, and the factors responsible for its deposition from the bile have long been matters of debate. Certain aspects of the problems involved have been considered in previous communications from this laboratory. The present paper is concerned with the total calcium output in the bile and the influences modifying it. Knowledge on these points is fundamental to an understanding of the genesis of calcium stones.

Hoppe-Seyler has published analyses of the bladder and liver biles of dogs. He gives figures on the amounts of calcium present in a single instance,—41 mg. of calcium per 100 cc. of bladder bile and 31 mg. per 100 cc. in the liver bile. According to Jankau's more extensive observations, there exists between 13 and 17 mg. of calcium in every 100 cc. of fistula bile of the dog, an output unincreased by the administration of lime salts by mouth. Lichtwitz and Bock studied the calcium output.
content of human bile obtained from patients with biliary fistulas, after operation. The amount of calcium varied from 4 mg. to 9 mg. per 100 cc. bile. The literature contains no observations upon the effect of normal influences on the calcium of the bile. The investigators above mentioned examined isolated samples of the secretion or specimens which represented but a small portion of the 24 hour output. The liver bile, collected as it was from exposed fistulas in which the gall bladder formed a link, was subject to the concentrating influence of this viscus and to infection and must often have been contaminated with fluid derived from an inflamed duct system. For the purposes of the present work a method for the permanent intubation of the common duct has been employed whereby during weeks or months the entire yield of bile may be obtained, in a sterile condition, and from uninflamed bile passages. The influence of the gall bladder was ruled out by severing its neck between ligatures.

The following method of determining the total calcium was employed.

Duplicate 5 or 10 cc. portions of the 24 hour bile were pipetted into platinum dishes, evaporated to dryness, and then ignited with the aid of a little nitric acid. The residue was dissolved with 2 or 3 drops of hydrochloric acid and taken up in about 40 cc. of water containing a drop of phenolsulfonphthalein solution. The solution was neutralized by adding enough ammonia to develop a slight pink color of the indicator. The mixture was boiled until the color began to turn again to yellow, owing to the evaporation of the ammonia, and 10 cc. of a saturated solution of ammonium oxalate was added. For about half a minute thereafter the mixture remained perfectly clear and then very fine crystals of calcium oxalate began to appear. The boiling was continued for a minute or so and further precipitation allowed to take place overnight at room temperature. The precipitate was collected on an asbestos filter mat and washed free of ammonium oxalate, after which the mat was transferred to a beaker and 10 cc. of 50 per cent sulfuric acid followed by 100 cc. of water was added. The whole was heated to boiling, the oxalic acid titrated with N/100 KMnO₄, and the amount of calcium in the sample calculated from the amount of KMnO₄ required.

This is a modification of Kramer and Howland's method. It was not necessary to use the small volumes of reagents which they employed, because of the larger amounts of calcium dealt with. Shohl has shown that calcium, under the conditions mentioned, is

7 Rous, P., and McMaster, P. D., J. Exp. Med., 1921, xxxiv, 47.
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quantitatively precipitated as calcium oxalate even in the presence of phosphates and magnesiurns and that correct values are obtainable by the volumetric method.

Normal Variations in the Calcium of the Bile.

The calcium output from the liver was followed in five dogs for periods ranging from 17 to 60 days following the operation for intubation of the common duct. Under ordinary circumstances, when the animal was eating well, the amount of the element excreted per cc. of bile varied little from day to day and from individual to individual (Charts 1 to 4), a constancy rendered the more striking by the large concomitant variations in the bilirubin output. In contrast to this finding, the total 24 hour yield of calcium varied enormously as would inevitably follow from the fact that the bile quantity is subject to large physiological variations from hour to hour or from day to day, whereas the calcium per cc. remains, as just stated, relatively unchanged.

Throughout the natural and induced vicissitudes of bile excretion by the animals, the total amounts of the secretion and of calcium tended to vary together. The correspondence was especially noteworthy when the bile volume underwent an abrupt and considerable increase as when food had been given after a period of fasting or low intake (Charts 1,B, 2,A, and 4,B). When the bile amount diminished greatly below the normal average, as during prolonged fasts, there occurred a well marked increase in the concentration of calcium per cc. which compensated, in some part but far from wholly, for the lessened total amount of the vehicle of excretion (Charts 3,B, 4,A, 4,C, 6,A, and 7,A). Such compensation,—if one may use the term,—has been found to be practically perfect in the case of bile pigment, every diminution or increase in the amount of bile being attended by a reciprocal increase or diminution in the concentration of pigment per cc., so that the total output for the 24 hours remains the same. The calcium concentration undergoes changes, upon food diminution or withdrawal, which are roughly similar to those of bilirubin, as the charts show (Charts 3,B, 4,A, 4,C, and 7,A), and this

Chart 1. The chart shows (a) variations in the total bilirubin and bile calcium outputs such as ordinarily occur following intubation of an animal; (b) variations following the relief of an intercurrent obstruction to bile excretion. It will be observed that the calcium content per cc. of bile varied little from day to day despite the wide fluctuations in bile quantity. The importance of the phenomenon, as affecting the total calcium output, is especially noteworthy in the days immediately following relief of the obstruction, when the total amount of bile for the time being, enormously increased, and the total calcium, likewise increased, to approximately the same proportion. B. M. B. = beef, milk, bread.
CHART 2. To show the constancy of the calcium output per cc. of bile and the consequent parallelism between the intercurrent changes in bile volume and total calcium output. The bilirubin output, by contrast, varied inversely with the total bile amount. On the 46th day of intubation the animal was anesthetized with chloroform for 2 hours. There followed a marked temporary drop in bile volume and calcium output, and, as ordinarily after such poison, some lessening in the bilirubin concentration.
is also the case when the volume of bile increases as when feeding is resumed. But the findings as a whole prove clearly that the total output of calcium toward the end of a fast of several days duration is well below the normal average.

**Chart 3.** To show the variations consequent on altered food intake and calcium administration. During the first 4 days after intubation, the bile output was low as is the rule at this time. On the 5th to 7th days inclusive, much bile was put out and the total calcium output increased almost proportionately. Between the 8th and the 12th days the animal ate little. The bile volume became low as also the total calcium output, whereas concentrations of calcium and bilirubin became unusually high. On the 13th day a large feeding with beef liver resulted in an abrupt increase in bile volume. The administration of 80 gm. of calcium lactate by mouth on the 16th day was without demonstrable effect on the calcium excretion. The drop in bile volume and calcium output on the 17th day is attributable to the low food intake. The concentrations of calcium and bilirubin were greatly increased.
When there occurs a large fluid output from the liver, the concentration of calcium in the bile does not alter much, whereas that of bilirubin, as already stated, usually undergoes a diminution directly reciprocal to the increase in bile quantity. These facts are illustrated in Charts 2A and 3. During a pronounced cholorrhea, the excretion of calcium per hour is strikingly increased. The greater the rise in the fluid output above the average, the more directly proportional to it does the calcium output become (Charts 1B, 2A, and 3A).
Effect of Calcium Administration on the Calcium Content of the Bile.

The question whether variations in the lime salts of the diet influence the output of the element in the bile has long been under discussion. The effect of a diet very rich in them was tested on an animal the bile of which had been studied during many weeks (Dog 16). A preliminary fast of 3 days was instituted, extending from the 49th to the 51st day of intubation. On the 52nd day and thereafter until and includ-


**Chart 5.** (A) Calcium lactate by mouth failed to alter the total calcium of the bile. The change in concentration is attributable to the lessened food intake. (B) Another instance of the changes following chloroform administration; anesthesia for 2 hours. An anomalous effect is to be seen in the temporarily heightened calcium concentration.

ing the 58th day, a diet was given consisting of 1,500 gm. of bread, milk, and lean meat in fixed proportion, plus 200 gm. of bone meal. The dog ate well of this mixture and always took over 1,000 gm. of it per day. The amounts eaten and the bile findings are given in Chart 4, B. As will be seen, the diet had effect in a large output of bile with the usual concomitant increase in calcium. But the increase is no greater than that on days when the animal put out the same quantity
of bile on a diet similar save that it lacked the bone meal, as Section A of Chart 3 shows. Evidently the changes in the calcium output were in this animal at least dependent rather on changes in the volume of the vehicle of excretion than on the presence of bone meal in the diet.

**Chart 6.** The effects of fasting, liver feeding, and the intravenous injection of a calcium salt on the character of the bile. On the 19th day three intravenous injections of 10 cc. each of 10 per cent calcium chloride were given.

Dogs 16 and 20 were given large doses (80 gm. and 40 gm.) of calcium lactate by stomach tube. The data as concerns the bile are recorded in Charts 3,B and 5,A. In the case of Dog 20, the addition of the salt to the diet had no observable effect on the calcium output from the liver. Dog 16 ate very little food the day after the dose of calcium was given and in consequence the output of bile was unusually
small. The calcium output fell markedly but did not differ in this particular from that of other dogs eating little and not fed lime salts (Charts 4,A and 6,A). There can be little doubt then that the administration of calcium lactate by mouth to dogs losing bile is without effect on the excretion of calcium by the liver.

Three experiments involving intravenous injections of calcium chloride were carried out. Dog 26 was given three 10 cc. injections of a 10 per cent solution of CaCl₂ in water at 2 hour intervals on the 19th day of intubation. Dog 29 received the same amounts on the 12th day, and Dog 16 two such injections at an interval of 5 hours on the 56th day. The results as concerns the bile are recorded in Charts 6, B, 7,B, and 4,B. Introduction of even these large amounts of calcium into the blood stream had no demonstrable effect to increase the excretion of the element by the liver. The slight variations to be observed in the charts are well within the ordinary range.
The Gall Bladder and the Bile Calcium.

There has been much debate as to the influence of the gall bladder on the calcium content of the bile. In a series of animals in which the common duct was obstructed by severing it between ligatures, the calcium content of the stasis bile found in the gall bladder was compared with that of the original contents of the organ as procured through a catheter run up through the common duct at the initial operation. The operative procedure has already been described. The calcium, bilirubin, and carbonate findings are given in Table I.

The enormous pigment concentration within the gall bladder occurring in some of these instances is attributable in the main to the functional activity of the gall bladder to concentrate the bile, though certain other factors discussed in another paper may have been responsible in minor degree. The calcium content of the stasis bile in

TABLE I.

<table>
<thead>
<tr>
<th>No.</th>
<th>Days of obstruction</th>
<th>Character of bladder contents</th>
<th>Bilirubin per cc. bile.</th>
<th>Ca per 100 cc. bile.</th>
<th>CO₂ per liter bile.*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>Watery, clear, yellow-brown.</td>
<td>0.47 mg.</td>
<td>22.8 mg.</td>
<td>1.76 gm.</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>Pale amber, clear.</td>
<td>0.56 mg.</td>
<td>53.3 mg.</td>
<td>1.37 gm.</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>Amber, clear.</td>
<td>0.42 mg.</td>
<td>38.0 mg.</td>
<td>0.63 gm.</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>Dark brown, flaky.</td>
<td>1.03 mg.</td>
<td>28.5 mg.</td>
<td>0.79 gm.</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>Pale yellow.</td>
<td>0.3 mg.</td>
<td>30.7 mg.</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>Pale yellow.</td>
<td>0.18 mg.</td>
<td>30.7 mg.</td>
<td></td>
</tr>
</tbody>
</table>

*The CO₂ was determined by the method of Van Slyke (Van Slyke, D.D., *Proc. Nat. Acad. Sc.*, 1921, vii, 229). The figures in this column indicate that the gall bladder is able to reabsorb this compound to a remarkable degree, as in the case of No. 2.
contrast to that of bilirubin was never very great. Indeed, the concentration per cc. was well within the normal range for animals taking little food. While there was usually some increase over the concentration in the specimen obtained at operation, in only one instance was there so much as a doubling, and that the one in which the bilirubin underwent a 58-fold increase. If the bile elaborated immediately after operation held the usual amount of calcium—and all the evidence from the fistula animals of the present work would go to show that this is the case—then one can only account for the failure to find an abundance of the element in the inspissated stasis bile by assuming that its salts passed out through the wall of the gall bladder. Hoppe-Seyler\textsuperscript{12} has shown that sodium and potassium do so pass out and Brand\textsuperscript{13} that the freezing point of gall bladder bile, no matter how thick, is the same as that of liver bile, a relation maintained through the removal of salts and water from the organ by the processes of osmosis and diffusion. A considerable portion of the calcium of dog bile is present in the form of carbonate\textsuperscript{1} which should lend itself well to such elimination. That most of the calcium present in bile exists in the form of dialyzable compounds is evident from my findings.

\textit{The Calcium Content of “White Bile.”}

In a previous paper from this laboratory\textsuperscript{14} the fact has been brought out that the bile ducts do not, like the gall bladder, act to concentrate the bile but instead tend to dilute it with a secretion of their own which is colorless and devoid of bile salts and bilirubin, even when the animal is deeply jaundiced. The “white bile” found above an obstruction in a duct system cut off from the ordinary connection with the gall bladder consists of such pent-up secretion. The calcium content of this fluid has considerable interest. For the purpose of obtaining specimens the common ducts and cystic ducts of three normal dogs were cut between ligatures. The operations were performed aseptically, under ether. Between 2 and 3 weeks later the animals were

\textsuperscript{12} Hoppe-Seyler, F., Physiologische Chemie, Berlin, 1878, pt. 2, 302.
\textsuperscript{13} Brand, J., \textit{Arch. ges. Physiol.}, 1902, xc, 491.
\textsuperscript{14} Rous, P., and McMaster, P. D., \textit{J. Exp. Med.}, 1921, xxxiv, 75.
chloroformed and the contents of the dilated ducts were removed, cultured in bouillon and on agar, and analyzed for calcium. The results are presented in Table II.

It will be seen from the table that the calcium content of the "white bile" was at the lower limit of the range encountered in ordinary fistula biles.

Under the circumstances of progressive obstruction to the outflow of bile from the liver, the amount of the secretion formed and its content in bilirubin both progressively fall off.\(^1\) In Dog 18, Chart 1,\(A\), such obstruction developed through a kink in the tubing, which was later relieved. The findings are anomalous in that the bilirubin concentration per cc. rose as the obstruction increased instead of falling as happens usually. After the obstruction was relieved the bile had a very low pigment content, and low calcium concentration.

<table>
<thead>
<tr>
<th>Period of obstruction</th>
<th>Character of duct contents</th>
<th>Results of cultures</th>
<th>Calcium content per 100 cc.</th>
<th>Range, under ordinary conditions, of the calcium contents of the bile of six fistula animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>37 days (\text{days})</td>
<td>Light yellow.</td>
<td>Sterile.</td>
<td>10.6 mg.</td>
<td>(7-52) mg. per 100 cc. of bile.</td>
</tr>
<tr>
<td>22 days</td>
<td>White tinged with yellow.</td>
<td>&quot;</td>
<td>10.0 mg.</td>
<td></td>
</tr>
<tr>
<td>25 days</td>
<td>Light yellow, watery.</td>
<td>&quot;</td>
<td>7.7 mg.</td>
<td></td>
</tr>
</tbody>
</table>

In another animal in which only the bile from approximately one-third of the liver was collected—by intubation of the duct from this portion—while the remainder of the secretion reached the intestine by way of the ordinary channels, there was transient and incomplete obstruction in the collecting system. The bile changes are recorded in Table III.

It will be seen that whereas the bile quantity and the bilirubin concentration had fallen off greatly when the obstruction was at its height, the calcium concentration had diminished to but a negligible degree. It was still within the lower limit of the range that is "normal" to fistula animals. The total output of calcium, on the other hand, was greatly lessened, by corollary, to the lessening of the bile amount.

“White bile” is met with under yet another set of experimental conditions. It has long been known that animals given toluylenediamine or phosphorus may at the height of liver injury yield only a glairy, approximately colorless fluid. This change in output lasts but a few hours as a rule and is usually preceded and followed by the elaboration of a heavily pigmented, thick secretion. Chloroform poisoning, on the other hand, as recent observations in this laboratory attest, results in a progressive and sometimes practically complete suppression of bile with, as the fluid output lessens, a marked diminution in bilirubin such that the final cubic centimeters of secretion are practically colorless. This ultimate fluid is thin and resembles in general character the “white bile” which assembles above an obstruction. The animal may remain in good condition throughout the changes which endure only 48 to 72 hours at most, and are rapidly recovered from; but in instances in which bile suppression is complete, death is more usual. The findings in dogs in which the suppression was not quite complete are illustrated in the charts. It will be seen that in one instance (Chart 2,B) the calcium concentration of the bile remained practically stationary throughout the disturbance, whereas in the other (Chart 5, Section

<table>
<thead>
<tr>
<th>Days after intubation</th>
<th>Appearance of the bile</th>
<th>Calcium per 100 cc.</th>
<th>Bile per hr.</th>
<th>Bilirubin per cc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Dark brown, clear, watery</td>
<td>12.7</td>
<td>2.79</td>
<td>0.555</td>
</tr>
<tr>
<td>3</td>
<td>“ “ “ “ “ “ “</td>
<td>13.6</td>
<td>2.49</td>
<td>0.521</td>
</tr>
<tr>
<td>7</td>
<td>Very light brown, clear, watery</td>
<td>12.7</td>
<td>0.42</td>
<td>0.239</td>
</tr>
<tr>
<td>8</td>
<td>“ “ green, “ “ “</td>
<td>11.4</td>
<td>1.21</td>
<td>0.114</td>
</tr>
<tr>
<td>9</td>
<td>“ “ “ “ “ very watery</td>
<td>11.5</td>
<td>1.48</td>
<td>0.092</td>
</tr>
<tr>
<td>10</td>
<td>“ “ brown, “ “ “</td>
<td>12.9</td>
<td>3.09</td>
<td>0.113</td>
</tr>
<tr>
<td>11</td>
<td>Light brown, clear, watery</td>
<td>12.6</td>
<td>2.81</td>
<td>0.135</td>
</tr>
<tr>
<td>12</td>
<td>“ “ “ “ “ “</td>
<td>12.9</td>
<td>3.33</td>
<td>0.181</td>
</tr>
<tr>
<td>13</td>
<td>“ “ “ “ “ “</td>
<td>13.5</td>
<td>3.17</td>
<td>0.267</td>
</tr>
<tr>
<td>14</td>
<td>“ “ “ “ “ “</td>
<td>12.9</td>
<td>2.75</td>
<td>0.301</td>
</tr>
</tbody>
</table>

B), in which recovery was complicated by a supervening obstruction within the tube system, the calcium concentration rose somewhat during the period when the suppression was at its greatest.

The evidence at hand does not permit of the conclusion that the "white bile" secreted after liver injury and that formed on progressive obstruction to outflow are identical in source with the "white bile" of frank obstruction; that is to say, are duct products. But the fact that they tend to be weak not only in pigment and salts but also in calcium may be not without significance in this relation.

DISCUSSION.

In the animals of the present study the calcium of the fistula bile varied under normal conditions from 0.0061 to 0.0578 per cent. Hoppe-Seyler found 0.031 per cent calcium in his single instance and Jankau's figures vary between 0.013 and 0.017 per cent. These workers had noted that the average percentage under normal conditions is approximately the same as that in mucous secretions of other derivation and they supposed the calcium not to be secreted by the liver like the bile constituents proper but by the lining of the bile passages. They made no observation on the variations in calcium output under physiological conditions. These variations as described in the present paper leave no doubt that most of the calcium must be eliminated through the liver, unless indeed one is willing to suppose that the mucous membrane of the biliary passages varies in its activity to put out calcium synchronously with the activity of the liver tissue proper to put forth bile. The calcium concentration of that "white bile" which is an undubitable secretion of the bile passages is always low as is the volume output. But when the bile secretion itself is much diminished as the result of an ordinary physiological influence, fasting, the calcium concentration is frequently great.

That the amount of calcium in the duct secretion proper should be so small is a point worth emphasizing because many writers have referred the calcium of cholelithiasis to this source. Needless to say the present findings throw no light on the content of calcium in the secretion of inflamed duct systems.
Jankau\textsuperscript{4} reached the conclusion from observations involving an intermittent and far from complete collection of bile that calcium feeding does not increase the output of the element. The present findings with the total bile prove this to be the fact. Jankau fed his animals diets rich in calcium and assumed that it would be absorbed from the intestine, since previous workers\textsuperscript{18} had found an increase in the urine after administration of calcium salts by mouth to normal dogs. In view of later work it is questionable whether an animal with a bile fistula can absorb much calcium from the food. Telfer\textsuperscript{19} noted that in infants in which there was a lack of bile in the intestine, owing to congenital obliteration of the ducts or syphilitic hepatitis, the calcium given in the diet was very largely combined with fatty acids to form soaps in the intestine and appeared as such in the stools. Other workers\textsuperscript{20} have described softening of the bones in animals with long-standing biliary or pancreatic fistulas,—a fact which would suggest a gradual depletion of the body content of the element. This depletion can scarcely be ascribed to the small amount of calcium leaving the body by way of the liver, for far more is excreted normally by way of the large intestine. Rather may one suppose that lack of bile or pancreatic juice in the intestine results in a lessened calcium absorption therefrom. But a metabolic disturbance within the organism cannot be ruled from possibility as the explanation of the bony changes.

In some of the experiments of the present work, uncertainties as to calcium absorption from the intestinal tract were avoided by administering calcium salts intravenously. Rey\textsuperscript{21} claims that the blood calcium remains elevated for more than 4 days after an intravenous injection of 0.8 gm. of oxide of lime. Salvesen, Hastings, and McIntosh\textsuperscript{22} find, however, that after the injection of 0.5 to 1 gm. of calcium, as calcium chloride, the calcium concentration in the blood is elevated

\textsuperscript{19} Telfer, S. V., Biochem. J., 1921, xv, 347.
\textsuperscript{21} Rey, J. G., Arch. exp. Path. u. Pharmakol., 1894–95, xxxv, 295.
for not more than 6 hours and in one case it was so for but 3 hours. They also find no increase in the calcium content of the urine or feces during the 6 days after intravenous administration, and conclude that the element must be stored somewhere in the body. Their observations on the blood will go far to explain the absence of any increase in the calcium output in the bile after intravenous injections, as in the experiments described in this paper, of as much as 0.25 gm. per kilo of calcium chloride—more than three times the greatest amount employed by Salvesen and his associates.

There is much diversity of opinion as to the source of the calcium of gall stones. Many have supposed that the mucous glands of the gall bladder add calcium salts in quantity to the bile. Dochmann compared the calcium and sodium present in the liver and bladder biles of the same dog and found the following relative amounts per 100 gm. dried substance.

<table>
<thead>
<tr>
<th></th>
<th>Ca</th>
<th>Na</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>Bladder</td>
<td>24</td>
<td>10</td>
</tr>
</tbody>
</table>

He infers from these figures that the gall bladder adds calcium to its contents. The stasis experiments of the present work show clearly that calcium is resorbed by the normal gall bladder, not secreted by it. Dochmann's figures are readily explained on the assumption that sodium salts pass out through the gall bladder wall with far greater ease than do those of calcium. Calcium would seem to hold a position, in this respect, intermediate between bilirubin, which is presumably not resorbed at all, and the sodium salts.

The present work does not suffice to answer the question whether, under pathological conditions, there are sources of calcium over and above the normal—as calcium in the exudates of inflamed bile passages or from abnormally overstimulated mucous glands. It does, however, provide some few facts toward an ultimate understanding of the circumstances under which calcium stones may be expected to form. For example, the element tends to be present in the bile in great amount during fasting. Other things being equal, one might

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However, there is to be noted during a fast, concomitantly with the increase in calcium, a change in the reaction of the bile,\(^6\) such as would tend to prevent the falling out of calcium salts. It is conceivable that, under circumstances of gall bladder derangement, the change may not be brought about and stones may occur in consequence. But there are so many other possible factors to be considered, owing to the complexity of the bile and of the influences of the channels with which it comes in contact, that this can be no more than a suggestion.

It is of interest to contrast the behavior of calcium with that of another constituent of bile, cholesterol, which is of extreme importance in relation to gall stones. Cholesterol, like calcium, becomes highly concentrated in the bile when the volume output is small,\(^2\) as during fasting. But cholesterol, in contrast to calcium, is greatly increased both in concentration and in total amount, by the administration of large amounts of it in the food. Whereas "white bile" always contains an appreciable amount of calcium, its cholesterol content is almost nil, indicating that there is but one important source of the latter—the liver cells—whereas calcium is secreted both by the liver cells and by the lining of the bile passages.

**SUMMARY.**

A day to day study has been made of the calcium content of the total liver bile of dogs intubated under sterile conditions. The concentration of this element in the bile is fairly constant under physiological conditions which do not involve wide fluctuations in the secretory output. It follows that the calcium yield for each 24 hour period in general varies directly with the amount of the bile. But when this amount becomes greatly lessened, as the result of fasting, the concentration of calcium becomes markedly increased, though not sufficiently so to compensate for the lessened volume. When the bile amount rises much above the average after the ingestion of food in quantity, the calcium content, unlike that of pigment, does not become correspondingly diminished, but tends to remain the same as ordinarily. Hence when great amounts of bile are put out, so too are rela-

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tively great amounts of calcium. This is not because of the increased ingestion of the element. For neither feedings with bone meal, nor the administration of large quantities of calcium salts intravenously or by mouth has any effect to alter the biliary output of calcium.

The normal gall bladder, far from secreting calcium into the bile, as some have supposed, acts to remove this element from the secretion, and removes carbon dioxide as well.

The "white bile," which is a specific secretion of the bile ducts, contains but little calcium, like the mucous secretions from elsewhere in the body. The concentration is only slightly greater than that in the blood plasma, and contrasts significantly with the high concentration to be noted in true bile of the fasting animal. Evidently the greater portion of the bile calcium must be secreted, not by the duct walls, but by the liver itself.

The findings have an evident bearing on the problem of cholelithiasis.