THE CONCENTRATING ACTIVITY OF THE GALL BLADDER.

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The experiments here to be reported were undertaken as the result of observations upon stasis bile collected after ligation of the common duct. The accumulation of pigment in such bile seemed to us to indicate that some part of the duct system possesses a concentrating faculty of considerable moment for pathological processes.

It is current knowledge that bladder bile is normally thicker than the secretion as it comes from the liver. The point finds a brief mention in text-books. But the significance accorded it may be judged from the fact that for quantitative studies of the bile from day to day the gall bladder is regularly utilized as a link in the fistulous channel connecting the hepatic duct with the body surface.

Maly[1] states that liver bile from the dog has 3.5 to 4.9 per cent of dry substance, and that from the bladder over 20 per cent. The latter fluid, according to Hoppe-Seyler's analyses which are quoted by Maly *in extenso*, yields far the greater quantity of bile salts. Brand[2] found 1 to 4 per cent of solids in the fistula bile of human beings, and as much as 20 per cent in the bladder contents. According to Hammarsten[3] a part of the water of the bile is abstracted and a mucinous nucleoprotein added by the bladder. It is probable that this organ empties itself only partially upon contraction, and the secretion may remain in it as in a sort of backwater to be acted upon over long periods of time. Because of uncertainty as regards these matters, comparative analyses such as those just given bear but obliquely on the problem of the rapidity of the changes undergone by the bile.

In the present paper we shall deal solely with the influence of the gall bladder upon the bile, reserving for an accompanying one the influence of the ducts.[4]

[1] Maly, R., in Hermann, L., Handbuch der Physiologie, Leipsic, 1881, v, pt. 2, 172.

[2] Brand, J., *Arch. ges. Physiol.*, 1902, xc, 491.

[3] Hammarsten, O., Lehrbuch der physiologischen Chemie, Wiesbaden, 8th edition, 1914, 411.

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

Method.

The best method of study will be one whereby a bile of known constitution is supplied through the normal channels to an intact gall bladder by the animal's own liver. It is practicable in the dog owing to the arrangement of the ducts. By means of a single ligature appropriately placed, a type sample of bile can be diverted for separate collection, while the remainder flows to the bladder.

The common duct of the dog is formed as a rule by the union of three large channels, and high up into the middle one the gall bladder empties. That on the right hand is derived from the caudate and right lateral and central lobes. By its entrance a few centimeters above the duodenum the common duct is finally formed, the other channels uniting much nearer the liver. If their derivative duct is tied just above where this final duct enters, all of the bile from the major portion of the hepatic tissue is pent up and directed into the gall bladder, whereas the secretion from the caudate and right lateral lobes still reaches the common duct and may be collected through a cannula. Here, in essence, is the plan of our experiments.

Owing to duct anomalies, the partition of bile effected by a ligature placed as described may vary considerably. We have made careful dissections at autopsy, tracing out each duct and ultimately determining by weight the amount of tissue delivering bile to either side of the ligature. The liver of the dog is so deeply cleft that usually this can be done accurately. But when a single lobe has ducts running to both sides of the ligature, as not infrequently happens, the partition of the tissue can be only approximately learnt.

Vigorous dogs with a wide costal angle were chosen. Under ether, a sufficient dissection was made to tie a small glass cannula into the common duct and to place higher up the essential, or partitioning, ligature, as we shall henceforth term it. In our first attempt a considerable segment of duct was freed and the gastrohepatic omentum subjected to trauma, with result that almost no bile was secreted in the 24 hours immediately following. Warned thereby, we handled the tissues of the later animals with great circumspection and met no other instances of the sort.

The bile taken as a type sample of the liver output was collected into a rubber balloon which was connected with the common duct by a short cannula and a soft rubber tube 4 to 8 cm. long and of about 2 mm. bore. The cannula was bound down into line with the duct—which was merely slit, not severed—by a thread about its shank and the lower duct portion, and thus obstruction from a kink or elbow was rendered unlikely. The balloon was left within the peritoneal cavity, and the abdominal wall closed completely in three layers. Asepsis was maintained throughout. The dogs bore the operation well, and remained in excellent condition throughout the term of experiment which was usually 24

hours. Often the animals ate largely soon after operation. All were ultimately chloroformed and immediately autopsied. Cultures were taken of the bile specimens, and pieces of liver from the regions separately drained were placed on agar and in bouillon. Infection was rare, despite the fact that the bile accumulated at body heat. Its occurrence is noted in the tables.

The pigment content of the bile was used as the index to concentration by the bladder. The sojourn of the bile for some hours at the temperature of the animal was found by repeated *in vitro* test to be without effect on the pigment, save for the conversion of a negligible portion into biliverdin when air was present. To prevent this change it was only necessary to deflate completely the collecting balloons prior to their introduction. Routine colorimetric estimations were made by Hooper and Whipple's[5] modification of the Salkowski method, whereby bilirubin and biliverdin are estimated together; but instead of a color wedge of artificial constitution, we have employed as a standard pure bilirubin, and in place of the Autenrieth instrument, a Duboscq micro-colorimeter. The bilirubin (Schuchardt) was in chloroform solution, 1 mg. to every 4 cc.; and, to prepare a standard, 1 cc. of this was made up in a volumetric flask to 10 cc. with Hooper and Whipple's acid alcohol, and allowed to stand 18 to 24 hours at room temperature, when the characteristic blue-green color described by these authors was found to have developed. The biles were treated likewise save that 1 cc. was made to 50 cc. with the alcohol. The bladder contents was often so concentrated as to necessitate some preliminary dilution with water.

Bile treated with the acid alcohol did not always go through the same color changes. Often the tint ultimately developed for the readings tended somewhat to the green, as compared with the standard, or again was pronouncedly more blue. The normal bladder bile removed at operation frequently yielded a gamut of purples, possibly as a result of the presence of bilicyanin,[6] and could at no time be read against the standard. This never happened with specimens obtained after operation. Fortunately for our work, the biles obtained from different portions of one liver during the same period always went through identical color changes and in consequence could be accurately compared; while such errors in quantitation as were involved in reading them against a standard of different tint were applicable to them in like degree. The results given in our tables are expressed in milligrams of bilirubin save for six early instances, in which the standard proved faulty. For them "color units" are employed instead. But "color units" could have been used to report the findings throughout, since the main significance of these latter lies in relative not in actual pigment quantity.

For a standard in the early experiments, a chloroform solution containing only 5 mg. of bilirubin per 100 cc. was employed, the stock solution advocated

[5] Hooper, C. W., and Whipple, G. H., *Am. J. Physiol.*, 1916, xl, 332.

[6] Hoppe-Seyler, F., in Hirschwald, A., Handbuch der physiologisch- und pathologisch-chemischen Analyse, für Aerzte und Studierende, Berlin, 8th edition, 1909, 371.

by van der Bergh and Snapper[7] in connection with the diazo reaction. A measured portion was dried on the water bath and the residual pigment taken up in acid alcohol. But it dissolved with a troublesome slowness, and furthermore the chloroform solution itself, after a month or two at room temperature, became greenish and much weaker as shown by the lessening color response with both acid alcohol and the diazo reagent.[8] With a concentrated stock solution having 1 mg. for every 4 cc., and kept in the ice box, the loss of pigment within 2 months is negligible. It can be followed in the colorimeter by a comparison of the acid alcohol standard made from it with a mixture of 10 cc. of copper sulfate solution (10 gm. to 100 cc. of water) and 0.075 cc. of potassium bichromate solution (1 gm. in 100 cc. of water). Indeed, an inorganic standard of the sort described will probably prove best in the long run for routine colorimetric purposes. Slight alterations in the amount of bichromate suffice to turn the color toward the blue or green without essentially altering its value.

Control Observations.

The plan just outlined could be useful for our project only on the assumption that the bile from different liver regions has approximately the same pigment content per cubic centimeter. This cannot be taken as a foregone conclusion, if for no other reason than because the individual liver lobes receive portal blood from different visceral sources as a number of workers have shown.[9] To determine the actual case was our first step.

In six animals the neck of the gall bladder was tied off and the contents of the organ was removed by aspiration. A partitioning ligature was then placed upon the main duct as usual and, just above, a cannula connecting with a rubber balloon was inserted for collection of the bile that in later experiments was to flow instead to the bladder. The usual sample bag was then connected with the common duct and the laparotomy wound closed. Some of the animals were full fed at the time of operation, while others had fasted for 24 hours. All had access to food afterwards, but in general only those took it within the first 24 hours that had previously been denied. The factor was without notable influence on the result, as the table shows.

The liver regions supplying the two bags varied considerably from animal to animal because of differences in the duct arrangement. In two of the six instances more tissue was tributary to the sample bag than to the upper one.

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[7] van der Bergh, A. A. H., and Snapper, J., *Deutsch. Arch. klin. Med.*, 1913, cx, 540.

[8] For a note on the changes in bilirubin kept in chloroform see Oppenheimer, C., in Fischer, G., Handbuch der Biochemie, Jena, 1909, i, 731.

[9] Bartlett, F. K., Corper, H. J., and Long, E. R., *Am. J. Physiol.*, 1914, xxxv, 36.

The table of results (Table I) has been arranged with reference to the question whether the bile in the sample bag gives any index to the pigment concentration of that elaborated by the rest of the liver. It will be seen that this question is answered in the affirmative. The "bile strength," that is to say the pigment per cubic centimeter, is nearly the same for both portions of secretion, despite wide variations from animal to animal in the total fluid output and its bilirubin content (compare Experiments 2, 4, and 5). This is a weighty point for it means that in the later work the bile strength can be used to gauge the concentration effected by the gall bladder.

The actual fluid in the upper bag and the calculated amount as determined from a knowledge of the quantity in the lower, or sample, bag and the relative pigment content of the two, assuming that there was an equal degree of concentration, differ but little, as would follow from the circumstance that the actual bile strengths of both proved to be nearly identical. A calculation of the sort was regularly employed in the later work to gain an idea of how nearly the real amount of fluid reaching the gall bladder approximated that which should have reached it, judging from the partition of the hepatic tissue.

Some marked discrepancies will be noted between the actual fluid in the upper bag and the theoretical quantity as worked out from the partition of tissue. The effect of the partitioning ligature could not always be exactly ascertained, as for example, when a single lobe possessed ducts draining to either side of it. But this will not suffice to explain certain cases. In Experiment 6 the rubber tube connecting said bag with the duct, being stiffer than ordinary, was at autopsy found sprung like a bow, with one end pressed upon the neighboring portal trunk and thus perhaps diverting blood to the region tributary to the lower bag with result in more active secretion there. Whatever the cause of the other discrepancies these had no significance for our project.

The fact may be noted in passing that four animals out of five, with the instance of Experiment 6 excluded, yielded to the upper bag a bile that was relatively, if slightly, richer in pigment per cubic centimeter. This was no accident, as will be shown further on. There too, in Tables IV and V, are data that indirectly corroborate Table I.

Concentrating Power of the Emptied Gall Bladder.

Observations on the bladder were now begun. In a first series of experiments the organ was washed with salt solution, and left to fill with bile as it normally might fill, assuming that it empties on normal contraction.

A small slit was cut in the main bile duct at the point where the partitioning ligature was later to be laid on, and, through a silk catheter thrust up into the neck of the gall bladder, all bile was withdrawn and the organ washed with 0.9 per cent salt solution until the rinsings came away uncolored. Due care was taken to avoid overdistention, and the final emptying was accomplished by gentle pressure with moist sponges. The catheter was then withdrawn and ligatures were placed on the duct above and below the slit, to close it and divert the bile, part as usual into a bag, and the remainder to the empty bladder.

It may be asked whether the brief washing was effective; for a concentrated residuum of bile such as might be left clinging next the bladder mucosa would certainly complicate the findings. No difficulty was experienced on this score. The wall of the bladder is translucent, with the color of the contents shining through, and the efficacy of the washing can be controlled by direct inspection. Furthermore, the original bile was retained for comparison with that accumulating later in the sample bag. Practically always it was weak in pigment, relatively speaking,—whence one may conclude that any remnant of bladder bile left after the washing would act, if anything, to dilute in this respect the fluid entering later.

The contents at autopsy of the gall bladder was always so very viscid as well as dark that it was removed by rinsing with distilled water, and still further diluted prior to the withdrawal of a type portion. On opening the animal a clamp was laid on the bladder neck to prevent any passage of secretion from the hepatic duct. As a guide in the dilution, the assumption was made that as much bile had originally reached the gall bladder as was called for theoretically from the quantity in the sample bag and the proportion of hepatic tissue supplying the two; and the actual bladder contents was brought up to this amount. The mixture of bile and water was shaken repeatedly, allowed to stand several hours, and shaken again prior to the removal of 1 cc. for treatment with acid alcohol and comparison with a standard. The results with duplicate specimens showed that an even distribution of pigment had been brought about.

In all save one of this series of experiments, and in Experiment 6 of Table I, pigment values are expressed in "color units," the unit being the amount of pigment in 1 cc. of bile from the sample bag.

The period of experiment ranged in the five animals from $22\frac{1}{2}$ to 49 hours. To our great surprise the gall bladder even at the end

of the longest period held but a few cubic centimeters of bile, far less than the amount necessary for normal distention; while after 24 hours, the organ was practically collapsed in two out of three instances, yielding only 0.77 and 1.4 cc. of fluid, and in the third case was but half full. Could one suppose that the hepatic tissue tributary to the bladder had failed to secrete as usual? Or had its output undergone concentration to an extent commensurate with the findings? The latter proved to be the case. The dark, syrupy or tarry, bladder contents had from 3.18 to 10.8 times the pigment strength of the fluid in the sample bag, with an average of 7.1 (Table II). The contents of the tributary ducts was always by contrast thin and weak in pigment, like the bag bile. There were only a few drops to be had from the ducts, too little for colorimeter readings, so dilutions with water were compared directly with similar dilutions of bag and bladder biles. Sometimes they were in addition tested for cholates by Hay's method. The results confirmed the pigment findings.

The Concentrating Power of Full Gall Bladders.

With the gall bladder emptied as in these experiments its whole concentrating influence is brought to bear upon the secretion arriving little by little, from the liver. In this favorable circumstance lay not impossibly one cause of our results. To determine the real case a series of animals was studied, of which the bladders were filled with bile of known pigment content, before the wash catheter was withdrawn.

The dog bile to be introduced was collected in bags under aseptic conditions and kept on ice for periods up to 48 hours. In one instance, that of the material derived from the upper bag of Experiment 5, Table I, it proved to have been infected with a micrococcus of dubious pathogenicity, and this organism was recovered in pure culture from the gall bladder into which the bile was put (Experiment 1, Table III). Less concentration was effected by the bladder in this instance than in any other of the series.

To fill the bladder under a known pressure while preventing the escape of any fluid into the peritoneal cavity, a ligature was placed upon the duct containing the catheter just above the slit in its wall, and this was tied down as the catheter was withdrawn, thus becoming one of the partitioning ligatures. The catheter itself was connected with a sterile funnel containing the bile. The pressure of a column of bile 60 to 100 mm. high proved just sufficient to effect a

normal distention of the bladder. This is less than the pressure withstood by the sphincter of Oddi.[10] Needless to say, the tributary ducts shared the pressure conditions. In Experiment 7, a pressure such as develops upon duct obstruction[11] —300 mm.—was used.

Our expectation was to find at autopsy a marked stasis with dilatation of all the passages above the partitioning ligature, owing to secretion into them of more bile than the gall bladder could cope with. But the event was quite another. So rapidly was fluid withdrawn through the bladder wall that the increments of hepatic secretion proved insufficient in most instances to hold the organ distended. It was found nearly collapsed in Experiments 1, 4 and, 7, while in Experiment 6, in which alone a normal distention was observed, its capacity was unusually small, only 3.5 cc. The amount of bile introduced at operation and, to a less extent, that removed furnished for each case an approximate measure of capacity.

The inspissating activity of gall bladders left distended (Table III) proved to be little behind that of emptied ones (Table II). On the average the concentration of pigment was 6.4 times that of the bag samples, with a range from 3.6 to 8.9. The bile collected from the tributary ducts was thin with relatively little pigment, showing, as in the animals with emptied bladders, that the secretion had not been elaborated in condensed form. The greatest concentrations—to bile strengths, 8.1 and 8.9 times that of the bag samples—were effected during only 18 and 22 hours respectively. The amount of secretion acted upon in these instances as calculated from the quantities of bilirubin in bag and bladder and the fluid content of the former,—assuming both bile portions to have had the same pigment strength originally,—was for the first case 59.8 cc. which was reduced to 7.4 cc. by an organ of 9 cc. capacity, and for the second 26.6 cc. brought down to 3 cc. by a bladder holding at most 4.3 cc.

In Experiments 3 and 4, the bile introduced at operation had previously been concentrated 3.5 times—it was the upper bag contents of Experiment 2, Table V—and was syrupy with mucus. These changes did not prevent a further inspissation in the bladder to pigment strengths of 4 and 5.9 times respectively that of the bag samples.

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[10] Judd, E. S., and Mann, F. C., *Surg., Gynec. and Obst.*, 1917, xxiv, 437.

[11] Herring, P. T., and Simpson, S., *Proc. Roy. Soc. London, Series B*, 1907, lxxix, 517.

In both Tables II and III it will be noted that the pigment found in the bladder frequently fell far short of the expected quantity (Table II, Experiments 4 and 5; Table III, Experiments 1, 3, 4, and 7). Must one suppose that part of the bilirubin reaching the organ had passed out through its wall, or was there diminished secretion above the partitioning ligature? Certainly pigment can pass the mucosa, for it has been observed histologically in transit;[12] but our observations upon the lymph indicate that the quantity thus removed is negligible. There is usually to be found coursing down the neck of the dog's gall bladder, and draining most of its extrahepatic portion, a large, turgid lymphatic yielding fluid in quantity when cut. We have frequently examined such fluid from gall bladders that held heavily pigmented bile. It was always practically colorless and failed to give positive reactions for bilirubin, though occasionally cholates were present, as shown by Hay's test. The direct passage of pigment into the blood stream cannot be ruled out, but it seems unlikely in view of these findings.

There remains the alternative of locally diminished secretion. The conditions in some cases would seem to have been highly favorable to this. The gall bladder in these instances was found at autopsy to be nearly collapsed, and so bound down by fresh adhesions between the adjacent liver lobes that its redistension could scarcely have been brought about by the normal secretory pressure. Under these cirsumstances, there may well have occurred a stasis in the tributary ducts at periods when secretion by the liver provided more fluid than the bladder could immediately concentrate. Direct proof of this was not obtainable because the necessary observations involved a severing of the very adhesions whereby the abnormal state was maintained. But it is interesting to note that the total output of bile pigment per kilo of animal averaged precisely the same (11.1 mg. in 24 hours, a normal amount) for the dogs of Tables II and III with a relatively small yield above the partitioning ligature, as for those of Tables I, IV, and V in which this was not the case. The fact suggests that whatever the cause of the small yield above the ligature, it resulted merely in a shift of the secretory activity with an unduly large output to the sample bag, and, by corollary, unwarranted expectations as to what should have been provided to the gall bladder.

The Bladder Utilized as a Duct.

The partitioning ligature of the preceding experiments could not be relaxed like the sphincter of Oddi. Fluid remained pent above it no matter how powerfully the gall bladder may have contracted. Here was an important departure from the normal; and the question arises whether the removal of fluid from the bile may not have been largely the consequence of pressure intermittently exerted by the bladder wall. For such reason another series of experiments was

[12] Aschoff, L., and Bacmeister, A., Die Cholelithiasis, Jena, 1909.

performed in which a partitioning ligature and sample bag were placed as usual, but the free tip of the bladder was connected by cannula with a second bag.

The tip of the bladder was seized with hemostats and a small slit made at the point where blood and lymph vessels were least abundant. The bile was removed by aspiration, and the usual flushing with salt solution; and a glass cannula with trumpet mouth and a least inside diameter of 2 to 3 mm. was fixed in place with a purse string suture. A rubber tube of the same bore, 4 to 8 cm. long, led from it to the rubber balloon. At autopsy this tube was clamped off as soon as the peritoneal cavity had been opened, to prevent the shifting of bile in either direction.

Four dogs were operated upon. The results are given in Table IV. The new cystic outlet was of somewhat larger caliber than the normal one, and at the most dependent portion of the bladder which was found practically empty at autopsy. The bile, urged by the secretory pressure and by gravity, had evidently run directly through, as through any other channel to the bag, being aided in two instances by a postoperative drawing together of the bladder wall which had much narrowed and shortened the organ. Nevertheless, the bile that had been submitted to it proved to be 2.3 to 4.8 times as concentrated as that in the corresponding sample bag. In view of such findings the results of Tables II and III cannot be attributed to the closed system existing above the partitioning ligature.

In the animals of Table IV the pigment content of the upper bag differed but slightly from the theoretical amount as calculated from the quantity in the sample bag and the proportion of tissue tributary to each. In this respect the findings were nearer perfection than in the control series of Table I. The absence of any cause for local portal obstruction such as was provided by the upper cannula in the dogs of Table I may have been responsible for this.

In final illustration of the concentrating activity, three experiments originally intended as controls will be reported, which were carried out prior to realization of the bladder capabilities. To obtain the bile from above the partitioning ligature in these instances, a cannula of large bore was thrust through a cut in the bladder wall into the neck of the organ and secured there. The duct from the right side of the right central lobe enters so high up that there is often no true cystic duct, and in order to avoid obstruction of this

tributary, the cannula was not pushed down to its level but left with a tiny pouch of bladder mucosa about its mouth. That the influence of the pouch was far from negligible is shown by the results. The upper bag yielded a bile syrupy with mucus and considerably richer in pigment per cubic centimeter than the thin, sample fluid (Table V).

It may be recalled that in the controls of Table I the bile from the upper bags was generally slightly the more concentrated. Now it so happens that in the dog the gall bladder wall extends some distance down the duct, the macroscopic character of the latter being often first evident below the entrance of the highest branch from the liver. Others have noted this before us. Indeed, both in the dog and in man a new gall bladder may develop out of the remnant of bladder mucosa left by a cholecystectomy that has failed to include the cystic duct.[13] It follows that a ligature placed on the neck of the bladder to block the organ off, as in the experiments of Table I, may frequently fail in some part of its function. To such a happening do we attribute the slightly greater concentration of most of the upper biles of Table I. For, as Table IV demonstrates, a transient exposure of the bile to but a fraction of the bladder wall results in a reduction of its bulk.

Influences of the Ducts.

For the purposes of the present study the duct system proper has been deemed without influence upon the fluid it conveys. But in view of the great activity of the gall bladder, is such an assumption warranted? For practical purposes it is, as we shall show in an accompanying paper. The ducts instead of withdrawing fluid from the bile tend to dilute it slightly with a watery product of their own.

Peculiar Character of the Bile Acted upon.

No such deeply pigmented biles as the gall bladder yielded at the end of our experiments are found under normal conditions. Normal bladder bile of the dog is often light yellow, and, at most, of a

[13] Rost, F., *Mitt. Grenzgeb. Med. u. Chir.*, 1913, xxvi, 710. Haberer, H., and Clairmont, P., *Verhandl. deutsch. Ges. Chir.*, 1904, xxxiii, pt. 2, 81.

medium brown tint, whereas that now referred to was always dark, and frequently brown-black. It may be recalled that the activities of the bladder had been brought to bear on but a fraction, and sometimes a small fraction, of the total secretion. But this is not the sum of the matter. For quantitation of the bag samples showed clearly that the liver had furnished an abnormal secretion, one extremely rich in pigment and small of bulk.

The bile of healthy dogs has ordinarily from $\frac{1}{3}$ to $\frac{1}{2}$ mg. of bilirubin in every cc., and practically never as much as 1 mg.;[14] whereas that of our animals contained after operation more than 2 mg. per cc. usually, only once less than 1 mg., and in one instance 6.8 mg. Such plenitude was attained almost wholly at the expense of the fluid output, as shown both by direct measurement of the latter and by the fact that the total pigment elaborated by the liver in the 24 hours immediately after operation was, if anything, only a little increased over the normal. The day to day output of bile varies greatly, a fact that Stadelmann[15] has emphasized. He gives figures for two dogs weighing 16 to 17 kilos which show that they secreted about 288 cc. of bile per 24 hours, that is to say slightly more than $\frac{3}{4}$ cc. per kilo in 1 hour. But the six dogs of our Table I yielded respectively $\frac{1}{18}$, $\frac{1}{11}$, $\frac{1}{3}$, $\frac{1}{4}$, $\frac{9}{10}$, and $\frac{1}{3}$ cc. per postoperative hour, amounts that are with one exception greatly below Stadelmann's average. The more indirect data of the other tables confirm the point thus illustrated. According to Stadelmann and Hooper and Whipple,[5] the normal bilirubin output is about 1 mg. per pound of dog in 6 hours, or for present purposes 8.8 mg. per kilo of animal in 24 hours. In our animals the pigment put forth after operation ranged from 8 to 13.4 mg. per kilo in 24 hours in uncomplicated cases, with an average of 11.1 mg. The two complicated cases (Experiments 7 of Table III and 5 of Table II) that were left from this computation yielded 7.3 and 15.4 mg. respectively.

One cause for the peculiar character of the postoperative bile at once suggests itself. Fasting animals, as is well known, yield but little bile, and this heavily pigmented and with a high percentage of solids.

In a dog followed by Stadelmann the secretion of the first 24 hours after food was withdrawn had a bulk only half that in the period immediately previous and in a second 24 hours less than one-third. The total pigment output, though,

[14] The pigment studies of Hooper and Whipple with fistula animals may be consulted upon this point.

[15] Stadelmann, E., Der Icterus und seiner verschiedenen Formen, Stuttgart, 1891.

underwent no change. The observation will explain many, perhaps all, of our instances. The food intake of our dogs and their water intake as well, in the preliminary and experimental periods together, was far below the normal. Nearly all of the animals that took food in the 24 hours following operation had fasted through a like period prior to it.

Nature of the Concentrating Faculty.

It would be highly interesting to know the exact composition of the scanty, dark, postoperative liver bile. For the withdrawal of fluid from it in the gall bladder is accomplished entirely through osmosis and diffusion, and the concentration thus achieved will necessarily vary with the bile character.

Brand[2] has found that both the hepatic and bladder biles have the Δ of the blood; and through comparative analyses he obtained clear evidence that the concentration effected in the bladder comes about at the expense of the inorganic salts which are removed as what is, practically speaking, a normal saline solution. Hammarsten[3] and others confirm this, in that they too find a less quantity of inorganic salts in bladder bile than in liver bile, but correspondingly more of substances having large molecules.

The fact that the limit of biliary concentration is the Δ of the blood will explain our observation that the gall bladder contents showed no greater degree of inspissation after 48 hours (Experiments 3 and 4, Table II) than after one-third to one-half this period (Experiments 1 and 2, Table II, and all of Table III). The shifting of constituents whereby a reduction of the bile volume comes about takes place so rapidly (Table IV) that it must be practically complete within a few hours. In the lack of comprehensive analyses, the suitability of the heavy, postoperative liver bile of our experiments for concentration in the gall bladder cannot be profitably discussed. But there is every reason to suppose that its limit of concentration would be reached far sooner than that of the normal secretion, which is, by contrast, watery.

Under the operative conditions we have employed, some obstruction to the passage of fluid away from the bladder must not infrequently have been caused. The large lymphatics derived from the viscus course close beside the main duct in the gastrohepatic omentum, and the slight dissection required to place the partitioning

ligature, together with the pull of the latter, cannot but have sometimes compromised such delicate vessels. As offsetting this in that it favored resorption of fluid may be put the preliminary cleansing of the gall bladder mucosa with salt solution. But in the course of many observations upon normal dogs we have only rarely encountered a mucous layer next the bladder wall. Usually the organ holds a thin, practically homogeneous syrup, and the mucosa is clean. That a moderate mucus admixture need not greatly hinder concentration is shown by Experiments 3 and 4 of Table III with a secretion already rendered syrupy and more than thrice concentrated by the gall bladder of another animal. Taken as a whole, the conditions of our experiments were probably rather unfavorable to the concentration of bile.

Bladder Fistulæ.

Practically all quantitative studies of the bile from day to day have been carried out on fistula animals, with the gall bladder as a link in the fistulous system. The bile runs from the ligated common duct through the gall bladder and out by a slit in its tip which is sewn fast to an opening in the abdominal wall. Our findings of Table IV show that this practice involves great possibilities of error as regards actual bulk of the liver secretion, since it may be much reduced in transit. Perhaps, though, the bladder wall soon loses its concentrating faculty, owing to pathological change. Instances have been described of temporary obstruction to a fistulous outlet in which the bladder was found filled, not with inspissated bile, but with hydropic fluid,[16] the product of a damaged mucosa.[12] Nevertheless, the possibility of concentration by the bladder must be kept in mind in reviewing the data of fistula experiments.

Functions of the Gall Bladder.

There appears to be little general realization of the physiological uses of the healthy gall bladder which has now become a favorite surgical trophy. Yet several attested purposes the organ has.

[16] Kölliker, A., and Müller, H., *Verhandl. physik.-med. Ges. Würzburg*, 1856, vi, 435.

Pawlow's assistants[17] have shown that an intermittent discharge of bile takes place into the duodenum during the passage of chyme from the stomach, but ceases with this. Thereafter, until food is again taken, only a very occasional spurt of secretion passes the sphincter of Oddi—about once an hour in the dog.[13] The first bile expelled into the chyme is recognizably bladder bile, being syrupy and usually darker than that coming later, though both escape under pressure, in small spurts or jets, at short intervals. After cholecystectomy a great difference is observed. Bile dribbles continuously from the ampulla of Vater[13] and during fasting may fill the duodenum and be voided as such in the stools.[18] The disturbance of function thus indicated is not without a bad effect on the digestive processes,[18] masked though this usually is; and Rost has described a striking anatomical change that is common in man and the dog after cholecystectomy; *viz.*, a general dilatation of the bile passages. His finding has been confirmed by numerous observers. The dilatation does not occur when the sphincter of Oddi is destroyed.[10] Rost applies the term "biliary incontinence" to the continuous escape of secretion into the intestine after removal of the gall bladder. The incontinence is associated with an abnormal relaxation of the sphincter[10] which latter, however, frequently recovers its tone as duct dilatation ensues.[13]

Such activities as are more or less directly illustrated by these facts fall into three categories. The gall bladder acts like a distensible bag interpolated into a rigid system of tubes, to minimize extremes of pressure when bile comes rapidly or in large quantity from the liver and its escape into the intestine is prevented by the sphincter. The bag in question is rendered capacious not so much through its size as by a singular ability to reduce the bulk of the fluid reaching it. Small wonder that after cholecystectomy the ducts dilate and the sphincter gives way![19] The organ is also propulsive, delivering bile to the duodenum when needed. But such service is subsidiary, if essential, to the storage of bile. During those periods when the duodenum is empty the bladder husbands the bile for future use, and through its concentrating activity is enabled to retain very nearly all of the liver output when the interval from one gastric digestion to another is not unduly long. An illustration of the point may be given:

[17] Bruno, G. G., Dissertation, St. Petersburg, 1898, and Klodnizki, Dissertation, St. Petersburg, 1898; cited by Babkin, B. P., Die äussere Sekretion der Verdauungsdrüsen, Berlin, 1914, 344. Bruno, G. G., *Arch. Sc. biol. St. Pétersbourg*, 1899, vii, 87.

[18] Hohlweg, H., *Deutsch. Arch. klin. Med.*, 1912, cviii, 255.

[19] Judd, E. S., *Ann. Surg.*, 1918, lxvii, 473.

The period of most abundant bile formation in a normal animal coincides roughly with that during which chyme leaves the stomach. Secretion has diminished markedly 10 or 12 hours after the ingestion of food,[15] and thereafter continues slowly to lessen. A normal dog of 9 kilos fed every 12 hours, that is to say under favorable conditions for secretion, will form, in every 12, about 90 cc. of bile.[20] Much less will be put out when a feeding is omitted. Now if such a dog be supposed to have a gall bladder holding 10 cc., which is well below the average capacity,[21] and the organ be endowed with the ability to concentrate the bile sixfold, which in view of our experimental findings is not too much to assume, there should be room in it for 60 cc. of liver bile. That so much will actually come from the liver is doubtful. The hourly small spurt into the duodenum during a fast is not indicative of a tensely filled gall bladder but may be directed to the maintenance of sterility of the passages.

In all likelihood the gall bladder has functions in addition to those outlined. Its importance as a reservoir is perhaps less in animals that, like man, eat frequently, than in species such as the dog that habitually go long periods without food. There may be reasons for the concentrating activity besides the reduction in fluid bulk. And the need for mucus in the bile is unexplained. The elaboration of mucus in quantity is, like the concentrating activity, a function of the bladder as distinct from the ducts. Indeed, the receptaculum chyli has been much too often considered a mere diverticulum in the duct system. The special character of its influence upon the bile deserves emphasis as demonstrating the highly purposeful differentiation of the organ. The fact that few ills follow upon removal of the normal gall bladder means merely that the body has adapted itself to the loss, not that the loss is unimportant. In this connection the surgeon would do well to remember that uncertainty as to function and confidence in readjustment are at best questionable motives for adventures in ablation.

[20] To judge from Stadelmann's instances.

[21] According to Mann (Mann, F. C., *New Orleans Med. and Surg. J.*, 1918, lxxi, 80), the average capacity for a dog of 8 kilos is 16.6 cc. The individual variation is great, as our Table III shows. Mann's figure indicates that we had to do with unusually small bladders, a view supported by our more recent experience.

SUMMARY.

The bile coming at one time from difierent portions of the liver of the dog has nearly the same amount of pigment per cubic centimeter. With this determined we have studied the power of the gall bladder to concentrate bile directed to it, using as criterion the pigment strength of a sample collected throughout the period of experiment from a duct branch. The extent and rapidity of the concentration are alike remarkable. A gall bladder emptied at the beginning of one experiment and left to fill from the liver, concentrated the 49.8 cc. of bile reaching it in $22\frac{1}{2}$ hours to 4.6 cc., that is to say reduced its bulk 10.8 times; while another bladder left distended with a bile of known constitution and receiving in addition fresh increments from the liver concentrated the secretion 8.9 times in 22 hours. A series of five emptied bladders concentrated the bile coming to them in about 24 hours on the average 7.1 times, or a little more than the 6.4 times of seven organs left full. The conditions in both cases were relatively unfavorable to the withdrawal of fluid from the bile because this takes place by osmosis and diffusion, with the ultimate Δ always that of the blood, and the secretion in our animals was notably rich in solids as an indirect result of the operation.

The rapidity with which fluid is withdrawn through the wall of the bladder may be judged from some experiments in which a bag was connected with the tip of the organ by a large cannula. Merely in its passage through the bladder the bile was concentrated 2.3 to 4.8 times. The finding indicates a potential source of error in observations upon samples of bile obtained from fistulous channels of which the bladder forms a part.

The bile ducts do not withdraw fluid from the secretion they convey but tend to dilute it, as we shall show in a companion paper. The restriction of the concentrating activity to the receptaculum chyli is good evidence that the latter has special significance for the organism. The nature of this significance is briefly discussed.

TABLE I.

Control Instances.

	Period.	Bag.	Lobes drained.	Weight of tissue drained.	Bile amount.			Pigment.			Bile strength.	Remarks.
					As calculated from		Actual.	Calculated total.	Actual total.	Amount per cc.		
					Tissue.	Pigment output.						
	hrs.			*gm.*	*cc.*	*cc.*	*cc.*	*mg.*	*mg.*	*mg.*		
Experiment 1; ♂; 12¾ kilos (fasting).	24	*Sample.*	Right lateral and caudate.	154.0			7.2		48.9	6.8	1	About 74 gm. of tissue in right side of right central lobe totally obstructed by the ligature; partition of tissue only approximately known.
		Upper.	Left central and lateral; papillary; left side of right central.	244.0	11.4	9.5	9.9	77.5	64.5	6.5	**0.96**	
Experiment 2; ♀; 8 kilos (fasting).	24	*Sample.*	Right lateral and caudate.	91.5			7.6		25.0	3.3	1	Very early pregnancy.
		Upper.	Remainder of liver.	161.0	13.4	12.9	10.3	44.0	42.4	4.1	**1.25**	
Experiment 3; ♀; 6½ kilos (full fed).	24	*Sample.*	Left lateral and central.	105.0			27.3		48.1	1.76	1	
		Upper.	Remainder of liver.	130.7	34.0	26.8	23.3	60.0	47.2	2.0	**1.15**	

Experiment 4; ♀; 7 kilos (full fed).	24	*Sample.*	Left lateral, half of left central; caudate and right lateral.	138.0			28.0		30.0	1.1	1	Partition of tissue only approximately known; very early pregnancy.
		Upper.	Remainder of liver.	94.0	19.1	24.4	19.0	20.4	26.1	1.4	1.28	
Experiment 5; ♀; 9 kilos (some food).	17	*Sample.*	Right lateral, caudate, and two-thirds of left lateral.	154.0			70.0		70.0		1	Partition of tissue only approximately known; pigment in units, not mg.; bile of upper bag infected.
		Upper.	Remainder of liver.	210.0	95.5	76.8	68.5	95.5	76.8		1.12	
Experiment 6; ♂; 8 kilos (fasting).	24	*Sample.*	Right and left lateral and caudate.	149.0			48.0		48.0		1	Pigment in units; probable interference with portal flow to tissue drained by upper bag; both biles syrupy.
		Upper.	Remainder of liver.	105.0	33.8	15.5	19.0	33.8	15.5		0.82	

Some of the animals had received no food in the 24 hours prior to operation. The fact finds parenthetical record in the first column of the table. All of the animals had access to food afterwards, but in general only those ate that had fasted previously.

TABLE II.

Emptied Gall Bladders.

	Period.	Bile.	Lobes drained.	Weight of tissue drained.	Kind of bile.	Bile amount.			Pigment.			Bile strength.	Remarks.
						As calculated from		Actual.	Calculated total.	Actual total (units).	Amount per cc.		
						Tissue.	Pigment output.						
	hrs.			*gm.*		*cc.*	*cc.*	*cc.*	*mg.*		*mg.*		
Experiment 1; ♂; 8 kilos.	22½	*Bag.*	Right lateral and caudate.	77.0	Golden brown, thin.			19.1		19.1		1	Hay's test yields similar findings; gall bladder half collapsed; right pneumothorax.
		Bladder.	Remainder of liver.	202.0	Dark brown, syrupy.	50.2	49.8	4.6		49.8		10.8	
Experiment 2; ♀; 9¼ kilos.	24	*Bag.*	Right and left lateral; left central; caudate.	198.0	Dark brown, thin.			15.0		15.0		1	Hay's test yields similar findings; gall bladder collapsed.
		Bladder.	Right central; papillary.	90.0	Brown-black, tarry.	6.8	6.65	0.77		6.65		8.6	
Experiment 3; ♂; 11¼ kilos.	46½	*Bag.*	Caudate; right and left lateral and left central.	296.0	Dark brown, thin.			47.5		47.5		1	Gall bladder half collapsed.
		Bladder.	Right central; papillary.	127.0	Brown-black, tarry.	20.6	22.8	4.9		22.8		4.65	

Experiment 4; ♂; 11 kilos.	49	*Bag.*	Left central and lateral lobes.	141.0	Dark brown, thin.			77.0		77.0		1	Gall bladder half collapsed.
		Bladder.	Right lateral and central; papillary; caudate.	196.0	Brown-black, tarry.	107.0	43.8	5.4		43.8		8.1	
Experiment 5; ♀; 4½ kilos.	24	*Bag.*	Right and left lateral; left central; caudate.	95.0	Dark brown, thin.			27.75		59.8	2.15	1	Probable stasis in ducts leading to gall bladder owing to adhesions which bind latter down; pigment in mg.
		Bladder.	Right central; papillary.	43.5	Dark brown, syrupy.	12.7	4.45	1.4	27.4	9.6	6.86	3.18	

TABLE III.

Filled Gall Bladders.

	Period.	Bile.	Lobes drained.	Weight of tissue drained.	Kind of bile.	Bile amount.			Pigment.			Bile strength.	Remarks.
						As calculated from		Actual.	Calculated total.	Actual total.	Amount per cc.		
						Tissue.	Pigment output.						
	hrs.			*gm.*		*cc.*	*cc.*	*cc.*	*mg.*	*mg.*	*mg.*		
Experiment 1; ♂; 7 kilos.	24	*Bag.*	Left lateral and central; right lateral; caudate.	173.0	Dark green, thin.			31.0		76.7	2.48	1	At autopsy gall bladder bound down by fresh adhesions; 10.5 cc. of infected bile containing 10.3 mg. of pigment had been left in it at 70 mm. pressure.
		Bladder.	Right central and papillary.	69.0	Brownish black, semi-fluid.	(12.4)*	(1.6)		(30.6)	(3.9)			
						22.7	11.9	3.3	40.9	14.2		3.6	
Experiment 2; ♀; 7 kilos.	18	*Bag.*	Right lateral and caudate.	67.0	Medium brown, thin.			25.5		14.3	0.56	1	9 cc. of bile containing 20.1 mg. of pigment left in gall bladder at 70 mm. pressure.
		Bladder.	Remainder of liver.	195.0	Dark brown, viscid.	(74.2)	(50.8)		(41.6)	(28.5)			
						83.2	59.8	7.4	61.7	48.6		8.1	
Experiment 3; ♀; 9 kilos.	24½	*Bag.*	Right lateral and caudate.	95.0	Dark brown, thin.			25.0		64.5	2.6	1	11 cc. of syrupy bile containing 25 mg. of pigment left in gall bladder at 100 mm. pressure.
		Bladder.	Remainder of liver.	256.0	Dark brown, viscid.	(67.4)	(8.9)		(174.0)	(23.0)			
						'78.4	19.9	5.25	199.0	48.0		4.0	

Experiment 4; ♀; 8¾ kilos.	25	*Bag.*	Right and left lateral and caudate.	188.0	Dark brown, thin.			37.75		67.7	1.8	1	11.8 cc. of syrupy bile containing 26.7 mg. of pigment left in gall bladder at 65 mm. pressure.
		Bladder.	Remainder of liver.	147.0	Very dark brown, viscid.	(29.5) 41.3	(13.4) 25.2	4.3	(53.0) 79.7	(24.1) 50.8		5.9	
Experiment 5; ♀; 6 kilos.	22	*Bag.*	Right lateral and caudate.	105.0	Dark brown, thin.			20.6		36.0	1.75	1	4.3 cc. of bile containing 5.4 mg. of pigment left in gall bladder at 70 mm. pressure.
		Bladder.	Remainder of liver.	180.0	Very dark brown, viscid.	(35.2) 39.5	(22.3) 26.6	3.0	(61.7) 67.1	(39.0) 44.4		8.9	
Experiment 6; ♀; 6½ kilos.	24½	*Bag.*	Right lateral and caudate.	59.0	Dark brown, thin.			8.3		27.1	3.3	1	3.5 cc. of bile containing 7.1 mg. of pigment left in gall bladder at 90 mm. pressure.
		Bladder.	Remainder of liver.	134.0	Brownish black, thick.	(18.8) 22.3	(20.0) 23.5	3.5	(61.6) 68.7	(65.4) 72.5		6.7	
Experiment 7; ♂; 7¾ kilos.	24	*Bag.*	Right lateral and caudate only?	98.5	Greenish brown, thin.			33.5		47.9	1.4	1	At autopsy gall bladder bound down by fresh adhesions; 4.5 cc. of bile, containing 7.9 mg. of pigment had been left in it at 300 mm. pressure.
		Bladder.	Remainder of liver.	163.0	Dark brown, viscid.	(55.5) 60.0	(5.9) 10.4	1.4	(79.4) 87.3	(8.4) 16.3		7.4	

* The figures in brackets = contribution of the liver to the gall bladder contents; those unbracketed = sum of this and of material introduced at time of operation.

TABLE IV.

Bladder Fistulæ.

	Period.	Bag.	Lobes drained.	Weight of tissue drained.	Kind of bile.	Bile amount.			Pigment.			Bile strength.	Remarks.
						As calculated from		Actual.	Calculated total.	Actual total.	Amount per cc.		
						Tissue.	Pigment output.						
	hrs.			*gm.*		*cc.*	*cc.*	*cc.*	*mg.*	*mg.*	*mg.*		
Experiment 1; ♂; 12 kilos.	24	*Control.*	Right lateral and caudate.	98.0	Thin, medium brown.			17.5		42.1	2.4	1	Gall bladder flaccid, contains 0.3 cc. of bile; cannula from it 3 mm. in least diameter.
		Bladder.	Remainder of liver.	249.0	Syrupy, dark brown.	44.4	49.5	16.2	106.9	119.0	7.4	3.1	
Experiment 2; ♂; 9¾ kilos.	24½	*Control.*	Right lateral and caudate.	118.0	Thin, medium brown.			10.75		23.6	2.2	1	Gall bladder shrunken, contains 1.4 cc. of bile pressed back into it at autopsy; cannula from it 3 mm. in least diameter.
		Bladder.	Remainder of liver.	255.0	Thin, dark brown.	23.2	25.7	11.2	51.0	56.3	5.0	2.3	

Experiment 3; ♂; 15¾ kilos.	9	*Control.*	Caudate and part of right lateral.	64.0	Thin, medium brown.			10.3		22.6	2.2	1	Partition of tissue approximate; gall bladder contains 0.6 cc. of deeply stained mucus; lower bag contents infected with an organism that does not alter the pigment; cannula 2.5 mm. in least diameter.
		Bladder.	Remainder of liver.	315.0	Ropy, brownish black.	50.7	47.4	9.8	111.1	103.9	10.6	4.8	
Experiment 4; ♂; 11½ kilos.	23½	*Control.*	Caudate and right lateral.	73.0	Thin, dark brown.			15.0		35.1	2.3	1	Gall bladder shrunken, empty; cannula 3 mm. in least diameter.
		Bladder.	Remainder of liver.	205.5	Syrupy, much darker brown.	42.3	41.1	10.25	98.8	96.1	9.4	4.1	

TABLE V.

Cannula in Bladder Neck.

	Period.	Bag.	Lobes drained.	Weight of tissue drained.	Kind of bile.	Bile amount.			Pigment.			Bile strength.	Remarks.
						As calculated from		Actual.	Calculated total.	Actual total.	Amount per cc.		
						Tissue.	Pigment output.						
	hrs.			*gm.*		*cc.*	*cc.*	*cc.*	*mg.*	*mg.*	*mg.*		
Experiment 1; ♂; 11¾ kilos (full fed).	20	*Lower.*	Right lateral and caudate.	200.0	Medium brown, thin.			43.0		43.0		1	Pigment in units, not mg.
		Upper.	Remainder of liver.	305.0	Similar color (thick ?).	65.6	56.5	40.0	65.6	56.5		1.41	
Experiment 2; ♂; 8 kilos (fasting).	21½	*Lower.*	Upper part of right lateral lobe.	43.0	Dark brown, thin.			5.2		3.4	0.66	1	Partition of tissue only approximately known. Stasis in ducts to upper bag?
		Upper.	Remainder of liver.	316.0	Dark brown syrupy.	38.2	100.0	28.8	25.0	65.6	2.3	3.48	
Experiment 3; ♀; 10¼ kilos (fasting).	26	*Lower.*	Right lateral and caudate.	115.0	Dark brown, thin.			12.5		42.3	3.4	1	
		Upper.	Remainder of liver.	245.0	Dark brown, syrupy.	21.3	33.5	20.0	90.0	113.6	5.7	1.68	

General Description of the Tables.

The most important data given relate to the relative pigment concentrations, or bile strengths, of the portions of secretion derived from the different liver regions of the same animal, that of the so called sample bag being taken as a standard. Table I shows that when the influence of the gall bladder is ruled out, the portions differ little in pigment value. The other tables present evidence that this value undergoes a manifold increase in bile submitted to the bladder.

Since the pigment strength is much the same for the unmodified secretion from different portions of a single liver, it becomes possible to ascertain approximately the quantity of bile reaching a gall bladder during a given period from the pigment accumulation in it as compared with that in a type specimen of the liver bile collected into a bag. Thus, for example, in Experiment 1 of Table II the bladder contained 49.8 units of pigment (a unit = pigment in 1 cc. of sample bile) in only 4.6 cc. of fluid, as compared with 19.1 units in the 19.1 cc. of the type specimen. It follows that 49.8 cc. of secretion had reached the bladder. Data obtained in this way have been given place in the tables. So too have figures on bile output and pigment quantity derived from a knowledge of the contents of the sample bag and the proportion of tissue tributary to it and to the bladder. In Experiment 1 of Table II the output of 77 gm. of liver, as collected in the sample bag, amounted to 19.1 cc., while 202 gm. supplied the bladder. It follows that the latter should have received 50.2 cc. of bile, an amount closely approximating the real one (49.8 cc.) as calculated out on the basis of actual pigment content. For the purposes of a comparison with this last, the total pigment that should theoretically have reached the bladder has been calculated out on the basis of the tissue partition and the pigment in the sample bag.

In the experiments of Table III the use of a foreign bile to distend the bladder has complicated the expression of results. To determine the amount of secretion and of pigment coming from the liver during the experiment, it was necessary to deduct from the ultimate findings the amounts introduced. This has been done. The figures in brackets represent the amounts of bile and of pigment furnished by the liver, and the unbracketed figures just beneath represent the sum of such quantities and of those introduced. Thus in Experiment 1, 10.3 mg. of pigment was put in the gall bladder and 14.2 mg. found at the close of the experiment. The liver then had contributed 3.9 mg. of pigment, or 1.6 cc. of fluid, judging from the pigment strength of that in the sample bag. Since 10.5 cc. of fluid had originally been introduced, the total acted upon by the gall bladder was 11.9 cc., and in the reduction of this to 3.3 cc. a 3.6 fold concentration was effected.