STUDIES ON THE TOTAL BILE.

I. The Effects of Operation, Exercise, Hot Weather, Relief of Obstruction, Intercurrent Disease, and Other Normal and Pathological Influences.

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By a method already described[1] the entire bile output of dogs can be obtained over long periods of time, unexposed to gall bladder activity, and uncontaminated with wound secretion or the product of inflamed ducts. Aided thereby we have studied anew several of the classical, unsolved problems of bile secretion. The first step, of necessity, has been to familiarize ourselves with the day to day variations in the bile due to intercurrent causes. To these, which are often striking and significant, the present paper will be devoted.

Method.

The dogs were kept throughout in roomy metabolism cages, and were given no yard exercise because of its possible influence upon the bile, a precaution justified by the findings, as will be shown. Long experience had convinced us that normal dogs kept and fed as described remain in good health. Following the operative insertion of the collecting apparatus, the bile was removed from the collecting balloon every morning at about the same hour. Cultures were made of it at once in bouillon and on agar plates. Its appearance and consistency were then noted, the quantity measured, and a 1 cc. specimen was mixed with Hooper and Whipple's[2] acid alcohol (49, 99, 199, or 399 cc., according to the bilirubin content of the specimen) and set aside for a colorimetric reading. The remainder was stored in the ice box. By duplicate mixtures with the acid alcohol, made on successive days, each specimen was quantitated at least once with those immediately preceding and following it, and against the same portion of inorganic standard.[3] Thus the individual readings became closely linked.

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[1] Rous, P., and McMaster, P. D., *J. Exp. Med.*, 1923, xxxvii, 11.

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

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

Just before the bile was removed from the balloon each day the hemoglobin percentage of the blood was ascertained by the Newcomer method with a specimen from a marginal ear vein; and immediately after the bile evacuation, food for the 24 hour period was given the animals. The routine bilirubin and hemoglobin determinations were made by different workers, each unaware of the other's findings, to rule out the subjective element.

Ordinarily the diet consisted of a mixture of cooked lean meat and bread in regular proportion; but, for special purposes, this was often varied with a ration of cooked lean meat and liver, or bread, milk, and bones. The amount eaten in the 24 hours, as of water drunk, was measured.

Each day the urine was tested for bilirubin (Gmelin reaction, and, for quantitative purposes, Hooper and Whipple's[2] modification of the Salkowski test). For the detection of urobilin and urobilinogen, the spectroscopic method of Wilbur and Addis[4] was employed, and for bile salts Hay's test. The stools were frequently searched for stercobilin (Schlesinger's reaction), to detect a possible reopening of the original duct channel; but it may be said at once that this complication was never encountered.

The Effects of Operation.

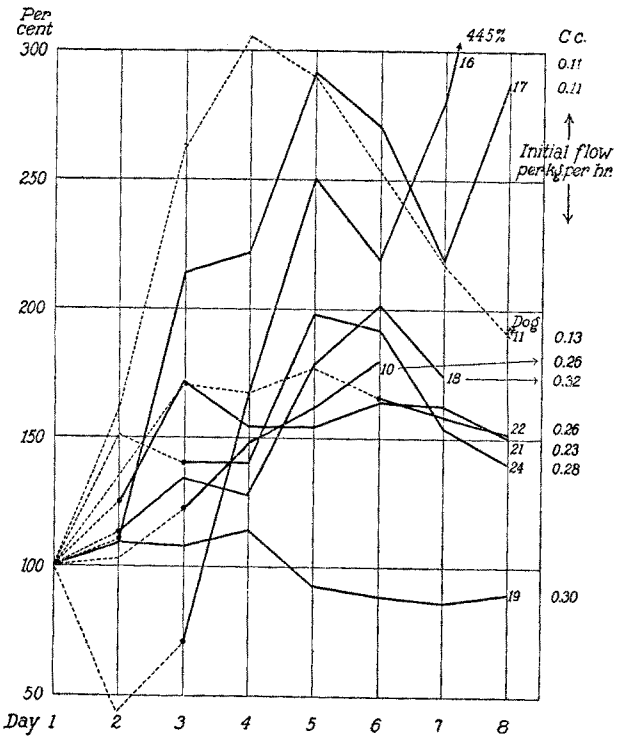
The literature contains no quantitative studies on the bile secreted in the period immediately after the operation whereby a biliary fistula is produced in animals.

During the first few days after the operative insertion, under ether, of our collecting apparatus and especially during the first 24 hours, the bile was nearly always much more scanty and more heavily pigmented than that secreted later on (Text-figs. 1 and 2). It was also more syrupy. The bile of the second 24 hours after operation was usually thicker than that of the first, sometimes even ropy, despite an almost regular, and frequently considerable, increase in amount; and on one occasion at least, a temporary obstruction at this time was referable to such cause (Text-fig. 3). Since recovery from the operation was rapid, and the animal during the second 24 hours was ordinarily in excellent condition, the peculiar consistency of the bile may be referred rather to a local reaction on the part of the traumatized ducts than to general influences.

In Text-fig. 1 the postoperative changes in the bile amount are expressed in terms of the output of the first 24 hours. The actual initial rate of bile flow per kilo per hour is given at the right margin of

[4] Wilbur, R. L., and Addis, T., *Arch. Int. Med.*, 1914, xiii, 235.

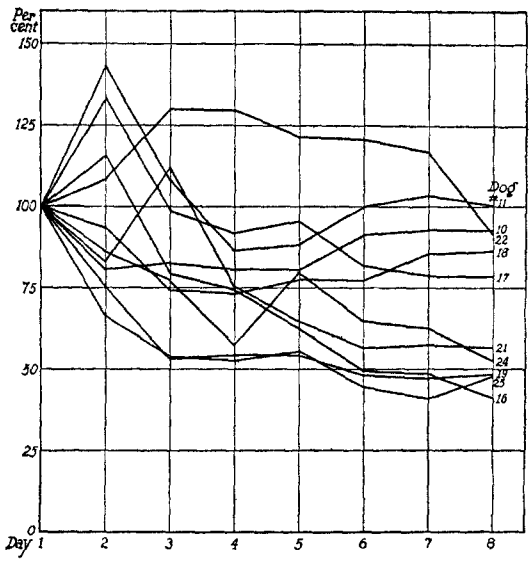
the chart. It will be observed that, in general, when this initial flow was least the relative increase later was greatest, as would inevitably follow from the fact that the later, or "normal," rate of bile flow is subject to much less variation than is the initial rate of flow.



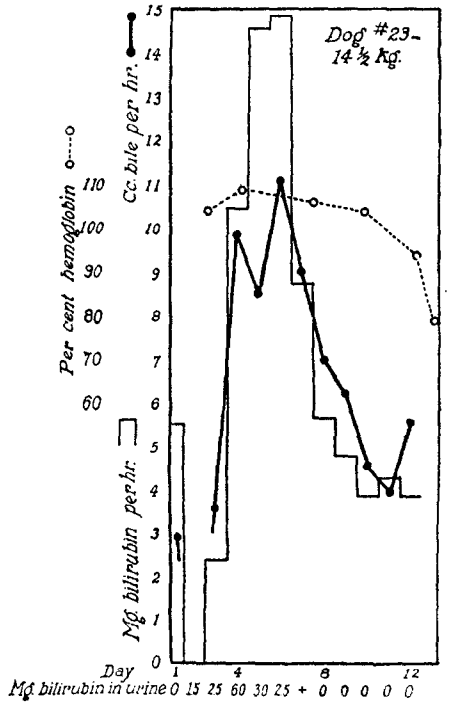
TEXT-FIG. 1. Postoperative changes in rate of bile flow as expressed in percentages of the 1st day's yield. The dotted ruling indicates that the animal was fasting.

For a day or so after the operation the dogs as a rule took little food. Since 1852 it has been known that fasting animals yield less bile than do those full fed.[5] Here was a possible cause for the small postoperative output. To determine whether it was the actual one, some of the animals were denied food for several days after operation,

[5] Bidder, F., and Schmidt, C., Die Verdauungssäfte und der Stoffwechsel. Eine physiologisch-chemische Untersuchung, Mitau and Leipsic, 1852.



TEXT-FIG. 2. Postoperative changes in bilirubin output as expressed in percentages of the 1st day's yield.



TEXT-FIG. 3. Postoperative obstruction due to viscid bile.

though given free access to water. The bile, at first scanty, underwent an increase later as in the well fed animals (Text-fig. 1). A lessened ingestion of food after operation will not, then, account for the findings. Nor probably will a water lack, for the animals always drank freely after operation, and the hemoglobin per cent of the blood did not change.

The scanty first bile was, as already stated, much more pigmented than the later secretion. The fact is well attested that with the increased pigmentation of the bile that follows upon a sudden liberation of hemoglobin in the body the quantity of the secretion may become temporarily less.[6] In certain of our dogs the operative trauma caused some extravasation of blood into the tissues, and from this hemoglobin must have been set free. In other instances, though, in which special attention was given to such a possibility and hemostasis carefully maintained, it can be practically ruled out; and nevertheless the bile was scanty.

Altogether the results incline us to the belief that the small output of bile immediately after operation is attributable directly to the influence of this latter upon the liver,—though the nature of the influence remains to be determined. Many authors have noted that in laparotomized dogs kept under ether for the collection of bile, none may be forthcoming;[7] and Hooper and Whipple have made the interesting observation that upon the introduction of a rubber drainage tube into a cholecystostomy opening bile secretion sometimes ceases for many minutes.[2]

One potential cause for the high pigment content of the bile in the first days after operation as compared with that secreted later has been mentioned above, namely a liberation of hemoglobin from extravasated blood. That this was occasionally at least an actual cause was demonstrated by the course of the bilirubin output in instances in which blood was known to have been lost into the tissues. A marked temporary rise in the output of pigment was then to be noted between the 2nd and 5th postoperative days, that is to say

[6] Stadelmann, E., Der Icterus und seine verschiedenen Formen. Nebst Beiträgen zur Physiologie und Pathologie der Gallensecretion, Stuttgart, 1891.

[7] For example, Wertheimer, E., *Arch. physiol. norm. et path.*, 1892, iv, series 5, 577; Neilson, N. M., and Meyer, K. F., *J. Infect. Dis.*, 1921, xxviii, 510.

during precisely the period when the extravasated blood was breaking down. A similar rise was observed later on, in two dogs, during the absorption of small hematomas resulting from jugular puncture.

The findings thus far described were obtained in animals yielding a sterile bile and rapidly recovering from the operative procedure. The results were far different in certain others in which the liver had been damaged with mercuric chloride carried into the peritoneal cavity upon the operator's gloves. The collecting balloon had been left within the abdomen in these instances, but its position may be dismissed from account in the present connection since in our experience bile is ordinarily secreted freely into bags so left. The mercury poisoning resulted in death of the animals after about 2 days; and there was outspoken parenchymatous and fatty degeneration of the liver at autopsy. In these cases almost no bile was secreted from the time of operation; in one of them none whatever during the 48 hours that elapsed before death. At the postmortem examination, in this case, the larger bile channels were found undilated, but in the lumen of each lay a thick cord of dark brown, semisolid, mucinous material. That the hepatic cells continued to secrete bile pigment was shown by a marked jaundice of the liver.

In several early experiments in which the collecting balloon had been left within the abdomen, infection of the biliary tract occurred; and in one of these in which a mixture of organisms was present, bile ceased to be secreted into the bag, and its place was taken by a clear, glairy, tenacious material like white of egg, obviously a product of the inflamed ducts. In this instance only a part of the liver, and this part atrophying as result of long standing local diversion of the portal flow,[8] was drained by the bag; and the pressure under which the bile was secreted was low, as always after such a change in the circulation.[9] But the finding suffices to illustrate one method whereby catarrhal icterus may be supposed to develop.

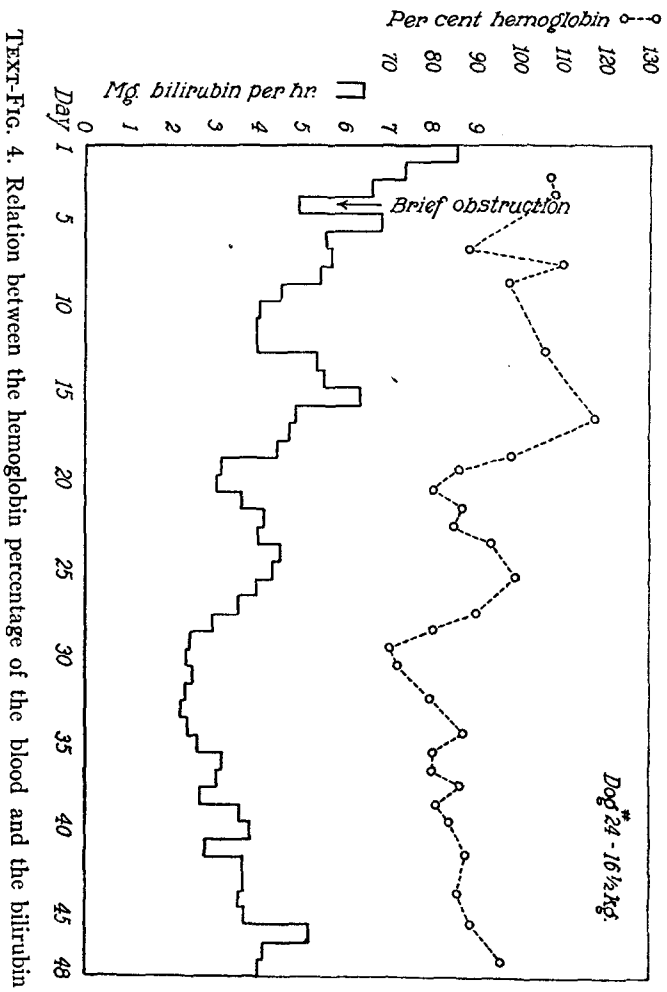
The Later Output.

Within 3 to 7 days as a rule the bile has increased to approximately the amount yielded during the weeks that follow if the conditions

[8] Rous, P., and Larimore, L. D., *J. Exp. Med.*, 1920, xxxi, 609.

[9] Rous, P., and Larimore, L. D., *J. Exp. Med.*, 1920, xxxii, 249.

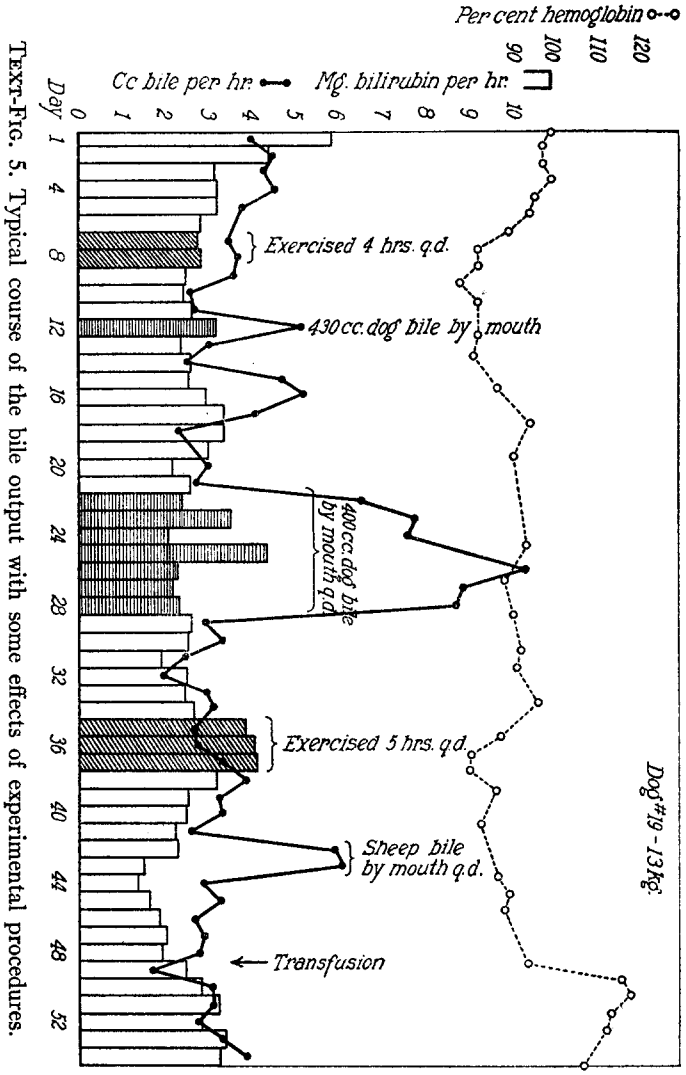
are not varied; but often as many as 8 or 10 days elapse before the pigment output falls to the "normal" of the succeeding period. The reasons for this will be discussed in a later paper; suffice it here that the gradual absorption of the hemoglobin from extravasated blood



TEXT-FIG. 4. Relation between the hemoglobin percentage of the blood and the bilirubin output.

is not the sole one. Always, despite an apparent lively health, a well healed wound, and a varied diet, there develops, more or less gradually, a mild but obdurate anemia of secondary type. The drop in the hemoglobin percentage incident thereto is accompanied by one

in the bilirubin output. That animals markedly anemic yield sometimes a bile notably poor in pigment is known.[10] But it is surprising to find that intercurrent variations in the hemoglobin percentage



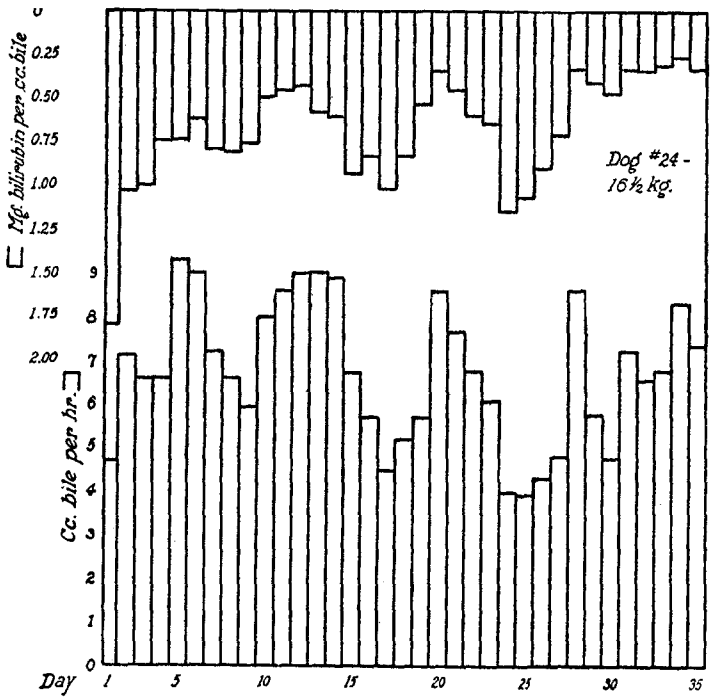
TEXT-FIG. 5. Typical course of the bile output with some effects of experimental procedures.

are closely paralleled by similar variations in the bilirubin yield. In many instances slow wave-like changes in both are to be noted (Text-fig. 4). Their importance in the interpretation to be accorded

[10] Whipple, G. H., and Hooper, C. W., *Am. J. Physiol.*, 1917, xliii, 258.

to data on the bilirubin output is obvious. In the absence of complicating influences, this output changes remarkably little from day to day, a fact which cannot be too much stressed. A typical curve, with incidental variations referable to experiment, is given in Text-fig. 5.

The bile quantity, by contrast to that of the pigment, undergoes sudden and great variations under normal circumstances. It follows,



TEXT-FIG. 6. Reciprocal relation between the fluid output of bile and its pigment concentration.

if a uniform output of pigment is to be maintained, that when the bile is copious it will be weak in pigment per cubic centimeter, and, when scanty, strong. This is strikingly true (Text-fig. 6). A like reciprocal relationship seems to exist normally between the quantity of the secretion and its mucinous constituent, judging from gross changes in the consistency of the fluid. A scanty bile is usually syrupy or even ropy, and a profuse one watery. The average specimen of hepatic duct bile, as we have observed it, is only tacky not syrupy.

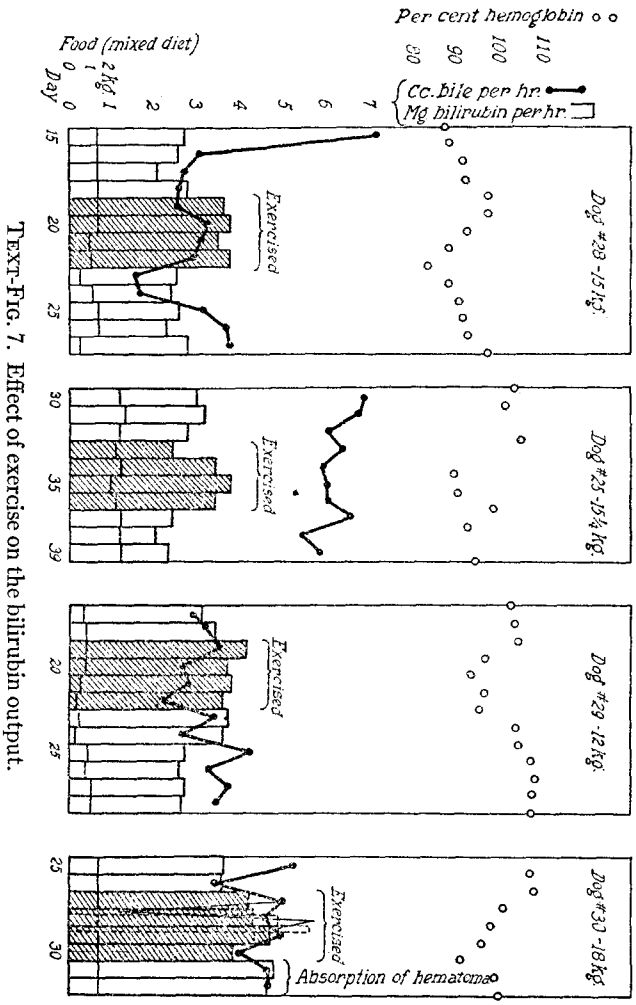
The Pigment Quantity.

Prior to the development of the mild anemia just mentioned, the bilirubin output corresponds closely with that recorded by Stadelmann.[6] This observer, studying three animals, found that the quantity of pigment in 24 hours varied from 4.2 to 10.2 mg. per kilo of dog and averaged between 6 and 7 mg. In nine dogs we have noted a range of from 5.2 to 11 mg. and an average of 7.4 mg. at such times as the hemoglobin percentage approximated the normal. These are to be considered as maximal figures for the individuals in question, since during much of the period of bile collection there was anemia and associated with it a low pigment output. Hooper and Whipple report an average output of about 9 mg. per kilo (1 mg. per pound of animal in 6 hours); but their animals had outdoor exercise each day, and, as will now be shown, may with reason be presumed to have made and broken down more blood.

The Effects of Exercise.

Broun[11] has shown that vigorous exercise of dogs previously kept under sedentary conditions leads to an increased blood destruction, as further that the daily turnover of blood in active animals must be greater than in quiet ones. Since bile pigment is supposed to come from destroyed blood, vigorous bodily activity may well influence the daily output. It was for this reason that our animals were kept caged. A number of experiments on the effects of running upon treadmills were carried out with them; and the results demonstrate plainly that such unprepared for exercise during 4 or 5 hours of each day causes, at the same time with the drop in the hemoglobin percentage already noted by Broun, a marked absolute increase in the bilirubin output (Text-figs. 5 and 7). Further data on the point will be recorded in a later paper. There can be no doubt of its general importance in relation to bilirubin figures. Curiously enough the quantity of the bile is not essentially altered by exercise. In the case of Dog 30 (Text-fig. 7) the output for the 6 hours in which exercise went on was separately examined on two occasions.

[11] Broun, G. O., *J. Exp. Med.*, 1922, xxxvi, 481; 1923, xxxvii, 113, 187, 207.



TEXT-FIG. 7. Effect of exercise on the bilirubin output.

The Bile Quantity.

That the bile quantity may vary greatly from day to day and even more from hour to hour is known to every student of the secretion.

According to Heidenhain's[12] summary, dogs secrete from 2.9 to 36.1 cc. per kilo in 24 hours. He considered 23 to 35 cc. as normal. Stadelmann[6] found a

[12] Heidenhain, R., in Hermann, L., Handbuch der Physiologie, Leipsic, 1883, v, pt. 1, 367.

range of from 8.8 to 21 cc., figures obtained by 12 to 24 hour collections from animals on a mixed diet. The hourly variations are frequently enormous. Bidder and Schmidt[5] demonstrated that fasting animals yield far less bile than those well fed, and Nasse[13] showed that the output is considerably greater on a meat diet than on one of carbohydrates (bread, potatoes). A host of later investigators has reaffirmed these facts. Voit,[14] Dastre,[15] and others have constructed curves indicating the dependence of the secretory rate on the time of feeding.

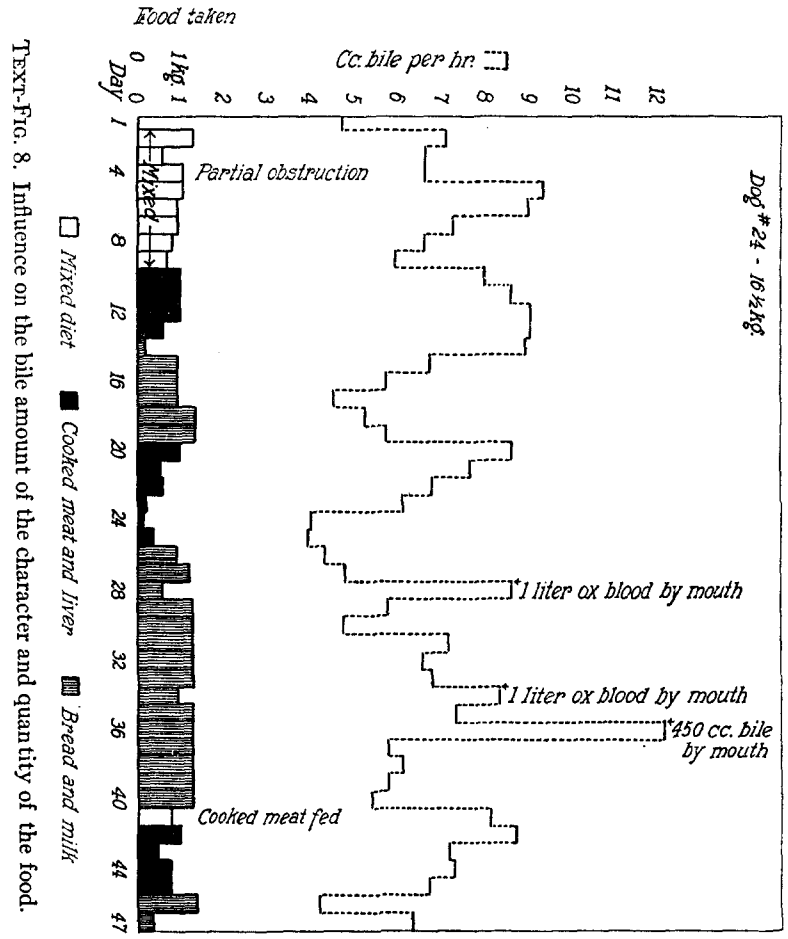
In our animals, lively, well nourished, and subsisting upon a varied diet, the average yield of bile was greatly less than that observed by any of the investigators just named. In seven uncomplicated instances the individual minimum ranged from 1 to 7 cc. per kilo in 24 hours, and the maximum from 8 to 14 cc. The minimum was registered on warm days when the animal ate little, or partook mainly of starchy food, and the maximum when meat, with sometimes a little cooked liver, had been given. The striking influence of the character of the food was over and over again manifest (Text-fig. 8). The average output on a mixed diet of bread and meat ranged from 3.5 to 9.5 cc. Some individuals consistently secreted less bile than others. For example, one vigorous dog of 13 kilos not infrequently put out as little as 3.3 cc. per kilo in 24 hours, never more than 9.6 cc., and averaged about 5 cc.; whereas another of 12 kilos yielded from 7 to 13 cc. with an average of 10 cc., or twice as much. In both cases the liver and duct system proved normal at autopsy.

During intercurrent illness unassociated with jaundice, the bile output may become extremely small. In No. 23, an animal of $11\frac{1}{2}$ kilos, yielding 6 to 7 cc. per hour when in health, only 0.23 cc. per hour, and this dark and ropy, was secreted during 27 hours of a severe coryza which was recovered from. It is difficult to be certain of the precise cause for such lessenings. Usually the dog has for some days eaten but little. They are, it is interesting to find, influenced but little by the administration through the stomach tube of a mixed dog bile that under better circumstances acts in the same animal as a marked cholagogue.

[13] Nasse, H., Commentatio de bilis quotidie a cane secreta copia et indole, Marburg, 1851, cited by Voit.[14]

[14] Voit, C., *Z. Biol.*, 1894, xxx, 523.

[15] Dastre, A., *Arch. physiol. norm. et path.*, 1890, ii, series 5, 800.

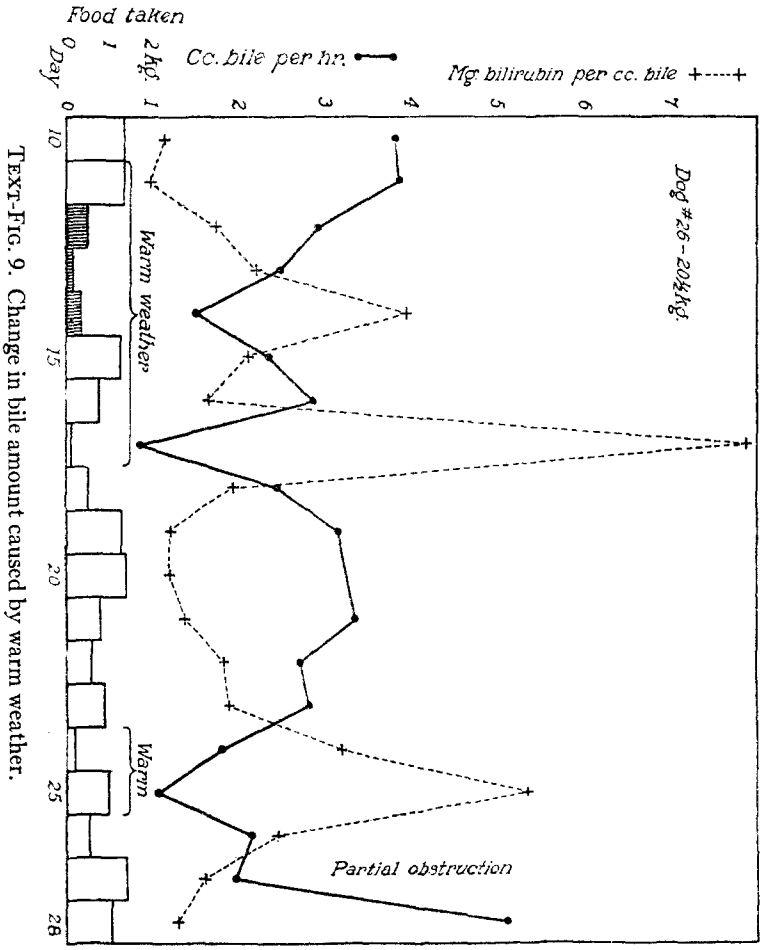


TEXT-FIG. 8. Influence on the bile amount of the character and quantity of the food.

The Effects of Hot Weather.

When the weather is hot the secretion of bile may strikingly diminish, even if the food intake remains considerable as is not usually the case. It was under such circumstances that our minimal "normal" figures were obtained. For example (Text-fig. 9), in a shaggy terrier of 20½ kilos the rate of secretion fell with the onset of warm weather from the already low quantity of 4 cc. per kilo in 24 hours to slightly less than 1 cc. The animal obviously suffered from the heat, panting

much and moving little; yet when its coat had been clipped, another period of warm weather, better borne, caused again a falling off of the bile to 1 cc. per kilo. The animal always had free access to water.



TEXT-FIG. 9. Change in bile amount caused by warm weather.

The Pigment Concentration.

During hot weather, when the bile output is notably small, the amount of pigment in it per cc. may be as much as 8 mg., whereas the average "normal" is considerably less than 1 mg., sometimes as little as 0.3 mg. Only through the elaboration of such different specimens is the bilirubin output maintained at the usual level.

After operations entailing some blood loss into the tissues, the scanty, first bile may contain as much as 4 mg. of pigment per cc. The total output later, when the extravasate is breaking down, may rise to 19 mg. per kilo in 24 hours, nearly three times the average "normal;" but, since the bile is then relatively copious, the pigment concentration per cubic centimeter is not great. Under other pathological conditions it is sometimes enormous. In a dog dying of pneumonia after 17 days of bile collection the black, ropy, last specimen held 19.7 mg. of bilirubin in each cc. Jaundice had developed. In Dog 23, during the coryza without jaundice, already referred to which caused the bile quantity to fall to about one-twenty-fifth of its "normal," the pigment concentration rose temporarily to 9 mg. per cc.

The Output after the Relief of Obstruction.

Most previous workers have noted far greater variations in the daily pigment output of healthy dogs than have we, a difference without doubt attributable to the methods they employed. Consecutive 24 hour collections over long periods of time have heretofore been undertaken only exceptionally, and then with the animal in a sling, that is to say under conditions which soon change it, as all admit, into an abnormal creature.[16] Collections for 6 hours or less out of each 24 have been the rule. And the fistula of Schwann has regularly been employed, an arrangement which favors, and is frequently followed by, recurring temporary closure of the cholecystostomy opening in the intervals between bile collections, with intermittent obstruction as result.[17,18] Under such circumstances large variations in the bilirubin output are to be expected.

We have studied the characters of the bile secreted after the relief of obstruction. The method employed for bile collection in the present work permits of total obstruction during several days merely by clamping the drainage tube for that period.

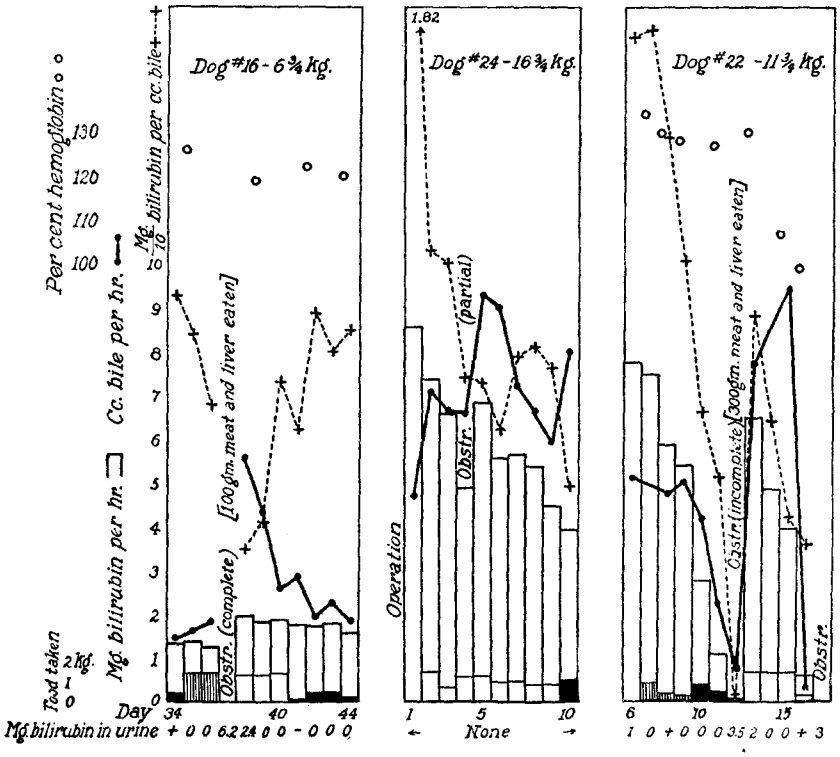
[16] The observations of Dastre by a balloon method constitute a special instance. Their results have no bearing on the subject now under discussion.

[17] von Kölliker, A., and Müller, H., *Verhandl. physik.-med. Ges. Würzburg*, 1855–56, vi, 435.

[18] Wisner, F. P., and Whipple, G. H., *Am. J. Physiol.*, 1922, lx, 119.

There exist in the literature no experiments upon the theme, though clinical records dealing with it are numerous, since nearly every surgeon of experience has had to produce a biliary fistula to relieve obstruction of the common duct. Such records usually state that a profuse flow of bile followed relief of the obstruction, with sometimes the further note that the bile was thin, and occasionally that a colorless fluid flowed forth at first, the so called "white bile" that is really a product of the ducts.[19]

Our first observations were made inadvertently.



TEXT-FIG. 10. The bile after relief from obstruction.

Dog 16,—a female fox-terrier of 6¾ kilos,—was given treadmill exercise for the first time on the 37th day of bile collection. During it the dressings slipped, bending the drainage tube sharply upon itself, with result that there followed 24 hours of total obstruction. Some characters of the subsequent bile output are recorded in Text-fig. 10.

Dog 24,—a mongrel, male hound of 16¾ kilos,—was accidentally subjected to partial obstruction through twisting of the drainage tube on the 4th day after operation, that is to say at a time when the bile pigment output was rapidly falling from the high postoperative level (Text-fig. 10).

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

Dog 22,—a white bull-terrier, male, of 11¾ kilos,—secreted bile in the usual amounts for some days after operation, and then the quantity rapidly diminished. On the assumption that angulation of the drainage tube within the abdomen was responsible several centimeters of the tubing was pulled forth. There followed an extremely copious bile flow during several days after which the obstruction reasserted itself, this time permanently (Text-fig. 10).

In each of these instances the bile flow was abundant upon relief of the obstruction, and there was a considerable transient increase in the output of bilirubin; but the fluid increase so far preponderated in two of the cases that the pigment concentration per cubic centimeter actually fell below the previous level. In the third case the conditions do not admit of a decision upon the point.

Systematic observations were now begun upon the consequences of obstruction, but unfortunately at a time when warm days were frequent, with, as result, notable intercurrent diminutions in the bile flow. Furthermore, extraneous circumstances made it necessary to kill several of the animals while in excellent condition, before the observations upon them had been completed. The data, nevertheless, are sufficient, and the findings clear-cut (Text-figs. 11 and 12).

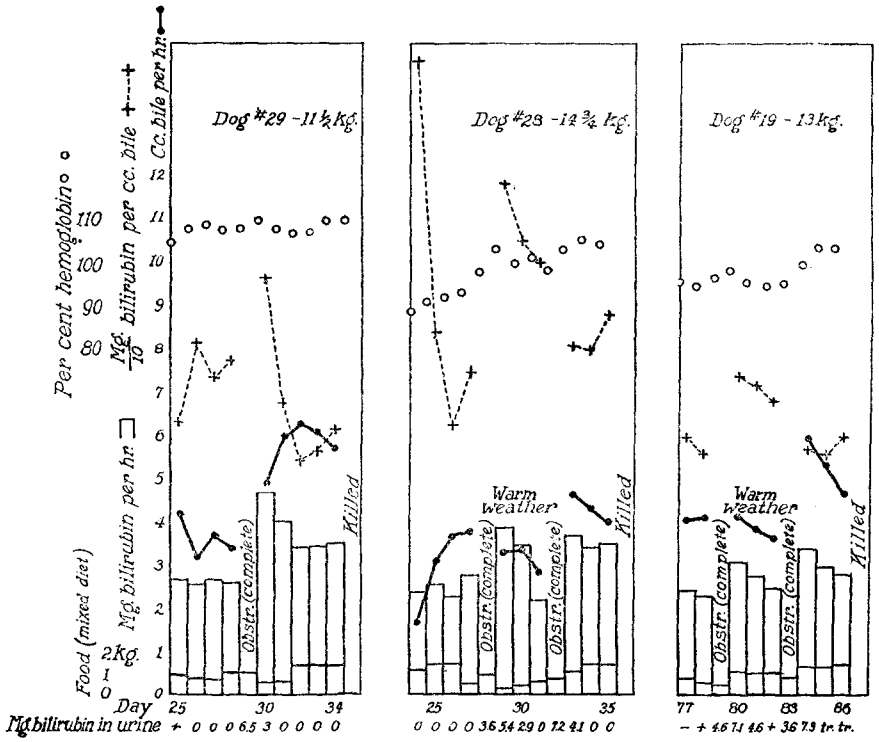
Obstruction was maintained for only 24 hours. It seemed inadvisable to use longer periods because of deposition of bilirubin in the tissues, malaise, blood changes, and other inevitable complications. In the dog the kidney threshold for hemoglobin is so low,—in contrast to the human threshold,—that bilirubin regularly appears in the urine before enough has accumulated in the blood to tint the plasma or give the diazo reaction.[20] Bile salts appear in the urine later than pigment. Bilirubinuria, then, is the most delicate indicator of the retention of bile within the body of the dog. It often appears in slight degree in normal dogs after a 24 hour fast. Fortunately our animals ate well.

The abruptly produced biliary obstruction appeared to cause our animals no discomfort whatever. During its continuance they usually retained appetite and were lively and cheerful. The development of jaundice after ligation of the common duct of dogs is ordinarily slow, bile pigment not occurring in the urine until the 2nd day, and occasionally even later.[21] But in the intubated animals of the present work, in which no operation was required to induce obstruction, bilirubi-

[20] McMaster, P. D., and Rous, P., *J. Exp. Med*, 1921, xxxiii, 731.
[21] Whipple, G. H., and Hooper, C. W., *Am. J. Physiol.*, 1916, xl, 349.

nuria became pronounced within the first 24 hours, demonstrating that some portion of the pent up secretion had already been resorbed from the liver.

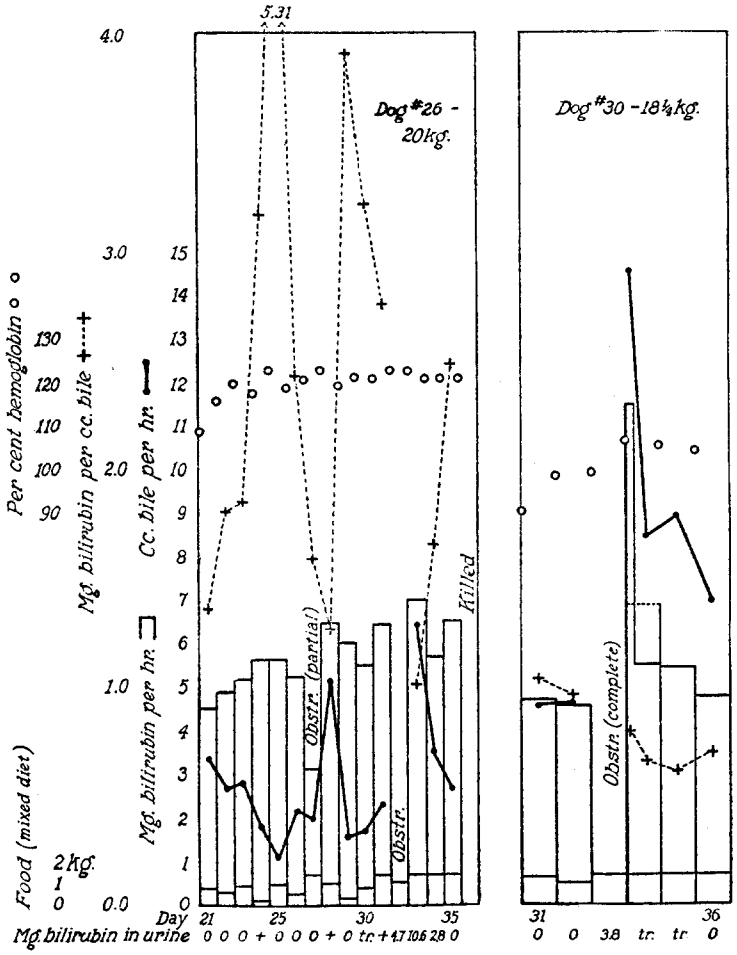
The charts show changes in the bile similar to those already remarked upon. The relief of obstruction was followed by a copious flow, save when the weather was warm; and the thin fluid had a low



TEXT-FIG. 11. The bile after relief from obstruction.

pigment concentration despite an increased total content of it. The fluid output often remained above the previous level for 48 hours, but tended, like that of the pigment, to fall rapidly to the previous level. It should be said that throughout the observations and a considerable preliminary period a standard ration of cooked meat and bread was fed, in order to rule out a possible influence of diet upon the

pigment quantity.[21] In one instance (Dog 30) the bile for the first 6 hours following the resumption of flow was collected separately. The amount secreted during this brief time was extremely large, and the



TEXT-FIG. 12. The bile after relief from obstruction.

bilirubin quantity also, but the pigment concentration per cubic centimeter was less than before the experiment.

A special interest pertains to the quantity of pigment put forth in the bile during the period of readjustment, since there are indications that bilirubin formation may be to some extent inhibited during

obstruction.[22] The interpretation of our data bearing on the point is complicated by the loss of a small amount of pigment in the urine, and furthermore by intercurrent variations in the hemoglobin percentage of the blood, which, as already stated, find a reflection in variations in the bilirubin output. Several of the experiments were performed at a time when the animals were slowly recovering from a mild anemia consequent on treadmill exercise performed while they were out of training. But allowing for these sources of error it would seem, as the charts indicate, that as much extra pigment was put out after relief of the obstruction as if its manufacture had been unimpaired throughout.

In two cases, obstruction was incomplete, entailing in the one instance (Dog 26, Text-fig. 12, first obstruction) the retention of an amount of pigment such as would ordinarily have been manufactured in about 12 hours, and in the other (Dog 24, Text-fig. 10) the yield of perhaps 8 hours. In neither case did bilirubinuria develop, and the existence of the obstruction might have gone unperceived had it been accidental in character. The point is not without importance in relation to the data obtained by bile collection from fistulas of the ordinary sort which are subject to intermittent closure.[23]

DISCUSSION.

In the present paper, as in those designed to follow it, attention has been centered on the amount of the bile and its pigment content. The bile salts are known to be derived, in part at least, from the food; and dog bile, unlike human, contains but little cholesterol, with several possible sources for it, among them the desquamated cells of the biliary tract and the food. Bilirubin has been generally supposed to have but one source, namely the hemoglobin of destroyed red cells, although recently Whipple and his collaborators have brought forward evidence which they believe shows that some of it is derived from ingested carbohydrates.[21] However this may be,—and the point is taken

[22] Whipple, G. H., Hooper, C. W., and Robscheit, F. S., *Am. J. Physiol.*, 1920, liii, 203.

[23] Pelkan, K. F., and Whipple, G. H., *J. Biol. Chem.*, 1922, l, 513.

up in a paper published herewith,[24]—the problem of the relationship between normal blood destruction and the bilirubin output remains an important one. The present communication may be looked upon as a preamble to work on the theme.

The quantity of bile secreted daily by our animals was on the average far less than that recorded by other observers, and in individual instances enormously less, despite the exceptionally good condition of the animal. This is attributable to several causes.

The observers referred to employed the open fistula method of bile collection, of which a heavily infected biliary tract[22] and cholangitis are the usual results.[23] And it is probable that sometimes a considerable part of the secretion which they collected as bile was actually the product of inflamed ducts. An instance in point is to be found in one of the dogs upon which Hooper and Whipple[2] made extensive observations (Dog 16–6). A correlation of the scattered protocols of the various experiments performed with this animal shows that between the 14th and 25th days after the operation whereby a fistula was established, bile secretion amounted to from 42 to 69 cc. in a standard 6 hour period, and averaged 58 cc. Throughout the later observations the amount steadily increased, without alterations in food or surroundings to account for the change, until, when 9 weeks had passed, the yield varied between 95 and 112 cc. with an average of 103 cc. for three successive collections. 2 days thereafter the dog died with convulsions. Pelkan and Whipple state that chronic cholangitis was usual in their animals.[23]

Many authors mention the frequency with which recurring biliary obstruction complicates bile collection by the open fistula method. Hooper and Whipple[2] refer in their autopsy protocols to a dilated condition of the ducts,—further evidence of obstruction. In the present paper it has been shown that 12 hours yield of bile may be retained without bilirubinuria, in other words without clinical evidence of obstruction, and furthermore that relief from obstruction is followed by a notably profuse flow of bile. Certain of the high figures on bile output previously reported can be referred to this cause. Yet another cause is to be found in the custom to let dogs lick away from the fistula orifice such bile as is secreted in the long periods between collections. For bile by mouth is a notable cholagogue. In this connection the fact should be stressed that none of the records

[24] Rous, P., Broun, G. O., and McMaster, P. D., *J. Exp. Med.*, 1923, xxxvii, 421.

on bile flow, neither ours nor the previous ones, can be considered as truly representative of the rate of normal secretion. For the diversion of the bile from the intestinal tract does away with an effective stimulus to further secretion.

If the biliary tract of dogs with an open fistula remained normal one would expect the bile obtained therefrom to be somewhat less in amount, not greater, than is yielded by the method we have employed, for the gall bladder forms a link in the tract of the open fistula, and this organ normally acts to concentrate the bile.[3] But under the circumstances of the fistula the gall bladder suffers with the rest of the duct system, and loses its concentrating faculty, as is clearly shown by the accumulation of "white bile" within the passages when the fistulous opening closes for a day or so.[17] The fluid accumulation in an hydropic gall bladder is admittedly the product of the diseased walls of the viscus. The same sort of fluid must frequently mingle with the bile from an open fistula and go to swell its total to a greater or less extent.

The ordinary variations in the rate of bile flow from day to day are great, and under exceptional circumstances, as we have shown, may be extreme. One of our dogs, weighing $11\frac{1}{2}$ kilos, secreted only 6 cc. during 27 hours of coryza, but a few days later, after recovery, put forth, during a period of bile feedings, 422 and 390 cc. on successive days, that is to say from 65 to 70 times as much. In this instance there were, for reasons which need not here be gone into, unusual fluctuations in the output of pigment, but alterations in the bile quantity almost as extreme as those mentioned often occur without change in the 24 hour pigment yield. As has already been pointed out, this is kept approximately constant by means of enormous variations in the pigment concentration of the bile per cubic centimeter. The more one follows the changes in quantity of the secretion in relation to the pigment content, the more one gains the impression that the variations in the fluid bulk of the bile are extraneous to, superimposed upon, the essential secretory activities of the liver in relation to the bile solids. No matter what the bile quantity may be, great or small, increased by a protein diet or bile feeding, or lessened by warm weather, fasting, or disease, approximately the same daily quota of bilirubin is put forth in it. And Rovsing's belief

that, during some diseases unassociated with biliary obstruction, the bile quantity may be greatly lessened,[25] is more than borne out by our findings. Yet though the bile bulk may dwindle for a time nearly to zero, the secretion of pigment continues at the normal rate.

Lack of bodily activity, or, to put the matter positively, a sedentary life, is held to be contributory to cholelithiasis in many human instances. It supposedly leads to a stagnation of bile, to prevent which patients are ordered exercise. Yet exercise, as the present experiments show, does not act to increase the bile quantity. Perhaps it accomplishes good by lessening the number of hours a day during which postures are maintained that may be attended by temporary biliary obstruction, with the evils consequent thereon.[19]

The influence of fasting to lessen bile secretion has frequently been emphasized in the past; but for practical purposes the emphasis should be placed on the obverse side of the phenomenon, that is to say on the effect of certain foods, notably meats, to induce a copious bile flow, and on the general importance of a frequent food intake if the bile is to be kept moving properly. We have often had occasion to note that one of the best of cholagogues, and a normal one, the bile itself, may fail to cause any considerable increase in the rate of flow when fed in large quantity, if the animal be eating poorly, though in the same dog under better conditions, the same material will give marked results. This being true it is not surprising to find, as we have further, that when disease or warm weather has caused a lessening in the flow, bile feeding often increases it little if at all. The unsatisfactory effects of flogging therapeutically a jaded organ receives in these facts a new illustration.

By contrast, as showing the happy natural solution of a problem in therapeutics, the course of events when a temporary biliary obstruction is relieved may be referred to. There is then an immediate need to rid the organism of retained bile constituents, and that is accomplished through the elaboration by the liver of a copious bile, one so much more abundant than the normal that it is thinner than this latter, despite its extra burden of solids, and less concentrated

[25] Rovsing, T., *Hospitalstid.*, 1915, lviii, 249, abstracted in *J. Am. Med. Assn.*, 1915, lxiv, 1460.

in bile pigment. Doubtless the bile salts, potent as cholagogues under experimental conditions, and largely retained in the organism during obstruction, are responsible for the cholorrhea. The changes in the bilirubin output during the period of readjustment suggest that the bile continues copious just so long as there is an accumulation of biliary constituents to be evacuated. Danger of a clogging of the passages during their removal is in this way avoided.

The marked rapidity with which bilirubinuria followed clamping of the drainage tube of our intubated animals, as compared with the delay following operative ligation of the ducts, calls for some comment. The difference is plainly due to the differing rates of bile secretion in the two cases. Afanassiew[26] has demonstrated that the delay in the appearance of jaundice after operation to close the common duct occurs because the dilated duct system serves as an emergency reservoir for bile. Only after this reservoir has been filled does the resorption of bile from the liver into the body fluids begin; and in freshly laparotomized animals, as we have found, the bile is scanty for some time, so the reservoir fills slowly. Thus it comes about that the jaundice is staved off for a longer period than after obstruction unaccompanied by operation in intubated dogs yielding a copious secretion. But even in these latter, as already mentioned, the bile yield of 12 hours may accumulate back of an obstruction without the development of bilirubinuria. It would be enlightening to know, in connection with day to day variations in the bilirubin output, the precise abilities of the liver to retain bile constituents within the parenchyma.

The changes in the bile that follow operation upon the ducts, more especially the viscid thickening of it, constitute a potentially serious menace of obstruction. Whether this menace ever asserts itself under clinical conditions we are not in a position to state. In one of our animals in which obstruction occurred from such cause, the tube through which the viscid bile had to flow was about 30 cm. long and only $2\frac{1}{2}$ mm. in diameter, that is to say much longer and narrower than is a distended common duct.

[26] Afanassiew, M., *Z. klin. Med.*, 1883, vi, 281.

SUMMARY.

By a method of permanent intubation the entire output of bile from dogs has been obtained in a sterile state over long periods of time, and studied quantitatively. The secretion of the first few days after the initial operation is scanty, contains more pigment than that secreted later, and is sometimes so viscid as to cause temporary obstruction within the collecting tube. The small amount and thick consistency of the fluid are referable to direct injury of the liver and ducts, while the abundant bilirubin is derived, in part at least, from the hemoglobin of extravasated blood. In some animals in which there was accidental mercuric chloride poisoning at the time of operation a suppression of the bile followed which, in one instance, was complete during the 48 hours before death. In a dog developing mixed infection of the biliary tract, an almost colorless fluid, glairy as white of egg, gradually took the place of the bile.

Not until a week or 10 days after operation does the bile acquire the character which it maintains later. The quantity of this later bile, as measured in successive 24 hour specimens, is greatly less than that recorded by previous investigators, a difference attributable to disturbing influences inevitable to the method of collection they employed. By our method, it averaged from 3.5 to 9.5 cc. per kilo of dog in 24 hours, though transient variations from 1 cc. up to 14 cc. were encountered. Some dogs give consistently far more bile than others. The frequently recorded effects of fasting to lessen the rate of secretion, and of a meat diet to increase it more than a carbohydrate one, were noted often. Contrary to expectation, vigorous exercise does not act to increase the quantity of the bile. During hot weather this may sink greatly, although the animal remains in good condition; and during intercurrent diseases unassociated with jaundice, the flow may almost cease. One of the best of cholagogues, bile by mouth, fails of effect under such circumstances. It acts best when the animal is healthy, the weather not oppressive, and the food intake abundant.

The bilirubin output, after the immediate effects of the operation have worn off, remains nearly constant from day to day, though often exhibiting slow, wave-like variations, each extending over a

week or more. These slow changes are synchronous with similar alterations in the hemoglobin percentage of the circulating blood. A recognition of them is important to studies of the bilirubin yield. A mild anemia, absent in controls maintained under like conditions, regularly develops in unexercised dogs completely deprived of bile, despite their apparent health, and as it does so the bilirubin output falls off. Prior to the development of the anemia the output averages about 7.5 mg. per kilo in 24 hours, a yield which closely corresponds with that recorded by previous workers. Vigorous exercise of animals previously sedentary causes an increased pigment output presumably as result of increased blood destruction.

Since the daily pigment output is approximately constant, whereas the fluid quantity undergoes frequent and great changes, it follows that the pigment concentration must vary inversely as the fluid quantity, and vary greatly. It does both. No matter how large or small the 24 hour specimen of bile may be within "normal" limits, therein will be found the customary quota of pigment. When, under pathological conditions, the bile flow almost ceases, the pigment concentration becomes extremely great. A similar reciprocal relationship between concentration and fluid quantity would seem to hold for the mucinous element of the bile. Scanty biles are ropy as a rule, and copious ones are watery.

Temporary obstruction was produced by clamping the outlet tube from the common duct, and the bile yield following upon its relief was studied. Secretion was for a time far more copious than usual, with a low pigment content per cubic centimeter, and tended to remain so until the accumulated pigment had been voided. As much extra bilirubin is put forth after 24 hours of obstruction as if the liver continued to manufacture the pigment without interruption during it. Obstruction for this length of time appears to cause no subjective disturbance in most instances, though bilirubinuria develops. Even this sign of bile retention may be lacking when obstruction endures but 12 hours.

The physiological and clinical significance of these facts is briefly discussed.