STUDIES ON UROBILIN PHYSIOLOGY AND PATHOLOGY.

II. DERIVATION OF UROBILIN.

RELATION OF THE BILE TO THE PRESENCE OF UROBILIN IN THE BODY.

BY PHILIP D. MCMASTER, M.D., AND ROBERT ELMAN, M.D.

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

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In this paper, and in others to follow, we propose to set forth facts regarding urobilin which have become evident through the use of a method peculiarly favorable to precise conclusions. The present work will be concerned with the problem of the normal physiology of the substance. In later communications the influence of pathological conditions will be taken up. Since the discovery of urobilin clinical interest in the substance has always been great. Yet at the present day the conclusions confidently drawn by workers with the substance far outrun actual knowledge of it. Few extensive experimental studies have been made and these are open to well grounded criticism.

By permanent intubation of the bile ducts of animals the total liver bile can be obtained in a sterile state during a period of months. This method has already been utilized for the study of bile constituents and it would seem especially suited for experiments on the vexed question of the relation of the secretion to urobilin. Variations of the method can be successfully put into application. Thus, for example, one may drain to the outside the bile from only one lobe of the liver, or may so arrange the intubation as to be able, at will, to collect the total bile, or have it flow as usual to the intestine.

PREVIOUS WORK.

The previous literature is crowded with articles written in advocacy of one hypothesis or another of urobilin formation. There are enterogenous, hepatogenous, histogenous, hematogenous, and even nephrogenous hypotheses of its genesis. The conflicting evidence has been admirably sifted and weighed by Meyer-Betz.\textsuperscript{9}

As far back as 1871 Maly\textsuperscript{10} succeeded in obtaining urobilin from bilirubin by reduction \textit{in vitro}. Since the work of Friedrich Müller\textsuperscript{11} it has been known that a bacterial reduction of bile pigment in the intestine accounts for part at least, of the urobilin of the stool. Recently Kämmerer and Müller\textsuperscript{12} have demonstrated that this change can be effected \textit{in vitro} by organisms of the type of \textit{B. putrificus} with the aid of \textit{B. coli} together with other aerobes in the presence of protein decomposition. It has been noted by many workers in the past that the feces of patients, or dogs, operated upon for the formation of bile fistulas and, hence, losing all the bile, became entirely, or almost urobilin-free in a short time. However, there was usually a reappearance of the pigment in smaller amounts a few days later. In spite of this discrepancy, the authors already mentioned, as well as Fischler,\textsuperscript{13} Wilbur and Addis,\textsuperscript{14} Adler,\textsuperscript{15} and many other workers in this field are agreed that the urobilin of the stool is largely derived from the bilirubin of the bile, and has an enterogenous origin in that it is formed out of bilirubin in the intestine.

The hypotheses concerning the derivation of the urobilin which is present in the bile itself, show no semblance of accord. The enterogenous derivation of the urobilin in the stool, has suggested to many that this pigment, when present in the bile, takes its origin from the same source. According to their belief urobilin formed by reduction of the bilirubin in the intestine, is in part absorbed and resecreted by the liver into the bile. The data for and against this view have been well summarized by Meyer-Betz.\textsuperscript{9}

The existence of urobilin in the bile has convinced some observers that the liver possesses the ability to manufacture the pigment, especially under the conditions of disease. Fischler\textsuperscript{13} studying dogs with ordinary bile fistulas believed he had been confronted with an occasional true hepatogenous formation of the pigment. The possibility of an hepatic derivation of the urobilin in the bile has recently been accepted by Whipple on the basis of his own work.\textsuperscript{16,17}

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In studies on the physiology of urobilin one great source of error has been constantly present, the faulty bile collection inevitable to work with the open fistula. The method has in general necessitated part time collections, which are by no means trustworthy as a gauge of the 24 hour output. Often the animals have been allowed to lick their fistula with the result that bile has reached the intestine in greater or smaller amounts thus accounting, as will be shown further on, for the reappearance of urobilin in the bile and stool. The secretion is obtained under conditions that favor infection, gall bladder inflammation, cholangitis, and hepatitis. Pigment changes in the bile due to action of the bacteria upon it cannot be ruled out, and some workers have held them responsible for the findings. The gall bladder is capable of modifying the bile greatly and the secretion studied has always been submitted to its influence. By the method we have employed all these difficulties are avoided.

EXPERIMENTAL FINDINGS.

It has long been known that under normal conditions in the dog and man urobilin is present in the stool and bile. We have regularly found it in the case of the normal dog. Our first problem has been to determine the normal variations of fecal and urinary urobilin and then to study in the same individuals the effect of bile deprivation.

For the preliminary observations healthy, vigorous dogs were employed. They were kept, throughout the period of study, in metabolism cages but were daily allowed a brief run in a small yard with a clean tile floor. The diet given consisted, in the main, of the standard bread and milk and lean meat used in previous experiments, that is to say, 1,050 gm. of bread wet with milk and 450 gm. of lean meat, for an animal of about 12 kilos. However, in many instances a free diet of table scraps and bones was given. This difference in ration appeared to be without effect on the urobilin output of the animals.

It was found that dogs which were let run for a few minutes every day soon acquired the habit of defecating then and then only, with result that the cage urine was not contaminated with feces. This proved of great advantage in the work. The findings when contamination occurred, as occasionally happened, were ruled out. The tiled floor of the small exercise yard was cleaned prior to

18 Rous, P., and McMaster, P. D., J. Exp. Med., 1921, xxxiv, 47.
the loosing of each dog. Such small amounts of urine as were voided in it were taken up into a pipette and added to the 24 hour specimen by an assistant, who observed each animal closely during the exercise period. The incidental loss of urine occasionally introduced a slight error into the urobilinuria readings but the complete separation of stool and urine removed a far greater one. In working with dogs intubated for total bile collection, the stool is urobilin-free as will be shown further on and fecal contamination of urine specimens does not present a difficulty.

Quantitative daily estimations of the urobilin in urine and feces were made as routine. The methods have already been described. It may be repeated here that separate determinations of urobilinogen and urobilin were not attempted, but the former was oxidized to the latter during the process preliminary to quantitation. Bilirubin was quantitated, in bile and urine, by a modification of the method of Hooper and Whipple. The hemoglobin percentage of the peripheral blood was followed by both Newcomer's method and that of Palmer.

Normal Urobilin Variations.

Great care must be taken in the interpretation of variations in the urobilin output by way of the stools. Changes in intestinal motility are responsible for startling differences in the readings. Both Wilbur and Addis and Dean have remarked upon the effects of constipation and diarrhea on the quantity of urobilin in human stools. Some of our experimental animals were given morphine sulfate for its constipating effect. The decrease in the amounts of urobilin of the stools passed after several days constipation, as thus brought about, was striking. The same phenomenon was many times noted incidentally to constipation occurring in the course of other experiments. The daily urobilin output even after defecation was recommenced, showed a very great decrease during 3 to 4 days, from that yielded in the period preceding the constipation. No protocols of such cases need be given here as there seems to be a general agreement concerning them. Whether the decreased elimination is significant of absorption of the pigment from the bowel, or its destruction therein, is not clear. Certain it is that the phenomenon acts to lessen greatly.
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The value of fecal urobilin estimations. When the passage through the intestines is more rapid than usual, greater amounts of urobilin are to be found in the feces, but again whether this is due to a lack of absorption or a lessened destruction is not clear.

The Effects of Total Bile Loss on Urobilin.

After the day-to-day variations in the output of urobilin in these normal dogs had been determined, we took steps to find what happens when the bile is diverted from the intestine and lost to the body. Some of the animals already studied were operated upon and the common duct was intubated by the method of Rous and McMaster, by which means all of the bile can be obtained in a sterile condition during months. The animals remained in excellent condition.

Operations were conducted under ether anesthesia. For some time previously the output of urobilin in feces and urine had been followed, and the hemoglobin percentage of the blood had been ascertained daily. After operation 24 hour specimens of the bile were collected from the balloon each morning and this bile, the urine, and feces were daily analyzed for bilirubin as well as for urobilin and the observations on the hemoglobin were kept up. Cultures were daily made of the bile, and centrifugalized sediments from it were frequently stained and examined for bacteria. All cases in which the hepatic bile became infected were ruled out.

At the end of the experimental period the animals were killed with chloroform and careful autopsies made with special reference to reconstitution of ligated ducts or anastomoses which might have developed around the cannulas. Both injection methods and dissection were utilized for the purpose. The few instances showing these causes of possible error were ruled from consideration, as were the very occasional ones showing pathological change in the liver or other organs.

Fourteen dogs with intubated common duct were observed. In twelve of these in which the bile remained sterile and total bile deprivation was effected, so that no pigment reached the intestine, the urobilin rapidly disappeared from bile and stool, and none was again found even though the observations were continued as long as 4½ months.

The day after the intubation a large amount of urobilin was regularly found in the bile, but it decreased rapidly, afterwards, and within 2 to 4 days all traces of it had disappeared. In the stools the pig-
ment disappeared almost as rapidly, the specimens being completely acholic and urobilin-free within 3 days after operation. Text-fig. 1 represents the findings in several animals chosen at random. In this chart the average of the urobilin findings in the feces of the days before operation, is plotted as 100 per cent, and on the days after operation, in terms of this figure. The output of urobilin of the bile on the 1st day after operation is plotted as 100 per cent and the sub-

Text-Fig. 1. The disappearance of urobilin from bile and feces of animals after intubation of the common duct.

Text-fig. 1. This illustrates the rapid decrease in the urobilin of the bile and stool after the operation for intubation of the common duct for the collection of the total bile. No bile pigment was allowed to reach the intestine. The bile and feces remained urobilin-free during the remainder of the period of observation. Each line represents the findings in an animal.

sequent changes in terms of this. The normal dogs showed no urobilinuria prior to operation, and after it the pigment was present in the urine for 1 or 2 days only and then was not found again.

In three of the fourteen animals urobilin persisted in the stools, always though in amounts too small to determine quantitatively, at the most in very faint traces. The bile of such animals was altogether urobilin-free. Realizing the possibility of the presence of
minute bile ducts which had escaped ligature and severance, or of the
development of small anastomotic branches, very careful dissections
and injections were made at autopsy but no such channels were found.
In the intestinal mucosa, however, were yellowish areas which yielded
the reaction for bile pigments with fuming nitric acid. Similar
findings of traces of urobilin in the stools have been reported by other
workers with bile fistula dogs and have been considered by some as a
typical finding.

Gerhardt25 stated that the cells of the intestinal mucosa, of bile fistula dogs
having traces of urobilin in the stool, showed globules containing bilirubin which
was apparently being secreted into the intestines. Fischler19 who cites Gerhardt
substantiates this finding. As will be seen later in the present work, these traces
of urobilin become far more noticeable in dogs with total biliary obstruction and
a resulting outspoken tissue icterus. In such animals the intestinal mucosa is
frankly yellow, a passage of bilirubin into the intestine can readily occur and
urobilin is demonstrable in the stool. Whatever the significance of faint traces
of urobilin in the stools of our fistula dogs, they were found in so few and the
amount of urobilin was so slight that it could scarcely play an important part
in the metabolism of the substance. Experiments to be reported below will
show that traces of blood in the intestine cannot be held accountable in any way
for this urobilin.

Needless to say, loss of the bile introduces an abnormal state of
affairs. In the further study of this pigment we made attempts to
approach the natural conditions closely.

The Effects of Partial Bile Loss.

To obtain samples of the bile while allowing most of it to flow, as
normally, to the intestine a modified form of intubation was employed,
a sampling intubation one may call it, whereby the secretion from
only one liver lobe, or at most from two small ones, was poured into
the balloon. In this way not more than 25 or 30 per cent of the bile
was lost to the animal, the remainder passing through the normal
channels into the intestine.

In nine cases the intubation was effected by joining one of the branches of the
hepatic duct to the collecting apparatus, as shown in the diagram, Text-fig. 2.

Text-Fig. 2. Diagram of the schemes of intubation of bile ducts for the collection of part of the bile.

Text-fig. 2. The diagram shows in continuous lines the arrangement for intubation of a single liver lobe usually used. When the individual ducts were small another scheme was occasionally employed (dotted lines). The common duct was ligated above the entrance of the duct from the lateral liver mass (right lateral and caudate lobes) and a cannula was inserted so as to drain bile from this mass only. A glass "bridge" cannula placed in the common duct conducted the bile from the main liver mass around the obstruction and passed it on toward the intestine. L, left lateral bile duct; C, common duct; D, duodenum.
It was necessary to use a cannula of only slightly smaller bore than ordinary. In two animals bile from the right lateral and caudate lobes was collected by intubating the common duct just below the entrance of the small duct from these, and then "bridging" the common duct with a glass tube in such a way that the bile from the main mass of the liver flowed around the obstruction made by the intubation, back into the common duct, and so into the intestine.

When the two cannulas had been placed they were tied together to avoid stoppage of bile flow by angulation of them.

Previous work from this laboratory has shown that samples of bile taken at the same time from different regions of the liver, have approximately the same volume per gm. of liver and contain equal amounts of bile pigment per cc. Knowing then the amount of bile and the bilirubin content in the daily sample yielded to the collecting apparatus, and, finding at autopsy, by injection methods, the exact amounts of liver tissue secreting into the balloon and into the intestine, respectively, the amount of bilirubin and bile put out by the entire liver on any given day can be estimated and so too with that portion entering the intestine.

Animals that underwent merely a sampling intubation, after the manner described, with result that most of the bile still flowed into the intestine, showed urobilin in the stool and bile at all times. Text-fig. 3, which depicts the urobilin findings in the bile and feces of four animals so treated, in which on the average 25 per cent of the bile was lost as a sample, should be contrasted with Text-fig. 1, of the dogs intubated for the collection of total bile. In this chart as in the previous one the urobilin output in the bile of the 1st day after operation and the average finding in the feces before operation are arbitrarily plotted as 100 per cent. Instead of the progressive fall in the urobilin of the bile that occurred with total bile deprivation, these sampled dogs showed first an increase in the pigment, then a fall, and by the 6th day after operation some return toward the initial amount. The early increase in the urobilin of the bile occurred at the time when the greatest amount of bilirubin was being secreted, that is to say, at the time when the largest amounts of this pigment were entering the intestine. For the bilirubin output of these dogs, like that of the common duct intubation animals, was greatest shortly after operation. That the increased amount of urobilin in the bile is not, like that of the bilir-
rubin, due to the absorption of blood lost during the operation is shown by the fact that the urobilin of the bile steadily decreases following the operation to drain the total bile by intubation of the common duct (Text-fig. 1).

In the eleven animals intubated for the collection of partial bile the normal fecal output of urobilin was determined daily in some cases for several weeks prior to the intubation. After it there generally occurred in the stool, as in the bile, an increase of urobilin from the 3rd till the 6th day, then an abrupt fall, followed by a rise again toward the normal preoperative output. It is significant that a complete return to this output did not occur even in experiments of long duration. The decrease in the stool urobilin roughly corresponded with

**Text-Fig. 3.** Continued presence of urobilin in both bile and feces after intubation for the collection of bile samples only.

Text-fig. 3. Each line represents the findings in a dog. More than 70 per cent of the biliary secretion of these animals entered the intestine, the remainder being collected for analysis. Urobilin was constantly present in both bile and feces.
the percentage of the total lost to the body in the sample. The correspondence was only approximate at best for variations in the intestinal motility from day to day can greatly change the urobilin content of the stool.

Text-fig. 4 shows the urobilin of the feces, before and after intubation for the collection of but 32 per cent of the total bile. The heavy horizontal lines show the average urobilin content of the feces before and after operation.

Text-Fig. 4. Decrease in the urobilin of the feces after intubation for the drainage of 32 per cent of the total bile.

Text-fig. 4. In this animal the bile duct to a single liver lobe (32 per cent of the whole) was intubated with result in the daily loss of this amount of the secretion. It will be seen that the decrease in the urobilin is roughly proportional to the percentage of the total bile lost. The heavy horizontal lines show the average urobilin content of the feces before and after operation. With the withdrawal of 32 per cent of the bile the urobilin of the feces fell 43 per cent.

The Urobilin Changes on Total Biliary Obstruction.

The effect of complete exclusion of bile from the gastrointestinal tract but without its removal from the body was studied in instances of total biliary obstruction produced by severance of the common duct between ligatures. Only those instances will be considered in which complete occlusion of the biliary system was maintained throughout the observations as proven at autopsy by injection methods and dissection. There were three such animals out of seven operated
All became heavily jaundiced, yet the urobilin of the stool fell off quite as rapidly as in the dogs from which the total bile was drained (Text-fig. 5). The feces of one became entirely urobilin-free and in the other two instances almost so, but by the 5th day after operation, when jaundice was well developed, appreciable amounts of the pigment, more than mere traces, had reappeared in the stools. At autopsy the intestinal mucosa of the totally obstructed animals was intensely yellow and gave the reaction for bile pigments strongly. It seems probable, then, that during jaundice, small quantities of bilirubin escape into the lumen of the intestine and are there changed to urobilin. Possibly this mechanism accounts for the very slight traces of the pigment found in the feces of animals deprived of the total bile. As will be seen later, small or large quantities of blood in

**Text-Fig. 5.** The urobilin content of the stools after total biliary obstruction.

Text-fig. 5. Plotted like the previous ones, but for the stool findings alone to show the changes in amount of the fecal urobilin of animals undergoing a total biliary obstruction. The urobilin of the stool fell off quite as rapidly as in instances in which the animals were deprived of all the bile by intubation of the common duct (Text-fig. 1) but small amounts of the pigment reappeared in the feces by the 6th day after operation. The continuous, broken, and dotted lines each represent the findings in a different individual.
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the intestine in the absence of bile, cannot account for the presence of this pigment.

The urinary findings possess much interest. After the operation for total biliary obstruction, urobilin appeared in the urine for 1 or 2 days in moderate quantities, but then disappeared, not to be found again. The following experiment, with others like it, to be reported in full later on, have shown that the practically complete lack of urobilin in the excreta of animals with total biliary obstruction is not to be explained as the result of changed conditions within the bowel, as distinct from an absence of bilirubin. For when bilirubin is fed as fistula bile free from urobilin to animals with biliary obstruction urobilin appears in quantity in both the stools and urine.

A dog in which total biliary obstruction was produced yielded, 3 days afterwards, feces and urine that were both urobilin-free. The stools of the 6th day contained small amounts of the pigment, less than 1 per cent of that found prior to operation. Up to this time there was no urobilinuria. Fresh sterile dog bile from another animal, containing 100 mg. of bilirubin and no urobilin was fed by stomach tube on 2 successive days. There followed at once a great rise in the intestinal urobilin to the original value, intense urobilinuria, and an increase in the bilirubinuria. The two latter phenomena were transient but the high fecal urobilin findings persisted for nearly a week with a gradual fall to the amount obtained before the feedings.

The Effect on the Urobilin Output of Bile Feedings.

In our experiments with vigorous dogs losing the bile as result of intubation urobilin was never found in this secretion save when there had been opportunity for bile pigment to reach the intestine in some way. There are three conceivable sources for the urobilin present under these latter circumstances. It may be formed as such by the liver and secreted into the bile, it may arise in other tissues and be brought to the liver in the blood, or it may occur in consequence of a reabsorption of urobilin from the intestine with its removal from the blood and secretion into the bile by the liver. The disappearance of the pigment from both bile and stool when bile is diverted from the intestine furnishes strong support for the latter explanation which is convincingly demonstrated to be the true one by experiments in which urobilin reappeared in the bile of completely intubated bile fistula animals, fed urobilin-free bile by stomach tube.
Eight dogs with intubated common duct, all with bile, urine, and feces free from urobilin, were fed by gavage sterile urobilin-free dog bile. At times the material came from the animals themselves and again from others. In every instance of such feeding the bile collected during the next 24 hours contained urobilin in greater or less amount. When the feedings were repeated for many days this pigment continued to appear throughout the period, and, persisted in the bile for 24 or 48 hours after they had been stopped. Text-fig. 6 gives the data for two such animals.

Dog A, 9 kilos, in excellent condition, was yielding, on the 38th day after intubation of the common duct, sterile bile containing no urobilin.
Day after day the bilirubin output had been uniform, on the 36th to 38th days, 55.1, 54.3, and 59.6 mg., respectively, of this pigment being eliminated in the bile. Now 128 cc. of urobilin-free bile from another dog, containing 100 mg. of bilirubin was given by mouth. The bile of the next 24 hours contained 30 mg. of urobilin and the bilirubin had increased from 59.6 to 81 mg. The following day, with no further bile feedings, a smaller amount of urobilin was eliminated, 2.7 mg. but more bilirubin, 85.3 mg. A day later a rapid fall to the normal bilirubin level took place with but a trace of urobilin present, and none 24 hours later.

Dog B, 154 kilos, in excellent condition after intubation of the common duct and on the diet of 1,050 gm. of bread, wet with milk and 450 gm. lean meat, yielded sterile bile, urobilin-free and of uniform bilirubin content. The stools and urine were likewise urobilin-free. On the 33rd and 34th days of the experiment, respectively, the bile contained 73.3 and 71.0 mg. of bile pigment. At this time, 34 days after operation, 104 cc. of sterile urobilin-free bile containing in all only 50.0 mg. of bilirubin was given by mouth. The hepatic bile of the next 24 hours showed 9.0 mg. of urobilin and 85.3 mg. of bilirubin, an increase in the latter pigment of 14.3 mg. Urobilin was now present in the feces in great amounts. A second bile portion containing 100.0 mg. of bilirubin and no urobilin was then fed; and the bile of the next 24 hours contained 51.0 mg. of urobilin and 102.8 mg. of bilirubin. In the stool the preoperative amount of urobilin was found and, in the urine a small quantity. Text-fig. 6 shows that after a further smaller feeding of urobilin-free bile, containing 50.0 mg. of bilirubin, 36.0 mg. of urobilin was eliminated in the hepatic bile of the next day, with 98.4 mg. of bilirubin. Bile feedings were then discontinued. Within 48 hours the bilirubin output had fallen to the usual level and the urobilin had disappeared.

In all, twelve bile feeding experiments were made upon separate occasions to nine of the dogs with common duct intubation, all of which had been yielding bile free from urobilin. In some instances a single feeding was given, in others several on successive days. In every case urobilin appeared thereafter in the fistula bile. Bile fed by mouth is subjected to gastric digestion and may be changed in some manner thereby. To avoid this difficulty as affecting the observations, a type of intubation was devised whereby, at will, all of the animal's bile could be collected or allowed to flow into the intestine through the papilla of Vater.

**Urobilin as Affected by Intermittent Bile Loss.**

Modifications of the ordinary bile fistula have often been attempted. As early as 186826 and 187027 Schiff described a cannulation of the

27 Schiff, M., *Arch. ges. Physiol.*, 1870, iii, 598.
gall bladder which permitted of the collection of bile from that organ at will. Since the secretion had access to the ducts as well as to the gall bladder, the quantity of bile obtained depended upon the functioning of the sphincter of Oddi for bile flowed into the intestine when this relaxed. It was assumed by Schiff that in the digestive interim between feedings, bile was retained in the duct system and could be collected from the gall bladder. He termed this an "amphibilous" fistula.

For us it was essential to obtain whenever desired, a complete bile deprivation or a passage of the secretion to the intestine. The type of intubation eventually adopted whereby the bile may flow either into a collection bag or into the intestine, but not into both, we will term an "alercursive intubation."

"Alercursive Intubation."

The intubation is illustrated in Text-fig. 7. The common duct was joined to two cannulas 1, draining from the liver and 2 directed toward the intestine, each with a soft rubber connection (A, E). To these latter, tubes of firmer rubber (X, Y) were jointed which led outside the body through punctured orifices, and ultimately to the collecting balloon. The bile could either be collected, by clamping the tubing at the point F, or be shunted back to the lower segment of the common duct by clamping at G. Under this latter arrangement the secretion simply made the passage of the tubing and reentered the common duct at a point not over 1 cm. from that at which it had left it. The sphincter of Oddi was not affected by this procedure, for the lower cannula was placed 1 ½ to 2 cm. above it.

This type of intubation proved highly successful, infection of the bile occurring seldom, despite the foreign system through which the secretion flowed. During the period of collection to the balloon, that is to say for several days often, bile remained stagnant within the efferent tubing and the lower portion of the common duct. In spite of this no ascending infection of the bile or tubing occurred. Evidently the physiological mechanism to prevent the passage of bacteria above the sphincter of Oddi is highly effective.

Nine dogs intubated successfully were used. During the first days after laparotomy, when extravasated blood was being absorbed
Diagram of the "altercursive intubation."

Text-fig. 7. The cannula I was placed in the common duct well below the entrance of the duct from the caudate and right lateral lobes. The bile flowed out the afferent tube A to a glass T-tube which led both to the collecting balloon B and to the efferent tube E. By clamping at F or G the bile could be shunted at will to the intestine or to the balloon. All collections were made under aseptic conditions as described in a previous paper from this laboratory. 2

A, afferent soft rubber tube; E, efferent soft rubber tube; X, afferent hard rubber tube; Y, efferent hard rubber tube; C, common duct; D, duodenum; F, G, clamps; T, T-tube; B, collecting bag.
bile was allowed to flow uninterruptedly, through the tubing into the intestine. The fecal urobilin after the usual postoperative rise, soon fell to the preoperative level. Later, for a number of days, the return flow of bile to the intestine was prevented and all of the secretion was collected into the balloon, just as in a common duct intubation animal. Outspoken urobilin changes took place as a result of these procedures. The typical findings as illustrated in a single animal are depicted in Text-fig. 8. They may be summarized as follows, for the group:

**Text-FIG. 8.** The urobilin changes caused by intermittent diversion of the bile stream from the intestine.

Text-fig. 8. For 3 days after the operation for "altercursive intubation" the bile flowed into the intestine. The stream was then diverted to the collecting balloon. Urobilin was present in the bile collected during the first 24 hours but not thereafter. After the animal had been losing bile for 8 days urobilin could no longer be demonstrated in the stool. On the 11th day of intubation the bile was again allowed to flow into the intestine. Practically at once urobilin appeared in the feces. The further observations of like sort have been charted. Each time the bile stream was diverted or again directed to the intestine the sequence of urobilin changes was the same.
During the first period of the intubation no bile was obtained for study, of course. That first collected when the secretion was diverted to the balloon contained urobilin. Thereafter its amount rapidly decreased though some was usually to be found for 1 or 2 days. Later none was ever present. The urobilin of the feces disappeared at about the same time as that of the bile and in both respects the animals came to resemble dogs deprived of the total bile—which indeed they were. After nearly a week the bile was allowed to flow once more to the intestine. In 1 or 2 days urobilin reappeared in the stool and continued to be present there in normal amounts. When, later, the total bile was again taken into the balloon and excluded from the intestine the same sequence of events occurred. The intermittent diversion of the bile was repeated many times in each instance, always with the same, highly characteristic result.

In passing it is of interest to note that when bile is taken daily in the collecting balloon, following a period in which it has been allowed to flow into the intestine, the amount of it, and of bilirubin as well, is at first relatively large compared with that obtained some days later. The decrease is gradual and ordinarily a constant level is reached after about 6 or 8 days of bile deprivation, a point which is not shown in the chart. The significance of this finding for the better understanding of the physiology of bile volume and bile pigment output, is an obvious one, to be taken up in detail in a subsequent paper.

Urobilin Is not Directly Derived from Bleeding into the Bowel or Tissues.

From these experiments it would seem that the presence of urobilin in the body is directly dependent upon the entrance of bile or bile pigments into the intestine. But may it not sometimes be derived from free hemoglobin especially when present in the gastrointestinal tract? Some investigators have assumed that blood pigment becomes changed to urobilin in the intestine; and workers supporting the histogenous hypothesis of urobilin origin consider it possible for various cells of the body to convert the hemoglobin liberated in interstitial hemorrhage, to this pigment directly, or so to change it that a urobilinuria results from absorption of the products.

In our experiments it was frequently noticed that the stools of animals intubated for the collection of total bile, though acholic and urobin-free, were often tinged with blood and gave a positive guaiac test, at times when the exceedingly sensitive test for urobin was negative. This was observed at times in six or seven out of fourteen dogs studied. At autopsy the affected animals showed slight hemorrhagic injection of portions of the intestinal mucosa.

To study the relation of the presence of hemoglobin in the intestine to urobin in feces and bile, two dogs with the common duct intubated and two others which were being deprived of their bile after "altercursive intubation," all yielding urobin-free bile and stools, were fed dog and ox blood in varying amounts, 25 to 100 cc. for 4 days. In the cases of dogs with the "altercursive intubation," the blood was injected directly into the duodenum through the efferent tube or fed by mouth on 4 successive days. The stools of these animals soon became black and tarry, and gave exceedingly strong guaiac tests but did not at any time contain urobin. The bile too remained urobin-free and the 24 hour bilirubin output in it remained unchanged throughout the experiment.

In the past authors have reported increases in the urobin of the bile or the appearance of urobinuria following interstitial, and even intracranial hemorrhage. The increase in the pigment has been taken to indicate a formation of urobin directly in those tissues, its passage into the blood and its secretion by liver and kidneys into the bile and urine.

Experiments to be detailed in full later, show clearly that no urobin is to be found under conditions involving blood destruction when all bile pigment is excluded from the intestine. Briefly, in animals intubated for the collection of total bile, marked hemolysis was produced by the introduction of distilled water, by withdrawing 200 to 300 cc. of blood, laking it with water, and injecting the centrifugated hemolyzed fluid intravenously, and by feedings of toluylenediamine. In three other instances large hematomas were produced. The animals used were receiving no bile pigment in the intestinal tract, the bile, feces, and urine were urobin-free, and the bilirubin content of the

 bile was uniform from day to day. After the hemolysis and interstitial hemorrhage no urobilin was found at any time in the excreta of any of these dogs. Enormous increases in the bilirubin elimination of the bile gave proof of the reaction of the liver to the masses of hemoglobin liberated in the body by both procedures. At times the hemolysis was sufficiently severe to cause pronounced hemoglobinuria but no traces of urobilin appeared in any of the excreta.

When similar experiments were done upon animals intubated for the collection of part of the bile only, so that the greater part of the bilirubin still reached the intestine, the large increases in the bilirubin content of the bile after the liberation of hemoglobin, were followed by, or often synchronous with, increases in the urobilin of bile and stool and at times a urobilinuria occurred. All these changes are plainly attributable to the increased amounts of bilirubin entering the intestine, there to be converted to urobilin.

In this paper it has been our intention to consider chiefly the normal physiology of urobilin. The findings just mentioned, relative to the effects of hemorrhage and blood destruction upon the pigment, will be reported more fully later.

DISCUSSION.

These experiments have yielded answers to a number of questions of prime importance in urobilin physiology. Together they prove that the existence of urobilin in the stool, and in the bile as well, under normal conditions depends on a delivery of the bile itself, or, to speak more precisely, of bilirubin to the intestine.

The earlier work of others has established the fact that urobilin can be obtained from bilirubin, in vitro, by reduction.\textsuperscript{10,11} That this change takes place in the intestine as well, by the action of bacteria, has also long been known. In recent years\textsuperscript{12} experiments upon the method whereby this ordinarily comes about in man have shown that certain of the intestinal flora, especially Bacillus putrefaciens and other bacteria possess this transforming ability.

The findings reported in the present paper point directly to an absorption of urobilin from the intestine and its secretion into the bile by the liver. An enterohepatic circulation of bile pigment has been demonstrated by previous work from this laboratory.\textsuperscript{4}
SUMMARY.

Methods have been developed whereby animals can be totally, partially, and intermittently deprived of their bile, without infection of this secretion or of the duct system. In all the instances considered the bile remained sterile throughout the period of the experiment. With this aid we have studied urobilin physiology.

We have been able to show that the normal presence of urobilin in the bile and feces of dogs depends on the passage of bile pigment to the intestine, either through the normal channels, or by abnormal ones, as when it is fed by mouth. Complete loss of the bile from the body resulted in the total disappearance of urobilin and urobilinogen from the bile, feces, and urine. Rarely very faint traces remained in the feces, the origin of which has been discussed. Partial loss of the bile resulted in a corresponding reduction in the urobilin of the dejecta.

Feedings of sterile urobilin-free dog bile to intubated dogs losing all of their bile and having no urobilin in it, or in feces or urine, were followed by the appearance of the pigment in the hepatic bile secreted shortly thereafter. When the feedings were stopped the urobilin soon disappeared.

Total obstruction of the bile flow caused disappearance of the urobilin of the bile and stool. Later as the animals became heavily jaundiced the pigment appeared again in very small quantity in the feces. Autopsy at this time showed that the intestinal mucosa was deeply tinted with bilirubin some of which undoubtedly had passed into the lumen of the bowel and had there been changed to urobilin.

Employment of the "altercursive intubation," by which an intermittent diversion of the bile stream of animals from the intestine to a collecting apparatus could be effected, showed that while bile pigment still reached the intestine urobilin was present in the bile secreted by the liver but that almost at once after the bile had been diverted from the gut urobilin disappeared from it. In relation to this finding it was noticed that in animals from which merely a small fraction of the bile was collected, that from a single liver lobe, while the greater portion reached the intestine there was most urobilin in the bile at times when most bilirubin was entering the intestine.