STUDIES ON UROBILIN PHYSIOLOGY AND PATHOLOGY.

IV. UROBILIN AND THE DAMAGED LIVER.

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Following the discovery of urobilin in 1868, recognition of it in the urine of patients aroused great interest. Urobilinuria was observed in a wide variety of clinical conditions, and many studies were reported of its pathological significance and diagnostic value. It was found present in noteworthy quantity in two general types of disease: those characterized by an increased destruction of red cells, and those involving actual damage to the liver parenchyma or occlusion of the bile ducts. Of great importance for the clinical interpretation of such a finding is the determination of what is responsible therefor within the organism. Despite the variety of hypotheses advanced concerning the physiology of urobilin in the normal and diseased animal, relatively little that is positive has been ascertained.

Earlier papers of this series have dealt with the origin and fate of urobilin under more or less normal conditions. The present communication will be concerned with the happenings when there has been direct injury to the hepatic cells or obstruction to the ducts.

We have shown that, under normal conditions, urobilin is formed in the dog only within the intestinal tract. Drainage of the entire bile quantity under sterile conditions, or retention of it by common duct occlusion results in the

prompt disappearance of the pigment from the excreta, and from the bile. The animal becomes in fact urobin-free. But whenever bilirubin enters the intestine, urobin is rapidly formed therefrom, part of which escapes in the stool, while another part is absorbed from the gut, either as such or in the form of related pigments, and makes its appearance in the bile. This secondary resorption and resecretion play an important part in the normal fate of the pigment, and, as will now be shown, in the findings when the liver has been injured.

Methods.

The present experiments were performed on the same series of dogs used in studies already reported. In some of the animals the common duct had been intubated according to the method of Rous and McMaster; in others a branch of the hepatic duct only was cannulated, but according to the same method, and the bile drained in this way from one or two liver lobes, whilst the remainder, from about two-thirds of the liver as a rule, reached the duodenum along the normal path. The preparation of the animals as well as the methods used for the daily collection and pigment estimation on urine, stool, and bile have been previously described. The influences of two general types of hepatic disorder were studied: the one brought about by the administration of toxic substances, the other by the occlusion of bile ducts. In judging the degree of liver injury in the animals which survived the principal criteria have been the nature and extent of the biliary disturbances as disclosed by the jaundice, or by changes in the bile and urine collected, or by both.

Hepatic injury was produced by the administration of chloroform, phosphorus, amyl alcohol, and toluenediamine. In general the amounts given were not large enough to cause a serious general intoxication. Aside from marked jaundice, there was, in many instances, no striking bodily disturbance. In the case of amyl alcohol, however, even moderate doses (2 cc. per kilo given with ethyl alcohol by gavage) resulted in a marked toxemia in the absence of jaundice. Use of it was discontinued for this reason. Yellow phosphorus was given by stomach tube in doses averaging 13 mg. per kilo dissolved in olive oil. Toluenediamine was given to the amount of 12 mg. per kilo, in aqueous solution, by gavage. It caused hemolysis and frequently a marked depression in the bile output and alterations in its constituents, 1 to 3 days after administration.

That chloroform has a specific damaging effect on the hepatic parenchyma is

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The dietary factor in the injury is an important one. In our animals, on uniform standard diets, the anesthetic was given always at the same time, 5 hours after feeding. As a rule, 75 cc. given by inhalation over a period of 45 minutes to a 10 kilo dog, causes marked changes in the bile, changes already described in a paper from this laboratory by Drury and Rous. The secretion is lessened in amount and in content of biliary constituents to such an extent, sometimes, that the fluid elaborated is a "white bile." The general condition of the animal is often good, despite the disturbance, but the margin of safety in such instances is narrow, at least in intubated animals losing all of the bile. In some animals the respirations would cease permanently at almost the first whiff of chloroform. It was found best, therefore, to bring the animal under anesthesia with ether, changing immediately thereafter to chloroform.

When liver disturbance was to be produced by biliary obstruction, the ducts were severed between ligatures under ether anesthesia, or the outlet tube of intubated animals was clamped. Not a few cases of biliary obstruction were studied in which, some time after intubation, the cannula had become plugged with mucus or stone, or the collecting tube had become bent sharply upon itself, thus effecting either a partial or a total obstruction, according as the tube was draining the entire liver or only a portion of it.

The findings after direct hepatic injury with the toxic substances just discussed, and those after biliary obstruction, will be examined separately. The data have been further subdivided into those obtained from dogs draining all of the bile, and those losing but a fraction thereof, since in the one case the body fluids and excreta are urobilin-free, while in the other bile and stool both contain the pigment, just as do intact animals.

Only animals yielding sterile bile were used in these experiments. The daily bile specimens were tested regularly for infection, every day or two, by incubating portions on agar. In addition, the stained sediment from centrifuged specimens was occasionally examined. When bacteria were found the dog was discarded for the purposes of this work.

Absence of Urobilinuria during Liver Injury under Conditions of Total Bile Loss.

The complete disappearance of urobilin from the bile and feces, 2 to 4 days after intubation of the common duct for bile collection,

has been reported in a previous paper. Under these circumstances of continued bile loss the bile and feces were found to remain urobilin-free for as long as 4½ months, the longest period of observation. Ten instances have now been studied of hepatic injury in animals rendered urobilin-free in this way. The results as regards urobilin were uniform in all, regardless of the injurious agent employed, or

![Graph](https://via.placeholder.com/150)

**Text-Fig. 1.** Effects of liver injury by chloroform in a dog losing the total bile.

The severity of the hepatic damage is indicated by the drop in the amount and bilirubin content of the bile, which, on the 3rd day after anesthesia, had become almost colorless. At the time of greatest disturbance there was a moderate bilirubinuria. No urobilin appeared in the bile or stool (see Protocol I).

the degree of liver damage it induced. In no instance did the pigment reappear in bile or stool despite the frequently intense icterus; and there was never any urobilinuria. Rarely, small amounts of the pigment appeared in the stool, a finding which is to be accounted for by the excretion from the jaundiced body fluids of small amounts of bilirubin into the large bowel.
Specimen Protocols.

I. Female dog, weight 9 3/4 kilos (see Text-fig. 1).

8 days prior to the experiment the common duct was intubated under ether. Recovery was uneventful, and the stools and bile became urobilin-free on the 2nd day. Chloroform was then given by inhalation for 50 minutes, 40 cc. being used. The 24 hour output of bilirubin for the period immediately preceding the anesthesia was 68 mg. The specimen of the next 24 hours contained only 34 mg.; and during

Text-Fig. 2. Effects of liver injury with phosphorus in a dog losing the total bile.

The hepatic disturbance, as indicated by the changes in the bile, was moderate, but bilirubinuria and tissue icterus developed. No urobilin was found at any time (see Protocol II).

The following 24 hours the liver excreted but 6.6 mg. On the succeeding day the collecting balloon held only 12 1/2 cc. of "white bile," and there was definite tissue icterus with moderate bilirubinuria. The stools remained clay-colored throughout the observations.

Now bile secretion was rapidly resumed. Within another week icterus had disappeared, the urine had become free of bilirubin, and the bile had the ordinary
character of the secretion elaborated some days after intubation. It was never
again so rich in bilirubin as shortly after operation, for reasons that have been
given elsewhere.\textsuperscript{13},\textsuperscript{14}

The urine at no time showed the slightest trace of urobilin, the stool remained
free from it, and none was present in the bile even at the height of the liver injury.

II. Male dog, weight 15 1/4 kilos (see Text-fig. 2).

Phosphorus was given, 200 mg. in olive oil, by stomach tube, 6 days after
intubation, under ether, of the common duct. The animal had become urobilin-
free on the 4th day after operation, and remained so. The bilirubin content
of the bile prior to administration of the phosphorus was approximately the same
from day to day, about 110 mg., during each 24 hours. Thereafter no serious
symptoms developed, and the biliary disturbance was only moderate, reaching its
height in 3 days, when the 24 hour bile specimen contained but 76 mg. of bilirubin.
There were scleral icterus and a mild bilirubinuria. The return to "normal" was
complete in 9 days.

Throughout the period of the hepatic disturbance the bile and urine remained
free of urobilin. The stool continued to show a trace of the pigment, never
exceeding 4 mg. per day.

\textbf{Urobilinuria Following Liver Injury under Conditions of Partial Bile
Loss.}

Eight instances of hepatic damage were studied in animals losing
but a fraction of the total bile as the result of the intubation of a small
hepatic duct. The effects of the injury on the bile could be followed
directly in the daily sample obtained. Under the experimental
conditions, with most of the bile still flowing to the intestine, urobilin
was a regular constituent both of bile and stool. The liver injury was
induced by chloroform, phosphorus, or toluylenediamine. Urobili-

\textbf{Specimen Protocols.}

III. Male dog, weight 12 1/4 kilos (see Text-fig. 3).

Under ether anesthesia the duct to the left lateral lobe was intubated, and 31
per cent of the liver drained, as the eventual necropsy showed. 6 days after
the operation the bile had come to contain approximately constant amounts of
urobilin and bilirubin from day to day, and the urine was urobilin-free. At this

time 200 mg. of phosphorus, dissolved in olive oil, was given by stomach tube. 3 days later the urobilin in the fraction of the bile studied had fallen to half its previous amount, and the output of bilirubin from 30 to 13 mg. in 24 hours.

Text-Fig. 3. Effects of fatal hepatic injury with phosphorus in a dog losing but a fraction of the total bile.

The urine was urobilin-free prior to the giving of phosphorus, but soon afterwards showed the pigment in increasing amounts. There were several days during which no urine was voided spontaneously. Urobilin quantitation was not attempted during this period, but tests were made on small, catheterized specimens obtained from time to time. Spontaneous voiding was resumed on the 12th day, by which time the liver was secreting only "white bile," devoid of bilirubin. The urine now became urobilin-free, just as when all bile is diverted from the intestine. With the bile suppression bilirubinuria developed and became intense (see Protocol III).

Catheterized urine specimens, procured on each of the succeeding 3 days, revealed the existence of an intense urobilinuria, whereas previously the pigment had been absent.

The biliary disturbance rapidly grew marked, eventuating in bile suppression;
the stools became acholic, and intense tissue icterus developed. Now the urine voided contained no urobilin, only bilirubin, and the disappearance of the urobilin was practically synchronous with the development of the bile suppression. The animal died next day, the 8th after the phosphorus had been given. Autopsy revealed a typical phosphorus liver, large, pale, and fatty. The bile ducts were

**Text-Fig. 4.** Effects of moderate liver injury from chloroform in a dog losing but a fraction of the total bile.

Evidence of the hepatic damage is to be found in the drop in the bilirubin content of the bile to half the ordinary amount. Since the damage never resulted in the development of "white bile," one may assume that the pigment was at all times reaching the duodenum (see Protocol IV). Urobilinuria developed promptly and persisted for several days. It may be recalled (Text-fig. 3, as also Text-fig. 5) that when there is complete bile suppression the urine, containing urobilin so long as bile continues to be secreted after liver damage develops, becomes urobilin-free.
practically empty, only the finer radicals containing a little dark, viscid bile. No signs of infection were anywhere visible, and culture from the liver on agar and in broth proved sterile.

IV. Male dog, weight 10 3/4 kilos (see Text-fig. 4).

Intubation of the duct from the left lateral lobe was performed under ether anesthesia, and thus we were enabled to collect daily the bile from 27 per cent of the liver. The secretion contained a uniform amount of urobilin and bilirubin from day to day. After 2 weeks, 60 cc. of chloroform was given by inhalation, the administration of the anesthetic lasting 50 minutes. 3 days later the 24 hour output of bilirubin in the bile sample had dropped from 11 to 4 mg., and biliary urobilin had likewise greatly diminished. Recovery, with reestablishment of a more normal biliary secretion, now rapidly took place, being to all appearances complete within another week.

The urine, previously urobilin-free, showed this pigment the day after the administration of chloroform, and there was pronounced urobilinuria for 5 days more, disappearing only on the 8th day. Bilirubinuria on the contrary was moderate and lasted but 4 days.

V. Male dog, weight 10 3/4 kilos (see Text-fig. 5).

30 days after the intubation, under ether, of the duct from the left lateral lobe, draining 27 per cent of the liver, toluylenediamine was given by gavage, 120 mg. in aqueous solution. 2 days later the bilirubin content of the bile had dropped to one-fifth of the previous quantity and, in the succeeding 24 hours, only 4 cc. of "white bile" assembled in the collecting balloon. Resumption of bile secretion soon followed.

Marked urobilinuria was found the day after the toluylenediamine had been given, and it continued for 2 more days. With the suppression of secretion, as indicated by the finding of "white bile," and the consequent failure of bile pigment to reach the intestine, it diminished and disappeared, only to reappear again upon resumption of the bile flow.

**Absence of Urobilinuria during Total Biliary Obstruction.**

When all of the bile is diverted from the intestine, and lost to the body, as after intubation of the common duct, urobilin disappears from bile, stool, and urine. But what will happen when the bile constituents are prevented from reaching the intestine and accumulate within the body, as happens during total biliary obstruction? Under such circumstances there is not only a general disturbance of the organism, but a progressively increasing injury to the liver, with secondary cirrhosis. Nevertheless, one finds but a transient initial

urobilinuria referable to the presence of the pigment in the intestine at the time when the obstruction is produced by operation. Thereafter the urine is free from the pigment, while the slight traces of it appearing in the stool have an obvious source in the bilirubin passing into the gut through the jaundiced mucosa. Where obstruction is induced in an animal previously rendered urobilin-free by intubation of the common duct, the pigment fails to appear at all, despite the development of marked bilirubinuria.

Text-Fig. 5. Effects of severe liver injury from toluylenediamine in a dog losing only a fraction of the total bile.
Practically complete biliary suppression developed in this instance. The initial urobilinuria, synchronous in appearance with the beginning of the liver derangement, disappeared when bile no longer reached the intestine, only to reappear when bile flow was resumed (see Protocol V).
VI. Male dog, weight 10 3/4 kilos (see Text-fig. 6).

Under ether anesthesia, the common duct was cut between ligatures, the cystic duct treated similarly, and the gall bladder evacuated. On the next day the urine contained bilirubin, and 3 days later tissue icterus developed, the stool becoming acholic at the same time, but containing traces of urobilin not exceeding 4 mg. per 24 hours.

Text-Fig. 6. Transient urobilinuria following the operation to produce total biliary obstruction.

The disappearance of the urobilinuria was prompt, and after the 3rd day none was found despite the persisting obstruction, intense jaundice, and marked bilirubinuria (see Protocol VI).

The urine was free from urobilin prior to the operation, but in the succeeding 24 hour period it contained 40 mg., during the second 24 hours, 11 mg., and none at any time thereafter. On the 6th day after operation the animal was noted to have developed a respiratory infection, and it was killed with chloroform. The usual picture of biliary obstruction was found: an enlarged, jaundiced liver and dilated, tortuous ducts filled with "white bile." Cultures from this fluid, as well as from the liver itself, in broth and agar, proved sterile.
VII. Male dog, weight 18 kilos (see Text-fig. 7).

The common duct had been intubated, under ether, 1 month prior to the experiment. The bile collected daily was sterile, showed no urobilin, and contained, on the average, 85 mg. of bilirubin in each 24 hour specimen. Urine and feces were urobilin-free.

Text-Fig. 7. Total biliary obstruction by occlusion of the outlet tube after common duct intubation.

Moderate bilirubinuria developed during the 2 day period of obstruction but no urobilinuria. The stools remained acholic and urobilin-free. Following the relief of the obstruction the bilirubin output of the bile was at first very large, but the secretion contained no urobilin (see Protocol VII).

On the 26th day of intubation total obstruction was produced by clamping the outlet tube for a period of 48 hours. Bilirubinuria developed within 24 hours after the onset of the obstruction, disappearing some 72 hours after it had been released. Urobilinuria failed to develop at any time, and the stools remained pigment-free. The bile, on resumption of flow, contained large amounts of bilirubin, 230 mg. during 24 hours, but the amount gradually fell off to the pre-obstructive level. Urobilin did not appear in it.
Development of Urobilinuria Following Local Biliary Obstruction.

The effects of obstructing bile flow from a portion of the liver only were next studied. Under such circumstances much bilirubin continues to reach the intestine and urobilin to be formed from it. The question whether the local obstruction will result in urobilinuria is an important one. We know that bilirubinuria fails to develop until the ducts from more than three-fourths of the parenchyma have been occluded.\footnote{McMaster, P. D., and Rous, P., \textit{J. Exp. Med.}, 1921, xxxiii, 731.} The present observations show that urobilin appears in the urine when obstruction of far less degree has been produced.

The obstruction was effected by clamping the outlet tube in dogs draining but a sample of the liver secretion. There were some instances as well in which stone formation or a twist in the tubing dammed back the bile. Ordinarily the obstruction involved about 30 per cent of the hepatic tissue. The finding in all cases was a moderate urobilinuria, without ever any bilirubinuria. The discrepancy cannot be laid to the existence of a higher kidney threshold for bilirubin than for urobilin, since in the dog there is practically no threshold for the first mentioned pigment.\footnote{McMaster, P. D., and Rous, P., \textit{J. Exp. Med.}, 1921, xxxiii, 731.} It can only be due to a failure of the unobstructed portion of the hepatic tissue to remove urobilin from the blood as completely as it does bilirubin.

Specimen Protocols.

VIII. Male dog, weight 11 kilos (see Text-fig. 8).
3 weeks prior to the obstruction, a duct had been intubated, under ether, draining 31 per cent of the liver, as autopsy later showed. The dog remained in excellent condition and continued to have urobilin in bile and stool.

The outlet tube was clamped during 48 hours, and then released. The urine previously free of urobilin was found to contain 10 mg. during this period, but never showed bilirubin.

IX. Male dog, weight 19 1/2 kilos (see Text-fig. 9).
An instance of exceptional interest will be recorded, in which the obstruction occurred shortly after a hemoglobin injection.

Under ether, the duct from the left lateral liver lobe was cannulated, and the bile from this approximate third of the hepatic tissue collected, while the secretion from elsewhere flowed uninterruptedly to the intestine. Urobilin continued present in the bile and stool. On the 6th day of intubation 150 cc. of blood was...
removed from a vein, laked with 200 cc. of distilled water, and the whole reinjected intravenously. There followed the expected tremendous increase in the bilirubin output. Bilirubinuria and hemoglobinuria were present for 2 days. For the sake of simplicity, these occurrences are not represented on the chart. After 3 further days, the bile from the intubated third of the liver was noted to contain very little bilirubin and urobilin, and to be much diminished in amount, indicating that obstruction was taking place. This obstruction was not a late result of the blood injection, or one involving the whole liver, but local and due to other causes,

![Text-Fig. 8. Urobilinuria following obstruction of about one-third of the liver.](chart)

The outlet tube draining the liver third was clamped for 2 days. Moderate urobilinuria resulted but no bilirubinuria (see Protocol VIII).

as the autopsy showed. In 3 more days it was complete, only 4 cc. of “white bile” being collected in 24 hours. There was no jaundice.

The chart depicts the changes in urobilin. Urobilinuria, absent during the initial period of derangement after the intravenous injection of hemoglobin, came on as the obstruction developed, only to disappear later. Bilirubinuria was entirely absent. The animal was killed to determine the cause of the obstruction, and the cannula found filled with deeply pigmented stones. The ducts drained by the cannula were dilated and held glairy “white bile.” Cultures from this latter proved sterile.
The fact is well attested\textsuperscript{15,16} that after obstruction of a duct from a portion of the liver the remaining parenchyma rapidly takes over the function of the occluded portion, and this latter gradually atrophies and disappears. The disappearance of the urobilinuria, despite the persisting obstruction, in the present instance, may, with good reason, be laid to a functional accommodation.

\textbf{Text-Fig. 9.} Urobilinuria following obstruction of one-third of the liver.

3 days prior to the development of the obstruction hemolyzed blood had been injected into a vein. No urobilinuria followed, yet it promptly appeared when the cannula draining the duct from a portion of the liver became plugged with stones. No bilirubinuria occurred at any time during the obstruction. Bilirubinuria and hemoglobinuria were found on the 7th and 8th days immediately after the blood injection (see Protocol IX).

\textit{Bile Feedings Induce Urobilinuria When the Liver Has Been Damaged.}

From the foregoing observations, it is evident that urobilinuria does not occur after liver injury when all bile pigment is excluded.
from the intestine, as, for example, when the common duct is drained or obstructed. But what will happen when the animals are fed bile by gavage? The test has been made on a human being by Müller, who found that he could precipitate urobilinuria in a jaundiced patient with common duct obstruction merely by feeding urobilin-free pig bile by mouth.

Ordinarily, the return to the animal of bile lost through an intubated

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**Text-Fig. 10.** Development of urobilinuria after bile feedings under conditions of total biliary obstruction.

The animal had an intubated common duct. The feedings (B) were begun before biliary obstruction was produced. They brought about the appearance of urobilin in the bile and stool, but not in the urine until after biliary obstruction had been effected by clamping the outlet tube from the liver. The pronounced urobilinuria (600 mg.) which then developed disappeared almost at once with discontinuance of the feedings and relief of the obstruction. Bilirubinuria was mild at most. Compare with Text-fig. 6 (Protocol VI),—the same experiment minus the bile feedings,—in which only bilirubinuria developed (see Protocol X).

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17 Müller, Fr., Verhandl. Cong. inn. Med., 1892, xi, 118.
common duct brings about the reappearance of urobilin in the bile and feces, as reported in a preceding paper. But in the absence of hepatic damage urobilinuria is not induced. In the experiments now to be described, in which the liver had been injured, urobilinuria regularly followed the feedings.

Text-Fig. 11. Urobilinuria following a bile feeding to a dog losing the total bile and with an injured liver.

The injury by chloroform did not of itself cause urobilin to appear in bile or urine (see also Text-fig. 1), but large amounts of the pigment appeared temporarily in both secretions after bile had been administered by gavage (B). The gavage was done at a time when the liver injury was at its height (see Protocol XI).

Specimen Protocols.

X. Female dog, weight 11 kilos (see Text-fig. 10).

Under ether anesthesia, intubation of the common duct had been performed. The bile collected afterwards remained sterile, and both stools and bile were urobilina-free. 9 weeks after operation urobilin-free bile was fed daily by stomach tube for a period of 10 days. The amounts given contained bilirubin in approxi-
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mately the quantity lost by the intubation. Soon after feedings were begun, urobilin reappeared in the bile and stool; there was, however, no urobilinuria. Beginning with the 9th day of the feedings, the outlet tube from the liver was clamped for 48 hours. A most striking urobilinuria ensued, 600 mg. of the pig-

Text-fig. 12. Marked urobilinuria following the administration of urobilin-free bile to a dog with total biliary obstruction.

At operation the common duct was ligated and cut and the gall bladder removed. Urobilin disappeared from the stool and urine in 3 days, but promptly reappeared in the latter within 18 hours after the administration of urobilin-free bile by gavage. There was no change in the bilirubinuria (see Protocol XII).

ment being excreted through the kidneys in 24 hours. There was only mild bilirubinuria. On release of the obstruction and resumption of bile flow, both pigments disappeared from the urine.

XI. Male dog, weight 18 kilos (see Text-fig. 11).

The common duct of this animal had been intubated under ether, 6 weeks
before. At the time of the experiment, urine, stools, and bile were urobilin-free, and uniform amounts of bilirubin were being excreted in the bile from day to day. A brief anesthetization with chloroform was carried out (50 cc. during 30 minutes). The biliary disturbance that followed was moderate. During the 2nd day after the anesthesia, the 24 hour bilirubin output dropped from 93 to 36 mg. The animal was still urobilin-free, as in other similar experiments already described (Protocols I and II). At this point, bile containing 100 mg. of bilirubin, but no urobilin, was fed by stomach tube, a dose which, as our experience shows, fails to cause urobilinuria in the absence of liver injury. In the present instance the urine voided in the next 24 hours contained 90 mg. of the pigment and the bile 45 mg.

The feeding was not repeated, and the urobilin in the secretions gradually diminished, disappearing wholly in 3 days.

XII. Male dog, weight 11 3/4 kilos (see Text-fig. 12).
Under ether, the common and cystic ducts were cut between ligatures, and the gall bladder evacuated. There followed, as always, transient urobilinuria, but soon the pigment disappeared from both urine and stool (see Protocol VI). On the 6th and 7th postoperative days, after the animal had become urobilin-free, bile which contained none of this pigment was fed by stomach tube; 100 mg. of bilirubin being administered in this way on each day. Large amounts of urobilin were avoided in the urine in the period immediately following, and only after 6 days did the urine become free of it. Small amounts appeared in the stools as well. The bilirubinuria of the persisting biliary obstruction appeared to be uninfluenced by the feedings of bile.

DISCUSSION.

Urobilin and urobilinogen will, of necessity, be considered together in this discussion of their behavior under pathological conditions. For, during our pigment quantitations, the latter is always oxidized into urobilin. In previous papers, we have shown that the normal presence of urobilin in the feces and bile depends upon the passage of bilirubin to the intestine, where it is changed to urobilin, and hence reabsorbed in part, to be taken out of circulation again by the liver and partly, at least, secreted into the bile. Complete bile deprivation, as when the common duct is intubated, results in complete disappearance of urobilin from bile and stool. Exclusion of bile from the intestine by direct closure of the common duct leads to similar results.

In the present paper we have shown that these facts hold true even under circumstances of severe liver damage, when unaccompanied by infection. Many workers suppose that the pathologically altered
liver cell can make urobilin, and that this explains the appearance of the pigment in large amounts when the liver is diseased.\textsuperscript{4,18,19} Some individuals have doubted the occurrence,\textsuperscript{4} but our observations would appear to be the first to prove that it does not take place. On the contrary, the liver loses, when injured, the normal power of taking care of the urobilin resorbed from the intestine, and thus of preventing urobilinuria. We have found that intubated dogs, losing all of their bile, remain urobilin-free after the most severe hepatic injury, as they also do during total biliary obstruction, when there is no passage into the intestines of bilirubin from the jaundiced organism (see Protocols I, II, and VII). Bile samples, also, from dogs with but a part of the liver intubated, and showing urobilin regularly in bile and stool, never contain increased amounts of urobilin as the result of hepatic injury; urobilin behaves in this respect like bilirubin, and in severe cases of injury when “white bile” is being secreted, is absent altogether from this secretion, from intestines, and from urine (see Protocols III and V). Urobilin in the bile, then, in instances of liver damage uncomplicated by infection, would appear to have the same source as in the normal animal and no other. It is not produced by the damaged but sterile liver. Whether it is produced within infected hepatic tissue will be discussed in another communication.

Urobilinuria during liver disease depends upon the formation of urobilin in the intestine, as is revealed in the evidence here reported. In our experiments it was never found even after the most severe hepatic injury in dogs losing the total bile, but appeared promptly after the feeding of an amount of urobilin-free bile far too small to cause urobilinuria in the absence of liver damage (see Protocols X, XI, and XII). In animals losing but a fraction of the bile, urobilinuria occurred promptly after liver damage (see Protocol IV) but disappeared in case there was bile suppression, with result that none reached the intestine (see Protocols III and IV).

The conception of urobilin pathology that accords with the facts observed in our experiments is that the liver, receiving urobilin or urobilinogen by resorption from the intestine into the portal blood

\textsuperscript{18} Fischler, F., Physiologie und Pathologie der Leber, Berlin, 1916, 185.

\textsuperscript{19} Whipple, G. H., \textit{The Harvey Lectures}, 1921–22, xvii, 103.
under normal circumstances, loses, when diseased, part or all of its ability to dispose of this pigment, which, instead of being removed from the blood, passes on into the general circulation and is excreted by the kidneys. The findings in clinical instances of catarrhal jaundice seem to support the conception outlined which is, of course, not new, but has been weakly supported. Urobilinuria is found only at the beginning and the end of catarrhal jaundice. The transient, early appearance of this pigment parallels our findings in complete obstruction in dogs (see Protocol VI). As the diseased condition resolves, and bilirubin is again discharged into the intestine, urobilin is again formed, absorbed, and, reaching a liver still too damaged to handle it, is passed on and appears in the urine. As the hepatic cells completely recover their ability, the urine again becomes urobilin-free.

The clinical occurrence of urobilin in instances of portal cirrhosis goes to confirm the conception outlined. Urobilinuria may occur in such cases without bilirubinuria, jaundice, or any evidence of excessive hemolysis or of biliary obstruction. The escape of portal blood directly into the general circulation by way of various anastomotic channels is an essential feature of the pathological picture of portal cirrhosis. Thus the urobilin absorbed from the intestinal tract may reach the kidneys before it does the liver, and hence appear in the urine. When there is biliary obstruction or hepatic damage in the absence of a cirrhosis some of the urobilin carried to the liver is passed on and so enters the general circulation, reaching the kidney. The mechanism of the urobilinuria is different; the result is the same.

How does urobilin behave under conditions of local biliary obstruction? It has been shown in this laboratory by McMaster and Rous that in dogs and monkeys over 80 per cent of the liver may be obstructed before bilirubinuria will result, and that in the dog more than 95 per cent of the organ must be occluded before jaundice occurs. We can now say that with urobilin the margin is far narrower. It has been shown in experiments described above that urobilinuria occurs regularly when local obstruction of one-third of the liver is induced, such experiments being easily carried out by clamping the outlet tube draining a small fraction of the total bile, while the rest goes to the intestine (see Protocols VIII and IX). The facts, as observed, cannot be explained by supposing a higher renal threshold.
for bilirubin than for urobilin, since in the dog, as already mentioned, bilirubin is, to all intents and purposes, not a threshold substance.

The importance of these relationships lies in their bearing on the interpretation to be accorded urobilinuria in the diagnosis and prognosis of liver disease. Urobilinuria is a valuable finding in that it makes manifest biliary disturbances of mild degree, perhaps not discernible in the urine in any other way. The presence of bile salts might be utilized but too little is known at present concerning its significance. Bilirubinuria occurs only when liver damage or obstruction is considerable. In such cases the finding of urobilin as well, would indicate, in the absence of liver infection, that bile is still reaching the duodenum (see Protocols III and V).

The evidence thus far assembled was obtained from the study of animals with uninfected livers, yielding sterile bile. How will the infection of the liver with urobilin-producing bacteria affect our conclusions? That one may actually produce urobilin in vitro by inoculating sterile urobilin-free bile is well known. We have been able to accomplish the same thing in vivo by introducing fecal bacteria into the biliary tract of intubated animals. Within 1 or 2 days thereafter the infected secretion is no longer dark, clear, and urobilin-free, but light brown, with a fetid odor, and it contains large amounts of urobilin. The possibility that urobilin formed in this way, in an infected biliary tract, may be resorbed therefrom and excreted in the urine will be taken up in detail in a paper to follow. It constitutes, theoretically at least, an extraintestinal origin of urobilinuria. But it must be clearly pointed out that the phenomenon, if it occurs, is extraneous, having nothing to do with liver function or with urobilin physiology as such, no matter how important it may be clinically.

The experimentally induced formation of urobilin in an infected biliary tract gives a ready explanation of certain discrepancies between our findings and those of others. Fischler always found urobilin in the bile of dogs with open common duct fistulas, and observed it to increase in amount after the administration of certain agents injurious to the liver, such as chloroform, amyl alcohol, and phosphorus. He concluded, therefore, that the diseased liver was able to produce urobilin. Analysis of the findings revealed a number of objections

to this conclusion, as Fischler himself pointed out. Our own recent work has given several obvious explanations. Not only does bile given by mouth,—and Fischler's dogs obtained bile by licking their fistula opening,—bring about the appearance of urobilin in the bile, but in fistulous tracts that are infected urobilin arises directly by a change from bilirubin.*

Whipple²¹ found urobilin regularly in the bile obtained from his open fistula animals during fasting periods, but then only. He believed it to have arisen by the action upon bilirubin of bacteria in the fistula tract. During a fast the bile flow is so sluggish that there would seem to be sufficient time for such bacterial activity to have effect. Very recently, Fischler²² has described some complicated observations in rabbits, which seem to show that the damaged liver may be able to make urobilin. The liver was injured by the production of "hypoglycemic intoxication," induced by the administration of phlorizin, often with adrenalin, to the fasting animal. Bile was excluded from the intestine by an operative occlusion of the common duct. Urobilinuria was found, especially after the animal had been rescued from the phlorizin intoxication by the use of glucose. Under the conditions, as Fischler describes them, the liver was drastically injured,—for that is a striking effect of sudden biliary obstruction in the rabbit,—while furthermore the jaundice gave opportunity for bilirubin to pass directly into the intestine from the gut wall. Moreover, no mention is made by Fischler of tests to ascertain whether the liver and bile ducts remained sterile. It is well known that bacteria frequently localize in damaged hepatic tissue.

SUMMARY.

A variety of evidence is presented, all of which supports the view that in the uninfected animal the intestinal tract is the only place of origin of urobilin, not merely under normal circumstances, but when there is biliary obstruction. Animals rendered urobilin-free by collection of all of the bile from the intubated common duct remain urobilin-free even after severe hepatic injury.

In our experiments urobilinuria was never found after liver damage except when bile pigment was present in the intestine. Thus, for example, it appeared during the first days after ligation of the common duct, but disappeared as the stools became acholic. When this had happened a small amount of urobilin-free bile, given by mouth, precipitated a prompt urobilinuria. After obstruction of the duct from one-third of the liver, mild urobilinuria was found, but no bilirubinuria. In animals intubated for the collection of a part of the bile only, while the rest flowed to the duodenum through the ordinary channels, liver injury caused urobilinuria, unless indeed it was so severe as to lead to bile suppression, when almost at once the urobilinuria ceased, though the organism became jaundiced.

The evidence here presented, when taken with that of our previous papers, clearly proves that urobilinuria is an expression of the inability of the liver cells to remove from circulation the urobilin brought by the portal stream, with result that the pigment passes on to kidney and urine. Urobilinuria occurs with a far less degree of liver injury than does bilirubinuria.

Our work has, for the most part, been carried out with animals having uninfected livers and bile passages. But the influence of cholangitis with infection has been briefly discussed in the light of some preliminary observations. The influence of infection on the place of formation of urobilin and on the occurrence of urobilinuria will form the subject of another communication.