UROBILIN: ITS CLINICAL SIGNIFICANCE *

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I. INTRODUCTION

The numerous studies made on urobilin and urobilinogen during the forty-four years since its discovery by Jaffé, while adding much to our knowledge of its chemistry and its clinical occurrence, still leave much to be desired as to methods for its estimation and as to its clinical significance. Some investigators are inclined to look on it still from the point of view of hemoglobin metabolism, while others consider it only in reference to hepatic insufficiency. Its relationship to the presence or absence of bile in the intestine has been a subject of considerable dispute, but in the main the contention of Friedrich von Müller, that it can originate only from the bacterial decomposition of bile reaching the intestine, is accepted. Nevertheless, the assertion of Fischler, that it can originate in the liver itself, seems to us probable. Whether it is then formed within the liver cell or is produced by decomposition of bilirubin in the biliary passages, it is impossible to state. A wide variability in point of view is reflected throughout the available literature. It has been our intention in entering on a study of this substance to endeavor to estimate its present value as an aid in diagnosis and prognosis, and we must frankly admit that it has been disappointing in some particulars, owing largely, perhaps, to the imperfections of the present methods of its estimation, and to its evident instability. Besides, when such variable factors as the bacterial fermentations within the intestine, the absorption from it, the activity of the liver, the elimination of bile, the eliminative power of the kidney, the rate of destruction of the red blood-corpuscles, the activity of the spleen, etc., have to be taken into account, it is not surprising that difficulty should arise in interpreting findings and in correlating the diverse views of many investigators and clinicians.

The interesting spectroscopic fluorescent and color tests for urobilin and its mother substance urobilinogen and their common occurrence in the body excretions, especially in disease, naturally stimulated much research as to their composition and origin and many clinical observations as to their significance. The Ehrlich aldehyd test, shown by

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Neubauer to be due to urobilinogen, received particular attention. The method of estimation with Citron's or other simple spectroscope of the urobilin and urobilinogen together proved with us to be fairly simple, and to lend itself readily to approximate quantitative studies based on the disappearance of the characteristic absorption bands between D and E, and B and F, with successive dilutions.

The term "urobilin" is not applied to a single body, but to a group of derivatives of blood and bile pigments containing the pyrrol nucleus. Throughout our work we have considered urobilin and its mother substance urobilinogen as practically the same, and have endeavored always to estimate them together in making clinical and experimental observations. The confusion between the two and the lack of a generally accepted theory of the origin of these substances is the cause of the present striking lack of uniformity in the interpretation of clinical and laboratory results.

The most prominent of the various theories as to the origin of urobilin are the hematogenous, the hepatogenous, the nephrogenous, the histogenic and the enterogenous. The latter is undoubtedly the most satisfactory, although it does not fully explain the origin of all urobilin within the body, for instance, that of some hepatic disorders (Fischler) and some cases of blood destruction and extravasation. In brief, these theories are as follows:

Hematogenous Theory.—This theory is that the urobilin can be, but is not necessarily, derived directly from blood pigment. It is based on the somewhat uncertain evidence of the long-known occurrence of urobilinuria in hemorrhage of the brain (von Bergmann), in blood extravasations (Kunkel), in extra-uterine pregnancy (Dick), in hemorrhagic ascites (such as the famous case of Gerhardt, in which there was also a carcinomatous closure of the ductus choledochus), after hemocytolytic processes, in sulphonal poisoning, in paroxysmal hemoglobinuria and in scurvy. While it is true that there is the possibility in many of these reported clinical observations of the existence of definite hepatic disturbance, still there is enough experimental evidence to warrant the assertion that at times urobilin can be derived directly from blood pigment without the intervention of either the liver or the intestine.

Hepatogenous Theory.—The principal tenet of this unsatisfactory theory is that there is failure on the part of the liver in its normal decomposition of hemoglobin, and that as a result of this insufficiency urobilin is formed and reaches the kidney through the blood-stream and is there eliminated, or is added to the intestinal contents by the bile. A somewhat strict interpretation of this theory is that there is a direct formation of urobilin within the liver cell, or at least within the bile passages from the bile pigment.

Nephrogenous Theory.—In brief, this is based on the fact that urobilin as such is very rarely found in the blood, and that it reaches the kidney in the form of bilirubin, which is reduced to urobilinogen in its passage through the renal epithelium. Herscher states that kidney substance can reduce bilirubin, but surviving kidneys with bilirubin circulating in their blood secrete no urobilinogen, but only bilirubin in the collected urine. The principal argument against this theory is that urobilin does not occur in many cases of jaundice.

Histogenic Theory.—This theory that hemoglobin or bile pigments are reduced in the tissues to urobilin and then reabsorbed and eliminated, offers an insufficient explanation of the various known phenomena.

Enterogenous Theory (Friedrich von Müller).—While not meeting all conditions, this theory is based on the explanation of the established facts of urobilin formation. In brief, it is based on the finding that bile pigment reaching the intestinal lumen undergoes a change into urobilinogen and urobilin caused by bacterial decomposition. It may then be absorbed into the capillaries of the portal system and so reach the liver parenchyma. If the liver is normal the major portion of the urobilin is broken up or synthesized to bile or blood pigment. A portion may be secreted in the bile and so be returned to the intestinal lumen to be reabsorbed or passed off with the stool. When the liver parenchyma is damaged, these changes in the urobilin may not take place, but it passes on into the general circulation. It reaches the kidney through the blood and is eliminated in the urine. This theory is principally sustained by the absence of urobilin in the urine in any quantity in cases of obstruction of the common duct.

We shall not stop for a complete discussion of these theories, since points of view vary, and there is no common agreement at present. We can safely assume that as a rule the presence of urobilinuria is indicative of the fact that bile is reaching the intestine, and that large amounts of urobilin in the urine usually mean that there has been either abnormal destruction of red blood-corpuscles, or an insufficiency of the liver to perform its normal functions. A large amount of urobilin in the stool indicates increased blood destruction. Urobilinuria frequently disappears in severe diarrhea. Although urobilin is normally present in the urine in small quantities, its presence cannot be ascertained by the ordinary qualitative tests — a positive reaction with Schlesinger's zinc acetate test or Ehrlich's aldehyd test is not found in normal urine. It is increased in many pathological conditions. It is commonly increased in infectious diseases, in diseases in which there is wide-spread hemorrhage, traumatic or otherwise, in pernicious anemia, malaria, cardiac decompensation, various forms of liver and gall-passage disease, and in numerous intoxications such as those due to alcohol, chloroform, lead and carbon monoxid.

II. SUMMARY OF QUANTITATIVE METHODS FOR UROBILIN ESTIMATION

Following is a summary of only the most essential points of the methods which have been used:

Almost all the work done has been with the urine. The methods may be divided roughly into those requiring a separation of the urobilin from the urine by means of precipitation with ammonium sulphate; secondly, those depending on the spectroscopic absorption bands of urobilin, and thirdly, those based on the property of fluorescence and lastly colorimetric methods depending on the violet color produced by copper sulphate in urobilin solutions.

1. Methods Involving Salting out of Urobilin by Means of Ammonium Sulphate.—Hoppe-Seyler in 1891 estimated urobilin in urine gravimetrically. The acidified urine was saturated with ammonium sulphate, and the precipitate extracted with chloroform and alcohol. The solution was evaporated, the residue dissolved in ether, filtered and evaporated, and the residue dissolved in alcohol, evaporated and weighed.

Viglezio in the same year recommended a method which depended on the salting out of the urobilin with ammonium sulphate and its solution in alcohol. The amount was gaged by noting how much of this solution was required to produce a certain grade of fluorescence or the appearance of the spectroscopic absorption bands, when it was added to a solution of zinc chlorid.

Studenski added copper sulphate to the urine, precipitated with ammonium sulphate and dissolved the copper compound of urobilin in chloroform. This color was compared with that produced by copper sulphate in a solution of urobilin of known strength.

Ladage recognized the necessity of taking the urobilinogen into account and recommended that iodin should be added to convert it to urobilin. The urobilin was then precipitated by saturation of the urine with ammonium sulphate, and the acid chloroform solution diluted until the spectroscopic absorption became invisible. In Fr. Müller and Huppert's method a mixture of barium chlorid and barium hydrate is added to the urine, and the precipitate is filtered off and washed with hot water. The excess of barium in the filtrate is removed by sodium sulphate, and the filtrate after neutralization with sulphuric acid is saturated with ammonium sulphate. The precipitated urobilin is filtered off, allowed to dry in the air and extracted three times after acidification with a warm mixture of alcohol and ether. This solution is then evaporated to a convenient bulk and the amount of urobilin estimated spectrophotometrically.

Charnas in 1909 gave us an alternative method to one based on the spectroscopic characteristics of urobilin, a gravimetric method in which after all urobilinogen in an ether extract of the urine has been converted into urobilin by exposure to sunlight, the urobilin is separated by water from the ether, and after filtration precipitated by ammonium sulphate, dried, dissolved in alcohol, the alcohol evaporated *in vacuo* and the residue weighed.

2. Spectroscopic Methods.—Gerhardt (1889) and Beck (1895) made estimations by means of the spectrophotometer using Vierordt's tables of the grade of light extinction in different regions of the spectroscope in normal urine and solutions of urobilin.

Saillet (1897) extracted the freshly passed urine with acetic ether. In another specimen the urobilinogen was converted into urobilin by exposure to light and then extracted. The two extracts are added together and diluted until the spectroscopic band disappears.

Conner and Roper (1908) made approximate measurements of the amount of urobilin in urine by noting the number of dilutions required to obliterate the urobilin band in the urine. They recommend the addition of a few drops of Lugol's solution to the urine in order to convert the urobilinogen into urobilin.

Auche (1909) found that a solution of urobilin of 1 : 200,000 prepared by a method he describes is sufficient to make the five absorption bands of potassium permanganate all of equal intensity, although without it some are lighter than others. He therefore superimposes the urine, which in some cases has to be extracted with a thymol-chloroform solution, over a solution of potassium permanganate, and dilutes the urine or its extract until all the bands are of equal intensity.

Simpson (1910) acidifies the urine with sulphuric acid and exposes it to light for some time in order to convert urobilinogen to urobilin. It is then diluted until the urobilin band disappears. This method gave fairly accurate results when a dilution of fifteen or more volumes was necessary. With smaller amounts the reading was apt to be obscured by other pigments.

Hausmann (1913) adds copper sulphate to the urine and dilutes until the spectrum becomes invisible.

Henocque, Hayem, Gautretet, Deniqués, Hildebrandt, Riva and Zoja have used one modification or another of these spectroscopic methods.

Charnas (1909) makes the urine alkaline, allows it to ferment at 37 C. (98.6 F.) for from twenty-four to forty-eight hours, acidifies with tartaric acid, and extracts with ether and petroleum benzin. To a portion of the ether extract an ethereal solution of paradimethylamidobenzaldehyd is added and two to three drops of absolute alcohol saturated with hydrochloric acid gas. After dilution if necessary with alcohol a spectrophotometric reading is made.

3. Methods Depending on the Property of Fluorescence.—Grimm (1893), Fischler (1906), Grigant and Monod (1909), and Descomps (1909) gage the amount of urobilin by the intensity of the fluorescence present in the zinc salt filtrate of the urine. Various devices are adopted to aid the conversion of urobilinogen into urobilin.

4. Colorimetric Methods.—Bogomaloff (1892) used a colorimetric method depending on the depth of the red-violet color produced in a chloroform extract of urine containing urobilin by the addition of copper sulphate.

Braunstein (1903) used a modification of this method.

Flatow and Brünell (1913) estimate the amount of urobilinogen in fresh urine from the depth of the red color given on the addition of Ehrlich's reagent. As a standard they use a phenolphthalein solution to which a few particles of metallic sodium have been added.

Brugsch and Retzlaff (1912) modify Charnas' method by extracting the alkaline urine first with ligroin. They make the final reading with Plesch's colorimeter, using a solution of Bordeaux red as the standard.

The Stools

Quantitative estimations of urobilin in the stools have been carried out by comparatively few investigators. Gerhardt at first used a somewhat complicated process of extraction and purification but later except in special cases simply extracted with acid alcohol and read spectrophotometrically. Auche extracts with alcohol, removes other pigments by shaking with ligroin and thereafter makes the same spectroscopic determination as he employs in urine extracts.

Simpson (1910) mixes the day's feces with water and adds enough dilute sulphuric acid to make the mixture distinctly acid. For one or two days full exposure to light is allowed to convert the chromogen into urobilin. Filtration is then carried out and the residue repeatedly extracted until the filtrate becomes colorless. Direct examination of the filtrates is made and the amount determined from the degree of dilution required to obliterate the spectroscopic band.

Brugsch and Retzlaff (1912) attempt to convert all the urobilin into urobilinogen by allowing the stools to stand for some time before extracting with ligroin to remove indol derivatives and then with acetic ether. After the addition of paradimethylamidobenzaldehyd they make a colorimetric determination as in their method with urine.

Serum

No quantitative work has been done with serum. The methods employed for the detection of urobilin in serum were reviewed by Conner and Roper. We shall refer therefore only to those which have appeared since their publication.

Morel and Monod (1908) add alcohol to the serum and heat to boiling for half an hour. The filtrate is concentrated and a drop of Obermeyer's reagent and an alcoholic solution of zinc acetate and acetic acid are added. After twenty-four hours the filtrate is examined for fluorescence.

Auche (1909) adds a solution of iodin in potassium iodid to the serum and afterward some zinc cyanate in ammonia and filters. The filtrate is examined spectroscopically.

Fromholdt and Nersessoff (1912) precipitate the oxalated plasma with alcohol, filter, concentrate, acidify, and shake with amyl alcohol, and then with alkaline water. The water is then made acid and shaken with amyl alcohol and examined spectroscopically.

III. DISCUSSION OF METHODS

In reviewing the literature on these methods, it was evident that the one main difficulty encountered by all investigators was the problem of separating urobilin and urobilinogen from the other coloring matters present in the excretions without at the same time destroying more or less of it. Many had frankly given up the attempt to attain any but the roughest purification, and had relied on the intensity of the physical properties of fluorescence or of light absorption. In doing so they had, of course, confessed that their results were not quantitatively accurate, for variations in the impurities present would undoubtedly affect the basis on which their determinations rested. Others have not remained satisfied with this, but have attempted to attain at any rate approximately pure solutions before estimation.

The question as to which of these two methods produced the better result depended on whether more was gained in accuracy than was lost by destruction of urobilin in the various precipitations, separations and evaporations required.

It appeared to us that there was one way in which the question could be answered. When undiluted urine is examined spectroscopically, nothing is seen, as a rule, except a general absorption of light, which becomes deeper and deeper toward the blue end of the spectrum. Even if urobilin is present, the absorption band cannot be clearly differentiated. When the amount of urobilin, however, is much larger than the other light-absorbing substances present, one finds that on simple dilution with water the band between E and F becomes more and more distinct, so that it at last stands out sharply. On still further dilution it begins to fade, and a more or less definite point is reached at which it becomes invisible when the full amount of light is allowed to pass through the spectroscope, but can still just be seen when the light is diminished. In some urines one may have to carry the dilution to forty or more times the original volume of urine to reach this point. At this dilution the disturbing effect of other substances is negligible. Such urines afford an

excellent means of deciding as to whether any particular manifestation leads to a loss of urobilin or not, because the number of dilutions required to remove the urobilin band before and after the procedure can be very readily determined. We were fortunate at this time in having under observation for several months a patient with bronzed diabetes in whose urine unusually large amounts of urobilinogen and urobilin were constantly present.

Practical experience with this method very quickly showed us that any and every tried means which may be applied to separate urobilin from the urine was associated with more or less loss. Extracts from precipitations with ammonium sulphate, separations by ether, chloroform or thymol-chloroform solutions, and processes of evaporation, with or without heat, all led to a greater or less diminution of the spectroscopic dilution value. Such methods are applicable in the endeavor to obtain pure urobilin, but it seems to us that they can have no place in any quantitative estimation, for the amount destroyed is too variable and too incapable of control. The one salient fact which has to be kept constantly in mind in choosing a method of estimation is that almost any manipulation is apt to be associated with more or less loss of urobilin. It is better to recognize from the first that ordinary chemical methods are not applicable to a substance which is so labile, and that their employment gives only a fallacious appearance of accuracy. Any gravimetric method for this reason seems to us to be entirely out of question. Solutions of urobilin or urobilinogen may no doubt be obtained in a state of sufficient purity to admit of spectrophotometric determination, but with the means which have hitherto been employed for this purpose the error by loss so far outweighs any increase of accuracy in the final determination as to make results obtained by such methods of very doubtful value.

Yet in the case of all urines in which the amount of urobilin was not exceptionally large and in dealing with stools, some preparation was necessary, for no direct reading could be made. After trying a large number of procedures, the conclusion was reached that the most innocuous of all was to mix equal parts of urine and a saturated solution of zinc acetate in alcohol and to filter. This is Schlesinger's qualitative test for urobilin. A small amount of urobilin may be absorbed by the precipitate, and at first we filtered under pressure and washed the precipitate with alcohol; but latterly we have found that the error is so small compared with other unavoidable sources of error that in routine clinical examinations we have simply taken the first fraction of the filtrate.

Another necessary procedure also soon became apparent. It has, of course, long been known that on adding acid to a urobilin solution the position of the absorption band is altered, but we had not gathered that the intensity was changed to the extent which we found in many urines.

Sometimes the increase in the number of dilutions required to obliterate the band was ten times as great in filtrates which had been made strongly acid as in the fresh filtrate. It was obvious that unless constancy could be attained in this point, no results of value could be obtained. The intensity of the spectroscopic characteristics of urobilin are as dependent on reaction as is the property of fluorescence. The difference is that to attain maximum fluorescence a nice balancing of reaction is required, whereas the strongest absorption band is obtained in a strongly acid solution and an excess of acid does no harm. All that is required, therefore, is to be sure that enough acid is present. For this reason alone the spectroscopic methods are to be preferred to those based on fluorescence in which large errors may arise from slight variations in reaction.

Still another essential point in any method is that the urobilinogen as well as the urobilin shall be taken into account, and that if possible the quantitative relationship between the two bodies shall be established so that the result can be expressed as a single figure. Urobilin is simply an oxidation product of urobilinogen; from the clinical or physiological points of view they have exactly the same significance. Urobilinogen is potential urobilin. In any specimen of urine or stool the relative amounts of the two are constantly changing, the amount of urobilin increasing and of urobilinogen decreasing the longer the exposure to light and air. In some methods this difficulty is met by keeping the extract for twenty-four hours exposed to light, and it is assumed that all the urobilinogen will then have been converted into urobilin. Others endeavor to hasten the process by the addition of oxidizing agents, and one method takes the opposite course, and by alkaline fermentation, attempts to convert all the urobilin into urobilinogen.

The only way by which urobilinogen can be determined, except gravimetrically, is by the spectroscope through the absorption bands of the pigment produced by its reaction with paradimethylamidobenzaldehyd. We used a solution containing 20 gm. in 300 c.c. of 50 per cent. concentrated hydrochloric acid. One c.c. was added to every 10 c.c. of the alcoholic zinc acetate filtrate. This gave a sufficient amount of acid to produce the maximum intensity in the urobilin band and at the same time brought out the urobilin spectrum, so that the dilution values of both urobilin and urobilinogen could be read in the same filtrate. The amount of urobilinogen could then be expressed as representing so many dilutions in the same way as with the urobilin.

The first point investigated was the effect of light on urobilinogen. It was found to vary greatly with the intensity of the light. A few hours' exposure to direct sunlight suffices to cause the complete disappearance of urobilinogen. With indirect light the process required a longer time; but after exposure to a diffuse light in a room with ground-glass win-

dows, there was found after twenty-four hours to be an almost complete disappearance of urobilinogen. The figures in Table 1 show the effect of leaving the zinc acetate filtrates of urine in diffuse room-light for twenty-four hours.

TABLE 1.—EFFECT OF LEAVING ZINC ACETATE FILTRATES OF URINE IN DIFFUSE ROOM-LIGHT FOR TWENTY-FOUR HOURS

Urobilinogen

Filtrate Examined at Once	Examined After 24 Hours in Room-Light
32	1
37	0
45	2
25	0
21	1
12	0
7	0
16	0
34	0
40	1
18	1
25	0
19	0
40	0

The effect on urobilin of a similar exposure to light could not be determined in the same way, since all fresh urine and stools contain urobilinogen which passes over in part at least to urobilin. It was invariably found, however, in these specimens in which by various means the urobilinogen had been removed, that light had a destructive effect on urobilin, as was shown by the diminution in the dilution value after exposure.

Under the influence of light, as the urobilinogen diminishes the urobilin increases. The question remained, however, as to how much loss of urobilinogen and urobilin occurred during this process. The precise degree of loss could be found only if we knew the quantitative relationship between urobilinogen and urobilin and the relative light-absorbing powers of the urobilinogen and urobilin pigments. These facts are not known, but an indication of the amount of loss of urobilin was gained by comparing the total dilution value obtained by adding together the urobilinogen and urobilin values in the fresh filtrates, in those which had been in darkness for twenty-four hours and in those which had been left in diffuse room-light for the same time.

After twenty-four hours in darkness there had been a diminution of urobilinogen from 371 to 301 and an increase of urobilin from 6 to 134. The light-absorbing power of urobilin must then either be greater than that of the urobilinogen pigment, or one molecule of urobilinogen must break up into two or more molecules of urobilin. In any case there is an increase of the total dilution values for urobilinogen and urobilin

together, associated with a diminution of urobilinogen and an increase in urobilin.

TABLE 2.—QUANTITATIVE CHANGE IN UROBILINOGEN AND UROBILIN UNDER THE INFLUENCE OF LIGHT

Fresh Urine		After 24 Hours in Dark		After 24 Hours in Room-Light	
Urobilinogen	Urobilin	Urobilinogen	Urobilin	Urobilinogen	Urobilin
32	2	28	11	1	26
37	0	30	12	0	32
45	0	40	12	2	37
25	0	22	8	0	17
21	0	19	14	1	16
12	2	10	7	0	3
7	2	6	3	0	5
16	0	14	7	0	15
34	0	30	12	0	21
40	0	35	13	1	30
18	0	14	10	1	16
25	0	14	9	0	16
19	0	7	4	0	8
40	0	32	12	0	24
371	6*	301	134†	6	266‡

* Total "Urobilin" 371 + 6 = 377.
† Total "Urobilin" 301 + 134 = 435.
‡ Total "Urobilin" 6 + 266 = 272.

In the filtrates exposed to light there is more than forty times as much urobilin as in the fresh filtrates and twice as much as in those kept in the dark, and yet in spite of that the total dilution value is much less, only 266 as compared with 377 and 435. If for a moment it is assumed that the diminution of urobilinogen and increase in urobilin in the filtrates kept in the dark represent the relationship between the two bodies, then 371 — 301 = 70 of urobilinogen should produce 134 — 6 = 128 of urobilin. But the disappearance of 371 — 6 = 365 of urobilinogen in the filtrates exposed to light led to an increase of only 266 — 6 = 260 of urobilin instead of the 667 which should have resulted. It is impossible to explain this decrease in total dilution value except as due to a destruction of part of the urobilinogen or urobilin or both. Since the actual amount of urobilinogen or urobilin destroyed by exposure to light cannot be calculated, and varies so greatly with different degrees of intensity of light and with other unknown conditions, the conclusion was reached that it was not a means which could be used in any quantitative method to convert urobilinogen into urobilin, and that as far as possible exposure to light should be avoided in urobilin estimations.

Attention was then turned to the effect of oxidizing agents which have been recommended especially by French investigators; but here

also the results were disappointing, for those reagents which were effective in oxidizing the urobilinogen were also very destructive to the urobilin.

Charnas states that if urine is made alkaline and left for from twenty-four to forty-eight hours in the incubator, all the urobilin will be converted into urobilinogen, which can then be estimated by the spectrophotometer. It is noteworthy that Charnas does not give any estimations indicating the decrease of urobilin and increase of urobilinogen during the process, nor does he bring forward any evidence to show whether or not any part of the urobilinogen or urobilin is destroyed by the fermentation which occurs. It has long been known that in freshly passed urine urobilinogen is present alone or at any rate in far greater amount than urobilin, suggesting that as it is excreted through the kidneys it is all in the form of urobilinogen; and it has been the universal experience that the longer a urine is kept the less urobilinogen will be found in it, so that the statement that in alkaline urine left exposed to the air at a temperature of 37 C. the reverse process occurs, and that instead of an oxidation a reduction takes place, is not one which can at once be accepted. We know that in the intestine there is a reduction of urobilin to urobilinogen, but there conditions are present much more favorable to reduction processes than those which are present in urine left exposed to air. Outside the body the formation of urobilin constantly goes on; but to bring about the reverse process the strongest reducing agents we know have to be employed. It seemed to us, however, that the question was one which could be decided by the simple spectroscopic method we have outlined.

Readings of the relative amounts of urobilin and urobilinogen in thirty specimens of urine before and after alkaline fermentation at 37 C. were made. In most of the cases fermentation was allowed to continue for twenty-four hours. The first twelve results are given in Table 3.

TABLE 3.—UROBILIN AND UROBILINOGEN BEFORE AND AFTER ALKALINE FERMENTATION AT 37 C.

Before Fermentation		After Fermentation	
Urobilinogen	Urobilin	Urobilinogen	Urobilin
15	5	0	11
12	0	0	9
26	9	7	0
15	5	0	0
12	0	6	0
7	0	8	3
26	9	0	2
19	3	5	2
0	3	0	11
0	5	30	0
17	6	0	1
10	5	50	2

Of these thirty specimens there was a diminution instead of an increase of urobilinogen after fermentation in all except three. In two there was a very considerable increase of urobilinogen, but both these specimens contained some bile, and as we found that urobilinogen was produced in decomposing bile we are inclined to ascribe these exceptions to this cause.

It seemed that these results, so completely at variance with the statement of Charnas as to the effect of alkaline decomposition in urine, might be due to the urobilinogen having become absorbed in the heavy deposit of phosphates in the fermented urines. The experiment was therefore repeated in ten urines, the readings before and after fermentation being made after the specimns which had been made faintly alkaline with ammonium carbonate had again been made acid with tartaric acid. Before fermentation a total reading of urobilinogen 18 and urobilin 27 was made. After twenty-four hours' fermentation, urobilinogen 13 and urobilin 16 was recorded, and after forty-eight hours, urobilinogen 12 and urobilin 4. We must conclude then that alkaline fermentation in the dark at a temperature of 37 C. not only does not quantitatively convert urobilin into urobilinogen, but that it has a directly destructive action on both.

Since there was no available way of converting all the urobilinogen into urobilin or vice versa, the only alternative was to make estimations of each separately. But could such results be added together and expressed by a single figure? Our knowledge of the chemistry of these bodies is not yet sufficiently advanced to enable us to be sure that one or the other may not be a condensation of the other so that one molecule of urobilinogen, for instance, may break up into two or more molecules of urobilin. No idea as to the relationship between them could be gained by noting the degree of increase in the dilution value of urobilin which followed a decrease in urobilinogen, for the factors concerned are too complex and variable to allow of any true idea being obtained in this way. For while the urobilinogen is being converted into urobilin, both are undergoing other changes and are losing the spectroscopic characteristics on which our determinations rest. In preparations of urobilin and urobilinogen made from bilirubin by Fromholdt's method we found such a lack of relation between the weight of bilirubin and the weight of urobilin produced, and such a variability in the relative amounts of urobilin and urobilinogen formed, that it did not seem likely that in this way either could we successfully determine whether or not urobilin and urobilinogen were equivalent in value. In dealing with two such extremely unstable substances whose presence can be detected only by a single physical property and which are constantly changing into other bodies which we have no means of estimating, the problem of their

exact relationship is one whose solution will probably not be definitely established until we have more knowledge of the decomposition products of hemoglobin. For urobilinogen and urobilin are only links in the chain of hemoglobin derivatives; they cannot be regarded as final end-products. The problem which we were attempting to solve was not the exact quantitative determination of urobilin, however, but simply the question as to whether or not any information of clinical value could be gained from urobilin estimations, in spite of the errors necessarily involved in any of the present methods. With this end in view the best that could be done was to add together the dilution values of urobilinogen and urobilin. We do not know that they are equivalent, but, on the other hand, we know that they are not very different in value. The proportion of the two substances varies so much in different specimens of urine, stool and bile that no comparison can easily be made if the dilution values for each are given separately.

As a result of this preliminary review we determined that the best method would be one which avoided all but absolutely essential manipulation of the material, one which was based on the most characteristic of the properties of urobilin, the spectroscopic absorption bands, and one in which the attempt to convert all the urobilinogen into urobilin or vice versa was abandoned and the results of separate estimations of the two were simply added together. Throughout this paper when the term "urobilin" is used apart from "urobilinogen," the sum of the dilution values of urobilin and urobilinogen is intended.

The following are the details of the methods which we finally adopted:

IV. METHODS

Urine

The method of collection of the twenty-four-hour urine has a considerable effect on the total spectroscopic reading. The vessel in which the urine is collected should be of dark brown glass and should be kept in darkness. Thymol crystals should be added, for even in cases in which no obvious fermentation had occurred we sometimes found a diminution in the total amount if no preservative was present. After measuring the amount of the twenty-four-hour urine, 10 c.c. are mixed with 10 c.c. of a saturated alcoholic solution of zinc acetate, and after a few minutes filtered. If a number of urines are being examined at the same time it is convenient to have test-tubes graduated to 10 and 20 c.c. Ten c.c. of the filtrate are taken and 1 c.c. of Ehrlich's solution[1] is added. It was found that this amount produced a sufficient concentration of acid in the mixture to give the maximum intensity of the urobilin band and contained enough of the paradimethylamidobenzaldehyd for the reaction with urobilinogen. The development of the urobilinogen band is not instantaneous. We found that as a rule it had attained its full intensity in a quarter of an hour. The action can be greatly accelerated by heating, but this is to be avoided. It is better to wait for an hour before making the reading, and during this time the solution should be kept in

1. Paradimethylamidobenzaldehyd, 20 gm.; concentrated hydrochloric acid, 150 c.c.; water, 150 c.c.

the dark. After three or four hours there is a diminution of the urobilin and urobilinogen in filtrates from urines, so the estimation should be made not later than three hours after adding the Ehrlich solution. We found that Citron's hand spectroscope[2] was the most convenient instrument to use. The filtrate was washed into a graduate and diluted with tap-water until first one and then the other bands of light absorption had disappeared when the full amount of light entered the spectroscope, but were still visible when the light was partly shut off. This gives a fairly definite end-point, and we did not find any great variation in the readings made by different persons. It is important of course that the light shall be always of approximately equal intensity. We made the readings in a dark room with a Tungsten electric bulb, holding the spectroscope close to the source of light. In highly colored urines one may be in doubt as to whether or not a trace of urobilin is present, for there may be so much general absorption of light as to obscure the urobilin band in the undiluted filtrate. There is no such difficulty with the urobilinogen band which lies between the red and yellow where there is no marked light absorption. With urines containing bile, if the amount of urobilin is not very large, it is necessary to add some fullers' earth and to leave the mixture standing for some time before filtration. If this is done the urobilin band can usually be read even in the undiluted filtrate. The dilution required gives the value for 5 c.c. of urine. If this figure is multiplied by the number of 5 c.c. quantities in the twenty-four-hour urine, the number of dilutions which would have been necessary if all the urobilinogen and urobilin in the twenty-four-hour amount had been concentrated in a volume of 5 c.c. is obtained. For instance, if in a twenty-four-hour urine measuring 1,000 c.c. a reading of ten dilutions for urobilinogen and of twenty for urobilin were made, the total urobilin would be $30 \times 200 = 6{,}000$. We tried to determine the dilution value of definite weights of urobilin prepared from bilirubin, but different preparations varied so much in their dilution value that it was obvious that we were not dealing with pure urobilin and we abandoned any attempt to express our results in milligrams of urobilin.

Stools

All the feces passed in the twenty-four hours were collected in the same receiver, the stools being protected from light. They were then washed into a large graduate and thoroughly ground up with water into a homogeneous paste, and water added to 0.5, 1, or if necessary 2 liters, depending on the quantity of stool. After thorough mixing, 25 c.c. were taken and 75 c.c. of acid alcohol (95 per cent. alcohol, 1,600 c.c.; concentrated hydrochloric acid, 25 c.c., and water, 800 c.c.) were added. The mixture was then put into a shaker for about half an hour. A considerable number of extractives were tried for removing the urobilin from the stools, but none were found so efficient as alcohol with hydrochloric acid. After thorough mixing in the shaker an equal quantity of a saturated solution of zinc acetate in alcohol was added and the mixture was filtered. After adding 2 c.c. of Ehrlich's reagent to 20 c.c. of the filtrate, the solution was put aside in a dark place until next day. The addition of zinc acetate is not absolutely essential, but in some cases we observed an intensification of the urobilin band following its use, and it is perhaps an advantage to make the urine and stool readings as far as possible under the same conditions. The development of the urobilinogen band was not always complete until six hours had elapsed, but thereafter there was no loss of urobilinogen or urobilin for a long time, although this was not the case with the zinc acetate filtrates before the addition of Ehrlich's solution. The reading was made in the same way as with the urine and the total amount calculated for the volume of stool after grinding up with water, the dilution of the 25 c.c. by acid alcohol and zinc acetate being taken into account. Instead of using tap-water for dilution of the final extract, 60 per cent. alcohol was required to avoid the development of a precipitate.

2. This can be obtained from Paul Altmann, Luisenstrasse, Berlin, Germany.

Bile

A measured amount of bile was mixed to a paste in a mortar with fullers' earth and left for some hours. An equal amount of the alcoholic solution of zinc acetate was then added and the mixture filtered under pressure, and the remainder washed with measured amounts of zinc acetate alcohol. In this way almost all of the bilirubin was absorbed, and though no doubt there was some loss of urobilinogen and urobilin, the comparatively small amounts found in the washings compared with the quantities present in the first filtrate seemed to indicate that the fullers' earth had very much less capacity for absorbing urobilin than bilirubin. Small amounts of urobilin or urobilinogen could no doubt be overlooked by this method. The Ehrlich solution was added in the same proportion as in the urine, but the dilutions had to be made with alcohol instead of water.

Blood-Serum

We have tried various methods, but so far we have not been successful in demonstrating urobilin with certainty.

V. THE EXCRETION OF UROBILIN IN URINE

Urobilin is a normal constituent of the urine, but the amount is so small under ordinary conditions that it can be demonstrated only by extracting large quantities of urine.

With the methods we have used, a positive result in twenty-four-hour specimens of urine indicates an abnormal increase over the amount usually present. We have only very rarely been able to find a faint and doubtful degree of absorption in the region of the urobilinogen or urobilin bands in the urines of persons in apparent health. When, therefore, urobilin is spoken of as being absent from urine, it is understood that an absence of an increased amount of urobilin is meant.

Saillet found in two normal persons that the quantity of urobilin excreted during the eight hours of sleep was only about one-tenth of the total twenty-four-hour amount. He gives two curves of the excretion during the daytime which show some relation to the body-temperature variations. He suggests a connection with the processes of digestion and of general metabolism.

In a case of bronzed diabetes with a large urinary excretion of urobilin, we had an opportunity to study the excretion from hour to hour. The patient was on a von Noorden strict diet plus 50 gm. of bread. His habits were very regular. He rose at 7 a. m. and went to sleep at about 9 p. m. Urine was passed every two hours during the daytime, and the amount of urobilin determined at once. The amount passed during the six or eight hours of sleep was kept in the dark and the urobilin determined at the earliest opportunity. In such freshly passed specimens, practically all the urobilin is in the form of urobilinogen, and the probable error arising from the assumption that the urobilinogen and urobilin readings represent equivalent values is avoided. For over a month, the two-hourly excretion was determined every day, with the result that there

was invariably found to be a marked diminution in the amount excreted at night. A graphic representation of the excretion during three consecutive days is given in Chart 1. The chart is typical of the results throughout the whole period. It shows the decrease of urobilin in the night urine and the great variation in the quantity from day to day and from hour to hour. The only constant feature is the fall during the night. Otherwise there is complete irregularity and the hour of maximum elimination occurred at any time during the day.

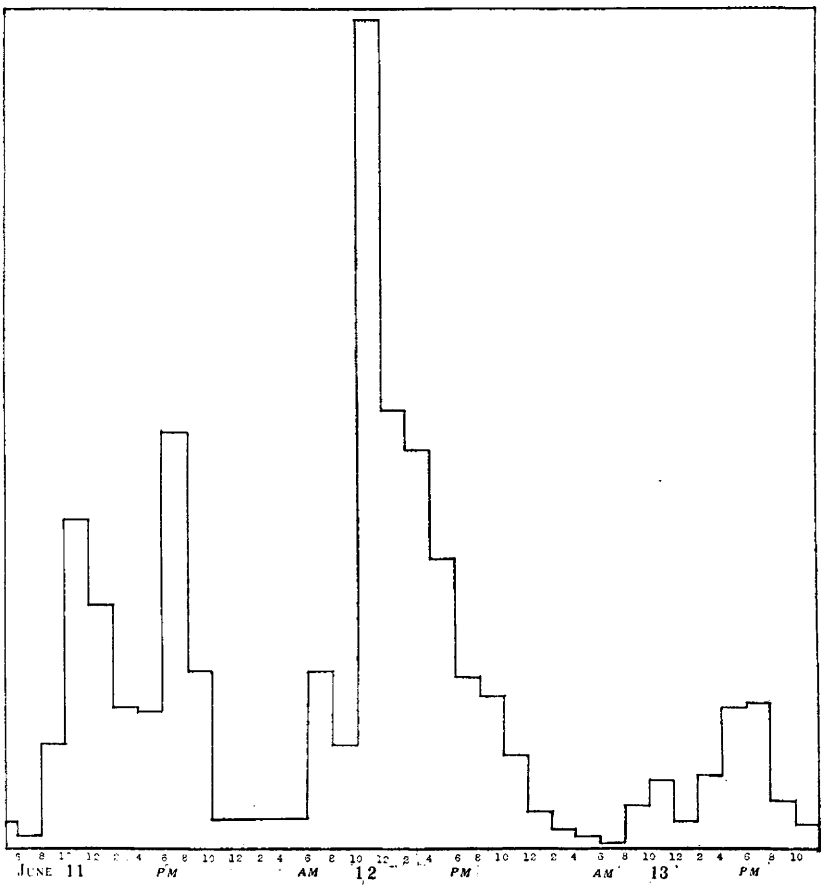


Chart 1.—Excretion of urobilin, June 11, 12 and 13.

When the patient remained up and took his meals during the night and slept in the daytime, the amount excreted in the night was much larger than in the day.

Charts 3, 4, 5, 6 and 7 show the relation between the excretion of water, total solids, sugar, chlorids and urea and urobilin. The relation was only a very general one, for the urobilin excretion shows irregular and wide fluctuations.

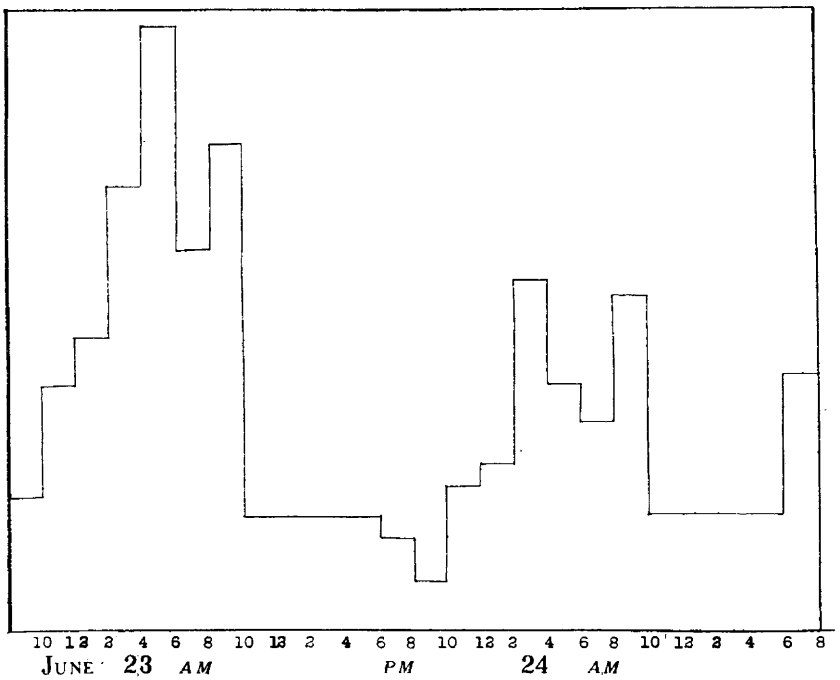


Chart 2.—Excretion of urobilin, June 23 and 24.

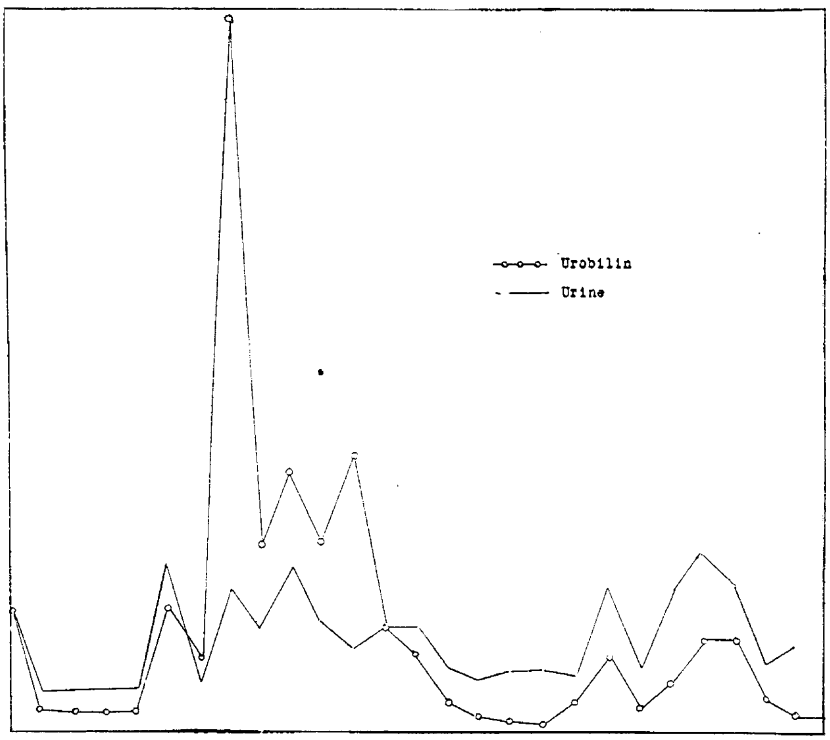


Chart 3.—Volume of urine and urobilin.

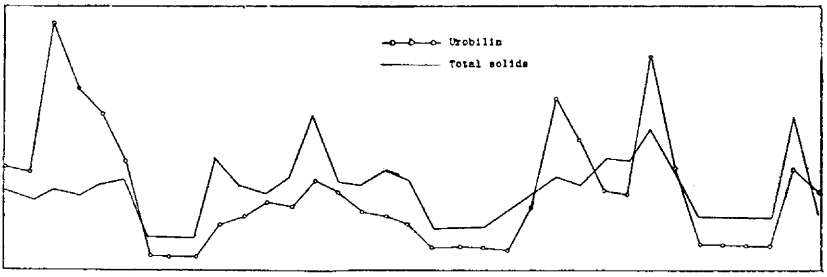


Chart 4.—Total solids and urobilin.

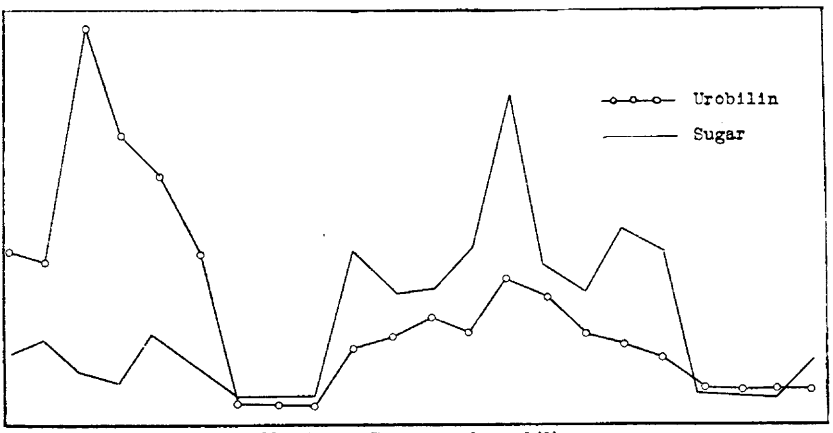


Chart 5.—Sugar and urobilin.

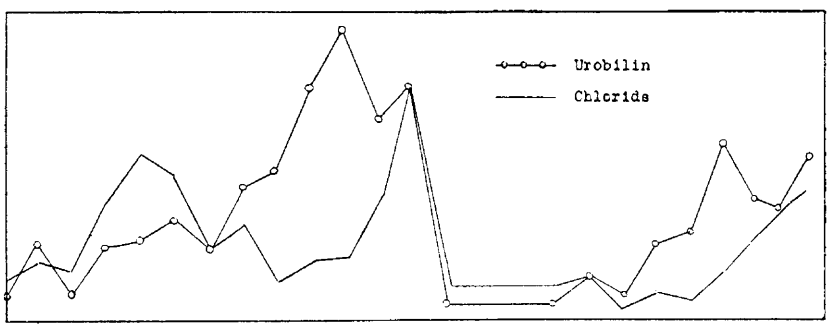


Chart 6.—Chlorids and urobilin.

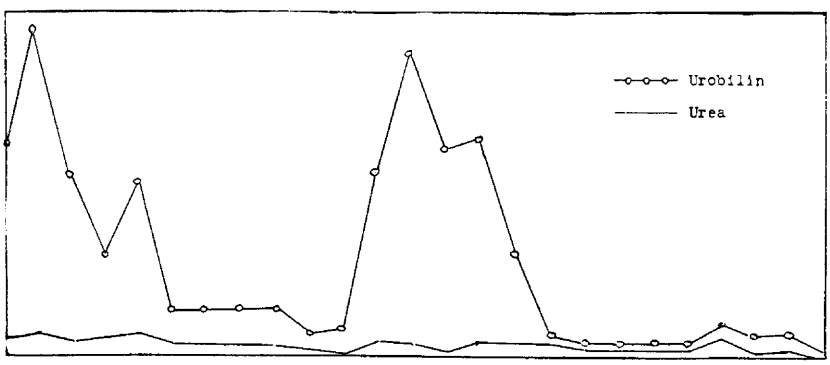


Chart 7.—Urea and urobilin.

These results appear to us to be of interest in connection with the question of urobilin excretion by the kidneys. It is generally agreed that most of the urobilin is formed in the intestine and is carried by the portal vein to the liver. There it is supposed to be either broken down further or resynthesized to bilirubin or hemoglobin (Brugsch) except for a very small part which is carried into the general circulation and is excreted through the kidneys. When the liver is diseased, the destruction or rebuilding of urobilin fails more or less, so that a greater quantity of the urobilin absorbed from the intestine passes by the liver to the kidneys.

The wide fluctuations in the quantities of urobilin in this case can scarcely be due to variations in the amount of absorption from the intestine. It is true that bile enters the duodenum only at intervals, but they are regular intervals when the periods of digestion are regular, and on these charts no signs of any periodicity during the daytime can be traced. In any case, the bile passes through the small intestine in about four hours, and as our findings as to the amount of urobilin in various parts of the intestinal tract show, the greatest quantity of urobilin is in the large intestine, not in the small. Urobilin is always present in the large intestine, and from this situation one could not expect any considerable changes from hour to hour in the amount absorbed. Urobilin is a readily diffusible substance, and since water is removed from the large intestine, it seems probable that urobilin will also be taken up by the blood.

Nor does it seem that changes in the excretory functions of the kidneys can account for more than a small part of the urobilin variations. The amounts of sugar and chlorids fall during the night mainly because their absorption from the alimentary tract almost ceases and not because there is any marked decrease in the eliminating functions of the kidneys. But urobilin is present in the large intestine in as large amount during the night as during the day, and if absorption is uniform one would expect uniform excretion, or at least no greater variation than might be ascribed to alterations in the circulatory and nervous mechanism of the kidneys. We have no evidence of any specific diminution in excretory power of the kidneys for any substance during the night.

If the absorption and excretion of urobilin appear to be too continuous and uniform to explain these charts, can we regard the variations shown in them as indicative of fluctuations in the power of the liver to keep the urobilin reaching it from the intestine from entering the general circulation?

The patient had an extremely large liver which post mortem was found to be cirrhotic and to contain a large amount of the pigment found in cases of hemochromatosis. During the day, when the work of the liver was increased because of the absorption of food products, it might

be imagined that the liver would be less able to cope with the urobilin brought to it, and the great increase during the daytime might be thus explained. But the wide and irregular fluctuations from day to day and from hour to hour, in spite of the fact that the meals were taken at regular intervals, remain to be accounted for. Possibly some other factor such as a formation of urobilin in the diseased liver may have played a part. In Saillet's two curves of hourly urobilin excretion in people in health, no such wide variations are to be seen.

Other possible factors which may play a part in alterations of the urinary excretion of urobilin are the changes which urobilin undergoes in the blood and tissues. The form in which urobilin circulates in the blood-stream is still unknown, and the question as to whether it may not there in part be broken up or synthesized to some other substance, or may not be stored in some form or other in the tissues, is still unsolved, and it is possible that in them an explanation may be found for these anomalies of urobilin excretion. At present, they can only be recorded.

VI. THE EXCRETION OF UROBILIN IN THE STOOLS

In ten adults in whom there was no reason to suspect any disturbance of hemoglobin metabolism, the average daily excretion in the stools was worked out. These cases, as shown in Table 4, showed variations between 3,307 and 8,737, and a general average of 6,475.

TABLE 4.—AVERAGE DAILY EXCRETION OF UROBILIN IN STOOLS OF TEN ADULTS WITH NORMAL HEMOGLOBIN METABOLISM

Length of Period of Observation, Days	Diagnosis	Average Daily Urobilin in Stools
8	Inguinal hernia	8,685
15	Fractured femur	3,855
3	Chronic appendicitis	6,080
3	Endometritis	3,307
6	Neurasthenia	4,887
10	Neuritis	8,702
15	Chronic arthritis	6,604
11	Neurasthenia	8,737
6	Fractured femur	5,840
6	Fractured tibia	8,053

The impossibility of gaining any idea as to the amount of urobilin excreted in the stools by an examination of a single twenty-four-hour collection is apparent when the figures for the daily excretion of any of the cases we have worked with are examined (Table 5). The variations, it is seen, are sometimes very large indeed.

To a certain extent, of course, this is accounted for by differences in the quantity of feces passed from day to day, but there are certainly other factors involved. We were particularly struck with the fact that on the days following a period of twenty-four hours during which no stool was passed, there was not as a rule any increase in the urobilin. If

the urobilin in the stools retained in the large intestine were absorbed by the blood-stream, one would have expected an increase in the urobilin in the urine on these days. But on reviewing the figures, no constant increase in the urobilin in the urine could be established. We were then inclined to attribute this apparent loss of urobilin to destruction within the intestine; but when the effect of keeping stools in an incubator at 37 C. was tried, we found to our surprise that there was no less marked loss of urobilin.

TABLE 5.—UROBILIN EXCRETED IN STOOL

Day	Urobilin in Stool			
1	2,408	4,800	16,640	5,120
2	10,880	3,360	5,280	13,440
3	*	3,840	7,680	2,560
4	7,360	960	1,440	*
5	4,480	*	4,800	3,840
6	1,280	6,080	15,360	14,400
7	7,680	10,240	25,920	*
8	4,000	8,000	8,320	35,200
9	*	*	3,840	1,536
10	720	3,200	5,760	3,200
11	7,040	11,200	6,720	7,040

* No stool.

The stools were thoroughly ground up in water and kept in the incubator. Every day, 25 c.c. were removed and extracted with acid alcohol. With the exception of the last stool, the figures show only such differences as lie within the limits of error of the method (Table 6). There was only a slight diminution of urobilinogen and increase of urobilin.[3]

TABLE 6.—EFFECT ON UROBILIN OF KEEPING STOOLS IN INCUBATOR

No.	Fresh Stool	After 24 Hours	After 48 Hours	After 72 Hours	After 96 Hours
1	15	16	16	..	..
2	3	3	2	3	3
3	12	19	14	18	11
4	57	62	65	66	65
5	20	21	20	18	24
6	60	55	48	53	60
7	3	3	3	4	4
8	65	60	55	51	48

So long then as stools are kept from exposure to light there does not seem to be a very rapid loss of urobilin. Nor, on the other hand, is there any increase such as might have occurred if the formation of urobilin in the freshly passed stool had been incomplete.

The absence of great destruction of urobilin in stools kept under these conditions outside the body does not, of course, preclude the possibility

3. Later experiments showed that there was a gradual diminution of both urobilin and urobilinogen, so that after a week about a quarter of the original dilution value had been lost. The greater constancy in the experiment given here was due to concentration by evaporation.

of such an action taking place within the bowel, where absorption and excretion are proceeding and where there is no exposure to air. It was thought that an indication of such a loss of urobilin either by absorption or destruction was obtained when the relative amounts of urobilin present in the contents at different levels of the intestinal tract were compared.

The feces obtained at post-mortem in five cases were ground up in so much acid alcohol as gave, so far as could be seen, an approximately equal consistency. The relative amounts of urobilin were as follows: duodenum, 1; jejunum, 1.2; ileum, 4.8; cecum, 12.3; descending colon, 5.5.

The relative decrease in the descending colon as compared with the cecum certainly suggests a loss of urobilin, but the number of cases examined was too small to permit of any definite conclusion. In all probability, both absorption and destruction are at work. The absence of increased urobilin in the urine on the days of constipation is not by any means conclusive evidence against the occurrence of absorption of urobilin from retained stools, for the absolute want of any relationship between the amount of urobilin in the stools and in the urine is a fact which must impress any one who makes such concomitant observations. No doubt the quantity in the intestine is a factor in determining the amount excreted by the urine, but except under conditions such as an unusually large hemoglobin destruction, when there may be a very great increase of urobilin in the stools, its effect is altogether overshadowed by other influences at work on the urobilin in its passage through the blood-stream, liver, bile, tissues and kidneys.

The interpretation of the results of urobilin estimations in the stools is a comparatively simple matter when compared with the complexities and obscurities surrounding the question of the elimination of urobilin in the urine, and we believe that from a clinical point of view, work on the stools will give results of much greater value and of much less doubtful significance than has been the case up to the present, when the attention of clinicians has been directed almost exclusively to the urine.

Our own experience in cases of pernicious anemia, and the reports of Simpson in cases of malaria have convinced us that, if constipation or diarrhea are avoided, the determination of the average stool urobilin over a period of several days is the best guide we have to the quantity of hemoglobin which is being broken down in the body. Its importance in anemias and in all cases in which excessive blood destruction is suspected does not need to be elaborated.

VII. THE EXCRETION OF UROBILIN IN BILE

Urobilin has been found in human bile post mortem by Jaffé, Müller, Tissier, Gerhardt, Beck and Braunstein. After the introduction of Ehr-

lich's reagent, urobilinogen was found in forty-three of forty-six cases by Kimura. Neither is constantly present, for in cases in which severe diarrhea had occurred, or in which there had been obstruction to the flow of bile into the intestine, they were not found. Austin and Ordway in a very careful study found urobilin present in 94 per cent. of fifty cases with biliary fistulas in which urobilin was present in the stools and urine. In seven cases in which no urobilin could be demonstrated in either the stool or urine, urobilin was present in the bile in two. Fischler found urobilin in the bile of normal dogs. After establishing a biliary fistula and obstructing the common bile-duct, urobilin disappeared from the bile, but it was still found, though in diminished amount, in the stools. When the liver was damaged by amyl alcohol, urobilin appeared in considerable amounts in the bile. He believes that the only way in which this can be accounted for is by assuming that the urobilin was formed in the liver itself.

Our experience with bile removed post mortem goes to show that the amount of urobilin is extremely variable. In Table 7 the value of the urobilin in 10 c.c. of bile is given in sixteen cases.

TABLE 7.—UROBILIN IN BILE REMOVED POST MORTEM

		Dilution Value in 10 c.c. Bile
1.	Pernicious anemia	3,940
2.	Pernicious anemia	80
3.	Pneumococcal meningitis	2,100
4.	Pyonephrosis	4,500
5.	Bronchopneumonia	.650
6.	Streptodiplococcal septicemia	0
7.	Cerebral embolism	620
8.	Aneurysm	300
9.	Aortic incompetence	45
10.	Diabetes	200
11.	Bronzed diabetes	1,800
12.	Carcinoma of liver (without duct obstruction)	45
13.	Tetanus	0
14.	Carcinoma of larynx	0
15.	Hypophysis tumor	0
16.	Adherent pericarditis and valvular disease	0

Of these sixteen cases there were five in which no urobilin was found by the method we used. But if larger amounts of bile had been used for extraction we might have found traces, and we therefore cannot say more than that the quantity was at least very small. No relation was found between the length of time between death and examination of the bile and the amount of urobilin found. In one case urobilin to a dilution value of 1,800 for every 10 c.c. of bile was present, although it was removed from the body within three-quarters of an hour after death. It does not seem that post-mortem decomposition of bilirubin can possibly explain the variations in quantity which were found.

On the other hand, there is no obvious connection between the nature of the disease and the amounts obtained. We are inclined to believe that the degree of absorption of urobilin from the intestine just before death is an important factor. In the two patients with pernicious anemia, estimations of the amount of urobilin present in the intestinal contents were made, and an enormous quantity was found in the case which contained so much urobilin in the bile, and comparatively little in the one which showed a low dilution value. In another case in which severe diarrhea had completely emptied the intestine, no urobilin could be detected in the bile. In a dog which died four days after the common bile-duct had been severed and in whose intestines no urobilin was found, there was also no urobilin in the bile, although it was found in the bile of normal dogs. These observations bear out the well-recognized importance of the intestinal source of biliary urobilin, and yet exceptions are sufficiently striking to make one hesitate to accept the exclusively enterogenous origin of urobilin.

In a few cases with biliary fistulas after operations for gall-stones the urobilin in the bile was found to increase as the bile began again to enter the intestine, but there was no definite qualitative relationship between the amount in the bile and in the stools. An exception was found in one patient whose case is discussed later, in whom urobilin was present in large amount in the bile while urobilin was practically absent from the stools. This was a case of gall-stones in which for a period of eight days after a gall-bladder fistula had been established, the total urobilin in the stools amounted to only 720 dilutions, while in the bile an amount equivalent to 35, 144 dilutions was found. The stools contained no more urobilin than we found in a case in which there was undoubtedly a complete occlusion of the common duct. Probably no bilirubin at all was entering the intestine. Later on when the bile again passed through the common duct, the urobilin increased in amount in the stools to 33,216 dilutions in seven days, but the percentage of urobilin in the bile, which still came from the fistula for the first four days of this period, was only 230 dilutions for each hundred c.c. of bile, in contrast with 1,360 when there was practically no urobilin in the stools. In cases of this sort it is difficult to avoid the conclusion that the urobilin has been formed in the liver itself.

VIII. UROBILIN IN SERUM

Urobilin is present in the intestine and is excreted in part by the kidneys. It presumably reaches the kidneys by means of the blood. One would expect to be able to detect it in the blood of those not infrequent patients in whom even more urobilin is found in the urine than in the stools. Yet there is no point in the whole subject on which there is more diversity of results and of opinions. The constant absence of uro-

bilin from the serum in cases of urobilinuria was a main support of the theory advanced by Gilbert and Herscher that urobilin was formed from bilirubin during excretion through the kidneys. Even long after the inadequacy of this hypothesis had been exposed by the work of Müller and the intestinal origin of the urobilin of the urine had been generally accepted, no one was able to demonstrate the presence of urobilin in the blood or serum. Müller and Gerhardt believed that it was not present in appreciable amount in the blood, and Schlesinger reported fifteen cases of very marked urobilinuria in which the serums gave a negative reaction for urobilin.

It was not until 1906 that Huber published a report in which he stated that urobilin was always found in serum when urobilin was present in the urine. He ascribed the negative results of Schlesinger to the fact that he had not added Lugol's solution to the zinc acetate filtrate in order that urobilinogen might be oxidized to urobilin. But considerable doubt was cast on Huber's results when it was pointed out that the green fluorescence characteristic of urobilin might be closely imitated by biliverdin, and that especially after the addition of Lugol's solution, the bilirubin normally present in serum might be oxidized to beliverdin. Biffi (1907) extracted oxalated blood with chloroform, examined the filtrate spectroscopically and found urobilin present in small quantities in a number of cases and stated that in lobar pneumonia it was almost constantly found. Moller could not confirm Biffi's findings as to the relative frequency of a positive reaction for urobilin in serum, but with zinc acetate and Lugol's solution found it in only two cases, both lobar pneumonias, and later with Biffi's method in two other cases, pneumonia and one of pernicious anemia.

Further evidence of the occurrence of urobilin in serum was given by von Jaksch, who stated that in fatal cases of pneumonia the serum separated from blood removed by venesection showed a green fluorescence which disappeared on exposure to light. Conner and Roper (1909), in a study of the relation between the bilirubin in serum and urobilin in the urine, repeatedly and carefully examined the serums of over sixty patients with urobilinuria, with uniformly negative results, until the serum of a patient dying of lobar pneumonia was encountered. This serum gave a markedly positive reaction for urobilin with all the methods. In nine other cases of pneumonia shortly before death the serum contained large amounts of urobilin. In three severe cases of pneumonia in which the patients recovered, urobilin was detected in small amount in the serum, but in fifteen other cases in which the patients lived, no urobilin was found. There was no parallelism between the amount of urobilin in the serum and in the urine.

Lehndorf (1913) could find urobilin in only two of the serums which he investigated. One was from a case of decompensated mitral stenosis and the other from a case of myocardial degeneration with pleurisy shortly before death. Fromholdt and Nersessoff (1912) by a somewhat complicated method and by extracting relatively large volumes of oxalated plasma, were able to demonstrate urobilin in the serum of seven out of eleven cases with urobilin in the urine. They record a doubtfully positive reaction in a case of occlusion of the common duct with no urobilin in either the stools or the urine. The spectroscopic band of urobilin became unmistakable after the addition of iodin, but they are inclined to think that this may possibly have been due to a conversion of bilirubin into urobilin.

Hildebrant (1909), who, after many negative results, found the spectrum of urobilin in the serum in a case of pneumonia shortly before death, believes that urobilin normally circulates in the blood as urobilinogen, because in this case the urobilin band appeared only after the serum had been exposed for some time to light and air. He obtained a red color on mixing Ehrlich's reagent with the serum before the urobilin had appeared. Moller, though he is inclined to agree with Hildebrandt, points out the technical difficulties of urobilinogen recognition in serum, since the hydrochloric acid precipitates the proteins. He obtained an apparently positive reaction in only two of his cases. In three cases Conner and Roper showed the characteristic red color and confirmed the presence of urobilinogen by finding the absorption band of the benzaldehyd compound. But in the other cases in which urobilin was present they did not find any urobilinogen.

Conner and Roper's spectroscopic recognition of urobilinogen is the only observation which can be taken as approaching to a proof of the presence of this body in serum, and in view of the substances of the indol group which may give a spectrum very similar to that of the urobilinogen compound, their statement cannot, in the absence of any data as to the position of the absorption band, be taken as decisive.

In fact, the evidence so far points in just the opposite direction and seems to indicate that urobilin, instead of circulating in the reduction form of urobilinogen, is present as an oxidation product which does not possess the spectroscopic characteristics of urobilin and so escapes detection. This evidence is all the stronger because it has been collected independently by three observers since 1903; none of whom apparently knew about the work of the others. Finding no urobilin in the blood of cases in which he expected it, Schlesinger tried the effect of adding urobilin to serum and to blood. He discovered that whereas it was readily demonstrated in the serum, it disappeared in the whole blood or in serum to which red blood-cells had been added. Clarens made the same

observation and in 1911 Roth and Herzfeld confirmed Schlesinger's experiment and suggested that the disappearance of the urobilin might be due to oxidation.

They found that oxygenated serum destroyed the urobilin and that in whole blood its disappearance might be delayed by carbon dioxid. In a few cases, though not in all, they were able to demonstrate the presence of urobilin in serum after it had stood for from twenty-four to forty-eight hours mixed with finely powdered zinc. Since urobilinogen is found in the urine of patients in whom no urobilin can be found in the serum, it seems that the kidneys must possess the faculty of reducing the oxidized urobilin. They were able to carry out only two experiments with kidney extracts, one of which gave a definite production of urobilin in a serum in which it had previously been found absent. It is noteworthy that urobilin was formed with an alcoholic kidney extract, so that the process cannot depend on any vital activity in the cells.

The conception of the oxidation of urobilin in the blood-stream is strengthened when it is remembered that the only cases in which urobilin has been with certainty found in the serum are those in which there was a great reduction in the available oxygen of the blood. They have been almost without exception cases of lobar pneumonia shortly before death.

The effect on urobilin of the blood and tissues opens up a new field for investigation and it is not improbable that along these lines facts may be discovered which will throw light on the present obscurity.

IX. ANIMAL EXPERIMENTS

The Excretion of Urobilin in Animals

No satisfactory estimations could be made on the stools of rabbits or goats because the amount of urobilin was too small.

The same difficulty, though to a lesser extent, was encountered in dealing with the excreta of cats and dogs. Even when the extract was made as concentrated as possible it seldom required more than a few dilutions to obliterate the spectra of urobilinogen and urobilin. We have not been able to find any systematic investigation of the pigments of the stools of dogs or cats, but it is known that cholecyanin,[5] a derivative of bilirubin which is not regularly present in human stools, is constantly found. Possibly the greater part of the bilirubin in the intestine of these animals may be excreted in other forms than urobilin. Certainly the acid alcohol extracts of the stools contained a large amount of light-absorbing material, so much so as to render the urobilin band somewhat obscure at the low dilution at which it had to be read. And not infrequently other absorption bands were seen. The method did not then give such definite and clear results as were obtained with human stools.

5. Fischler: Inaug. Diss., Heidelberg, 1906, p. 84.

It was soon found that such small amounts of urobilin as were detected varied considerably from day to day. No satisfactory explanation for this variation was obvious, but one possible factor appeared to be the length of time during which the feces remained in the intestine. Hard constipated stools as a rule contained very little urobilin. The following experiment shows how markedly the quantity of urobilin increases during regular action of the bowels as compared with a period of constipation.

Dogs B, C and D were normal. Dog A had had a biliary fistula and a closure of the common bile-duct. At the time of this experiment the fistula was closed and the common duct was again patent. Urobilin was present in the urine and was added to the amount in the stools. The figures (Table 8) represent the total dilution value of the urobilin excreted during ten days. In the first period the dogs were constipated; in the second sufficient cascara was given to obtain a stool every day without giving rise to marked diarrhea.

TABLE 8.—UROBILIN IN CONSTIPATION AND IN REGULAR BOWEL ACTION

	First Period of Ten Days		Second Period of Ten Days	
	Urobilin	Number of Stools	Urobilin	Number of Stools
Dog A	5,640	4	13,120	10
Dog B	14,400	8	19,200	10
Dog C	9,200	5	16,420	10
Dog D	12,860	7	37,200	9

In these four dogs the total urobilin during the period of constipation was 42,100 and in the period during which cascara was given, 85,940, more than twice as much. During another ten-day period castor oil was given, and the total amount passed was 51,250, but regular daily movements were not obtained. Constipation, therefore, in dogs diminishes the amount of urobilin in the stools. We are satisfied that this is true for man also though we cannot bring forward any systematic evidence. The diminution may be due either to increased absorption of urobilin from the intestine or to destruction of the urobilin. If increased absorption were the cause, the excretion of urobilin in the urine should be increased. In normal dogs or cats urobilin was never found in the urine in sufficient amount to estimate. But in Dog A, in which the common duct had been tied and a biliary fistula made, it was found six months later that communication between the bile-ducts and the intestine had become reestablished and that the fistula had closed. In this animal considerable amounts of urobilin were constantly present in the urine. The cause of this was found at death to be an extremely marked cirrhosis of the liver, which had developed probably as a result of infection through the fistula combined with the initial bile stasis.

TABLE 9.—UROBILIN IN URINE AND IN STOOL OF DOG A

Date	Urobilin in Urine	Urobilin in Stool
Dec. 2	160	No stool
Dec. 3	248	800
Dec. 4	192	800
Dec. 5	224	No stool
Dec. 6	21	400
Dec. 7	100	No stool
Dec. 8	160	No stool
Dec. 9	120	No stool
Dec. 10	240	No stool
Dec. 11	400	1,200
Dec. 12	520	No stool
Dec. 13	240	No stool
Dec. 14	280	800
Dec. 15	80	No stool
Dec. 16	200	800
Dec. 17	600	No stool
Dec. 18	160	No stool
Dec. 19	680	400
Dec. 20	480	No stool
Dec. 21	500	No stool

Increased absorption during constipation might be expected to show itself in a rise of urobilin in the urine in this case. Yet we were not able to establish any such increase in urinary excretion. The average amount of urobilin in the urine on the seven days in which urobilin was lost from the body in the stools was 273, and 272 on the thirteen days during which urobilin was retained in the intestine. This evidence would have been more convincing if we had succeeded in contrasting a period of regular evacuation with a period of constipation, but on giving cascara it was found to be impossible to be sure of excluding contamination of the urine with the stools. Still if the figures for the four days from December 3 to December 6, inclusive, during which three stools were passed, are contrasted with those from the 7th to the 10th when there were none, it is seen that a total of 685 dilutions of urobilin were present in the first period, and only 620 when the bowels did not act at all.

It seems probable, therefore, that destruction of urobilin plays a much larger part than increased absorption in the diminution of urobilin which occurs during constipation. This is only what would be expected of a substance which outside the body is so readily altered.

Diarrhea, unless it is so pronounced as to lead to the excretion of unaltered bile, does not seem to have nearly so great an influence in diminishing the quantity of urobilin.

The total excretion of urobilin per kilogram of body-weight in dogs which had been kept under exactly the same conditions and on the same diet for a long time was determined over a period of forty-one days (Table 10).

TABLE 10.—Excretion of Urobilin per Kilogram of Body-Weight

	Weight, Kg.	Dilution Value Per Kg.
Dog C	7.82	6,100
Dog B	9.95	5,220
Dog D	17.80	5,450

Dog A, with cirrhosis of the liver, which was also kept under the same conditions for the same time, and which during that period exhibited no obvious signs of ill health, weighed 4.02 kg. and excreted urobilin with a dilution value of 8,806 per kilogram body-weight. This is an increase of 58 per cent. over the average excretion of urobilin in the three normal dogs. It does not seem probable that an increased hemoglobin metabolism could account for this. It appears much more likely that there was either a diminished destruction of urobilin or an increased formation of urobilin from bilirubin. Constipation was present to a more marked degree in this dog than in the others, so that the increase of urobilin was probably even more marked than the figures suggest. A diminished destruction, if such occurred, must have operated in the body itself. If one of the functions of the liver is the synthesis of urobilin absorbed from the intestine into bilirubin, an explanation of the increase in urobilin excretion is afforded by the supposition that the diseased liver in this case allowed much of the absorbed urobilin to pass through it into the general circulation to be excreted in the urine. But the possibility of the increase in urobilin being due to a formation of urobilin from bilirubin in the diseased liver cannot be excluded. Outside the body we found that urobilinogen and urobilin were produced in considerable amounts in bile left at a temperature of 37 C. And it seems possible that such a breaking down of bilirubin may occur in stagnating bile especially if an infection of the bile passages exists.

In attempting to follow the effect of closure of the common bile-duct in dogs on the urobilin excretion, a difficulty was encountered in connection with the methods we used. In the alcoholic stool extracts a pink color was produced which apparently showed the spectroscopic absorption of urobilinogen. That in reality it was some other substance was shown by the difference in the color reaction with Ehrlich's solution and also by the fact that on exposure to light of the zinc acetate filtrate the absorption band still persisted instead of disappearing. In all probability this substance was indol or some derivation of indol. Von Moracewski found that in alcoholic stools there is an increase in indol which has an absorption band very similar to that of urobilinogen.

To avoid confusion with this substance we have taken no account of the urobilinogen either before or after the closure of the common bile-duct. This makes the total amounts given inaccurate, but our object

was to see whether or not the absence of bile from the intestine would completely stop all excretion of urobilin. As is seen from the two experiments reported below, urobilin disappears for a time, but it afterward returns in diminished though relatively not inconsiderable amount in the stools. In both these cases also urobilin after an interval was found in the urine. The impossibility of obtaining urobilin-free stools in dogs even though all bile is prevented from entering the intestine has been noted by Fischler. Brugsch and Yoshimoto found that urobilin reappeared after an interval in the stools of a dog with biliary fistula.

OBSTRUCTION OF COMMON DUCT

Dog E.—Section of Common Duct Removed

Icterus, acholic stools and bile in the urine appeared. At the post-mortem a distended gall-bladder and ducts were found. No communication with the intestines. A few patches of organized lymph were found on the liver and adhesions round the resected duct but no general peritonitis. Urobilin given as total amounts in periods of six days. (Table 11.)

TABLE 11.—Total Urobilin, Dog E, in Periods of Six Days

Period	Stools	Urine
BEFORE COMMON DUCT CLOSURE		
First	3,200	0
Second	2,320	0
AFTER COMMON DUCT CLOSURE		
Third	0	0
Fourth	512	185
Fifth	32	56
Sixth	320	0
Seventh	160	76

Dog F.—Section of Common Bile-Duct Removed

Uninterrupted recovery with development of jaundice. At the post-mortem the gall-bladder and ducts were found to be greatly distended. There was no communication between them and the intestine. The urobilin is given as a total of estimated dilution value for the stools and urine passed in periods of six days. (Table 12.)

TABLE 12.—Total Estimated Dilution Value of Urobilin, Dog F, in Periods of Six Days

Period	Stools	Urine
BEFORE COMMON DUCT CLOSURE		
First	9,312	0
Second	5,120	0
AFTER COMMON DUCT CLOSURE		
Third	0	0
Fourth	0	0
Fifth	576	396
Sixth	2,368	1,084

BILIARY FISTULA WITH OBSTRUCTION OF COMMON DUCT

In three other dogs the common duct was obstructed and a biliary fistula established. No jaundice developed.

Here also the amount of urobilin in the stools decreased, but did not disappear. Urobilin appeared in the urine of Dog A, which was afterward found to have

developed a cirrhosis of the liver. In the other two it was absent. The urobilin is given as the total dilution values of six-day periods. (Table 13.)

After death no communication was found between the bile-passages and the intestine in Dogs G and H.

TABLE 13.—TOTAL DILUTION VALUES OF UROBILIN, DOGS G, H AND A, IN PERIODS OF SIX DAYS

Period	Stools	Urine*
BEFORE OPERATION		
Dog G: First	1,920	0
Second	1,590	0
Third	3,524	0

* Urobilin was occasionally found in the urine, but this was due to contamination with bile. It was at first difficult to prevent the urine wetting the dressings and taking up some of the bile which was rich in urobilin.

AFTER OPERATION		
Fourth	240	0
Fifth	1,845	0
Sixth	560	0
Seventh	384	0
BEFORE OPERATION		
Dog H: First	1,744	0
Second	930	0
Third	632	0
AFTER OPERATION		
Fourth	64	0
Fifth	176	0
Sixth	688	0
BEFORE OPERATION		
Dog A: First	3,480	0
Second	3,256	0
AFTER OPERATION		
Third	728	0
Fourth	520	22
Fifth	280	68
Sixth	1,260	26
Seventh	632	239
Eighth	720	...

The presence of urobilin in the stools after closure of the common bile-duct cannot be explained as due to the excretion of bilirubin into the intestine from the blood, for it was also found in the stools of the dogs in which the bile was allowed to drain out of the body and in which there was no increase of bilirubin in the blood. The only possible explanation seems to be that urobilin itself was excreted from the blood into the intestine. Where was this urobilin produced? Urobilin is known to have formed in old hematomas in various parts of the body, so that the formation of urobilin from bilirubin in the tissues of the icteric dogs is a possibility. But the fact that it also was found in the excreta of dogs which were not jaundiced points to the liver as the place

of formation, for in the dogs with biliary fistula this was the only organ in which bile was present. Urobilin was found in the bile escaping from the fistulas.

We must conclude then that in dogs the intestine is not the only site of origin of urobilin, but that under certain conditions urobilin is formed in the liver. Whether or not this is a normal function of the liver is another question which will be discussed later.

These experiments, though few in number and inconclusive in their results, are yet sufficient to show the complexity of the subject and the number of factors which may play a part in excretion of urobilin. Variations in the amount of formation of urobilin in the intestine or in destruction after it has been formed, the degree of absorption from the intestine, the fate of the absorbed urobilin in the liver, whether it is rebuilt to bilirubin or is allowed to pass into the general circulation, the question as to a change of bilirubin into urobilin in the liver under certain conditions, and the possibility of the excretion of urobilin from the blood into the intestine are some of the points which have to be considered in interpreting the results.

In any case we must conclude that in dogs at least the theory of the exclusively enterogenous origin of urobilin does not suffice to meet the facts.

X. CLINICAL REPORTS

To enumerate all of the conditions in which urobilin has been found in excess clinically does not seem to us desirable as we wish to point out the significance of its occurrence in the few in which its clinical study has been found to be of most value. The following clinical reports are selected from a series of observations made by us during the last two years.

Diseases of the Liver without Much, if Any, Interference with the Entrance of Bile into the Intestine

TABLE 14.—SUMMARY OF CASES 1-6

No.	Case	Length of Period of Observation, Days	Diagnosis	Average Daily Urobilin Urine	Stools
1	I. N.	53	Portal cirrhosis	572	3,647
2	A. F.	10	Portal cirrhosis	241	3,088
3	C. D.	14	Cirrhosis (syphilitic)	456	3,451
4	C. B.	30	Bronzed diabetes	5,800	7,400
5	F. M.	4	Hanot's cirrhosis	2,034	3,360
6	F. O'B.	15	Abscess of the liver	1,713	4,047

CASE 1.—I. N., enlarged liver with ascites. Red blood-cells about 3,000,000 and hemoglobin 55 per cent. Remained during the time of observation in about the same condition. Fluid was removed from the abdomen on several occasions. Urobilin was never found in it. There was a slight grade of jaundice, the serum on one occasion showing six times the normal amount of bilirubin. One month after the estimations were discontinued the patient died and the liver showed a well-marked portal cirrhosis. Chart 8 shows the urinary and stool excretion

of urobilin. The stools were given as averages for periods of three days to diminish variations due only to changes in the motor activity of the colon and rectum. The considerable variations in the quantities in the stools and the fact that urobilin was not constantly found in the urine are the noteworthy points in this case.

CASE 2.—A. F. A typical case of portal cirrohosis.

CASE 3.—C. D. Small liver, large spleen, hematemesis melena. Glycosuria with polyphagia, polydipsia and emaciation. Positive Wassermann.

CASE 4.—C. B. Very much enlarged liver and spleen. Ascites. Pigmented skin. Glycosuria. At post-mertem the diagnosis of bronzed diabetes was confirmed by the finding of a deeply pigmented cirrhotic liver and pancreas. Chart 9 shows the great variations in the amounts of urobilin in the urine.

CASE 5.—F. M., A man aged 48 complaining of persistent slight jaundice of two years' duration. No loss of weight. No pain. General health good. Liver greatly enlarged, extends 10 cm. below costal margin. Spleen much enlarged, projects 12 cm. below costal margin. No ascites. Urine contains bile. Red blood-cells 4,400,000. Hemoglobin 86 per cent. Negative Wassermann.

TABLE 15.—DAILY UROBILIN IN CASE 5

Date	Urine	Stools
November 23	1,300	4,800
November 24	2,950	5,120
November 25	1,104	960
November 26	2,784	2,560

CASE 6.—F. O'B. Admitted with a temperature and polymorphonuclear leukocytosis, complaining of pain and tenderness in the right hypochondriac region. On the eighth day after admission the abdomen was opened and a large abscess of the liver drained.

In this group of cases the urobilinuria is evidently not due to an increased formation of urobilin in the intestine, for the quantities found in the stools are not above the normal limits. There is no evidence of any overflooding of the liver by urobilin derived from an increased destruction of hemoglobin. That the abnormal quantity of urobilin in the urine is to be ascribed to the pathological changes in the liver which were present to a marked degree in all these cases is suggested not only by the constancy of the association of urobilinuria with liver disease which has been noted by all observers, but also by the fact that in particular cases it is possible to observe the diminution and final disappearance of urobilin from the urine in those cases in which an abnormal condition in the liver is removed. The case of F. O'B. with a large liver abscess was a good example of this. During the week preceding the opening of the abscess the average daily excretion was 2,187. For a few days after the operation the quantity in the urine remained high and then dropped so that the daily average for the seven days after operation was only 244.

It will be noted that it is not possible to exclude pronounced disease of the liver by a failure to find urobilin in the urine on one examination. As may be seen from the chart of the case of I. N. (Chart 8, Case 1), who had a very marked cirrhosis of the liver, there was a period of five days during which no urobilin could be detected. And even in the

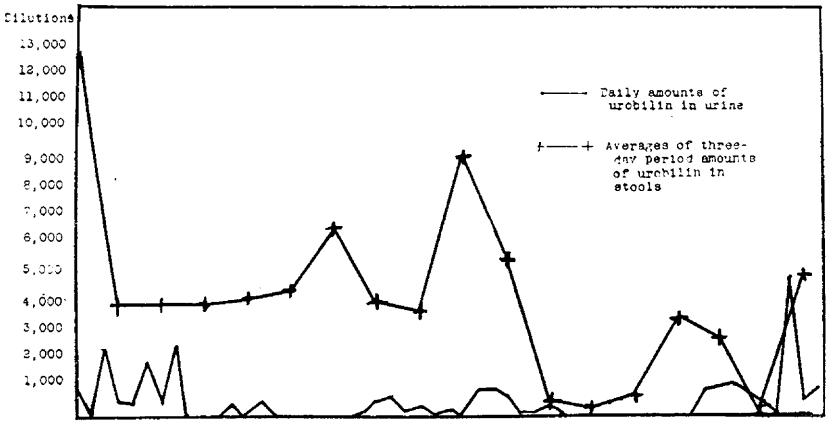


Chart 8.—Urinary and stool excretion of urobilin, Case 1.

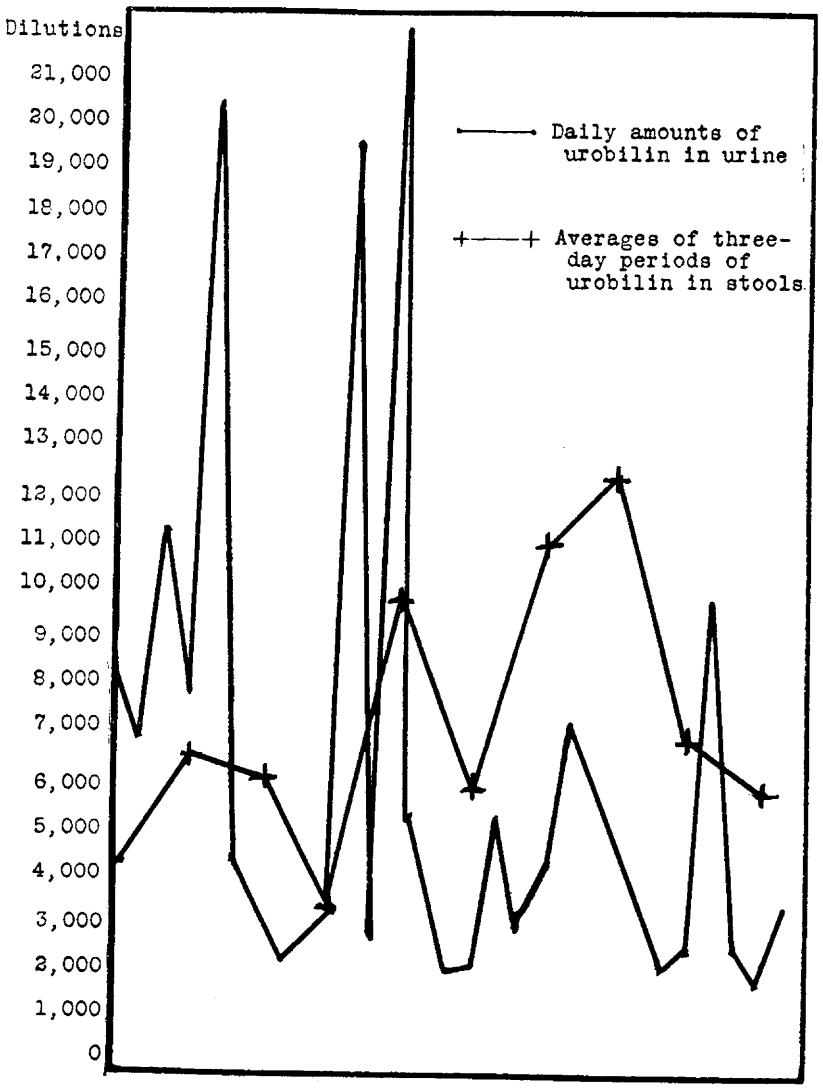


Chart 9.—Urinary and stool excretion of urobilin, Case 4.

patient C. B., in whose urine there was as a rule such a large amount of urobilin, none was found in one twenty-four-hour specimen. The fluctuation in the quantity of urobilin from hour to hour and from day to day makes it imperative to take the average of a series of twenty-four-hour specimens before an idea can be obtained as to the grade of urobilinuria in any particular case.

The study of a large series of cases of liver disease along these lines, with concomitant estimations of the urobilin in the stools and in association with other tests of hepatic efficiency, will be necessary before it is possible to say whether or not the degree of urobilinuria may be taken as an approximate guide to the amount of impairment of liver function.

Cases in Which Part of the Bile Entered the Intestine and Part Left the Body through a Gall-Bladder Fistula

TABLE 16.—SUMMARY OF CASES 7 AND 8

No.	Case	Length of Period of Observation, Days	Diagnosis	Average Daily Urobilin Bile	Urine	Stools
7	A. R.	18	Cholelithiasis	788	0	4,240
8	B. H.	13	Chronic cholecystitis	1,505	0	2,669

CASE 7.—A. R. This patient was operated on for relief of dyspeptic symptoms. No icterus. A thickened gall-bladder containing a large stone was found. The gall-bladder was drained. There was no jaundice following the operation.

CASE 8.—B. H. Operated on for symptoms of dyspepsia. No jaundice before or after operation. A moderately thickened injected gall-bladder with adhesions was found and drained.

In these cases there was no obstruction to the flow of bile through the common duct, and only a part of the bile escaped from the fistula.

The urobilin found in this fraction of the bile is of interest in connection with the question as to how much of the urobilin is simply excreted again into the intestine after absorption by the portal blood-vessels. To judge from these two cases, it would appear that a very large part of the absorbed urobilin is excreted into the intestine unchanged. But it must be remembered that neither of these was a normal case, and that the possibility of the formation of urobilin in the liver itself existed.

Complete Obstruction to the Entrance of Bile into the Intestine

TABLE 17.—SUMMARY OF CASES 9-12

No.	Case	Length of Period of Observation, Days	Diagnosis	Average Daily Urobilin Urine	Stools
9	F. W.*	34	Carcinoma of hepatic ducts	0	178
10	G. W.	5	Carcinoma of stomach	0	0
11	A. M.	6	Carcinoma of pancreas	0	0
12	B. M.	3	Carcinoma of duodenum	0	0

* Thirty-five gm. of dried bile were given to this patient by mouth in an attempt to confirm Müller's experiment in which he succeeded in producing urobilinuria in such cases. No urobilin appeared in the urine, but this may have been due to the form in which the bile was given. Fromholdt also obtained negative results with various preparations of bile and found that it was necessary to give fresh bile as Müller himself did. Some of the urobilin found may have been derived from bile given by mouth, but it was also found before it was taken and also more than two weeks afterward.

CASE 9.—F. W. This was a case which we have already reported in which there was a large liver, deep jaundice and clay-colored stools. At the post-mortem a large carcinomatous mass in the liver was found to have completely obstructed the hepatic duct.

CASE 10.—G. W. Jaundice had been present for two weeks before the estimations were made. Afterward, at operation a pyloric carcinoma obstructing the common duct was found.

CASE 11.—A. M. Deep jaundice of several months' duration. Post mortem a scirrhous carcinoma of the pancreas was found.

CASE 12.—B. M. At operation, a carcinoma of the duodenum was found which completely obstructed the ampulla of Vater.

In these four cases, no bile was entering the intestine except such traces as might be excreted from the blood. Bile was present in all the tissues of the body, and practically the only mode of excretion was through the urine.

The law of the enterogenous theory of the origin of urobilin, that without the entrance of bile into the intestine, no urobilin is formed, is borne out by the last three cases but not by the first.

In itself, we should not have been inclined to attribute much importance to this exception, if it had not been that Fischler and we ourselves had found urobilin in the stools of dogs with closed common ducts, and that several observers (Gerhardt, Hoppe-Seyler, Tsuchuya and Fischer and Meyer-Betz) have even found urobilin in the urine when no bile was entering the intestine.

Our method gives positive results in the urine only when the amount is distinctly larger than normal, so that urobilin may very well have been present. And it is noteworthy that we found urobilin in the fluid removed post mortem from the broken-down cancer tissue in the liver.

In the other three patients in whom no urobilin could be detected in the stools, the liver itself was not directly involved. It seems, then, that this case gives some evidence in support of the hypothesis that the diseased liver may itself form urobilin.

Cases in Which there was Obstruction to the Entrance of Bile into the Intestine and in Which Part of the Bile Left the Body through a Gall-Bladder Fistula

TABLE 18.—SUMMARY OF CASES 13 AND 14

No.	Case	Length of Period of Observation, Days	Diagnosis	Average Daily Urobilin		
				Bile	Urine	Stools
13	A. R.	5	Cholelithiasis	0	0	0
14	E. W.	8	Cholelithiasis	5,856	640	160

CASE 13.—A. R. Admitted with typical signs of acute cholecystitis. Slight icterus but no bile in the urine. The gall-bladder was drained. For the first few days the jaundice deepened and bile appeared in the urine. Thereafter, it diminished and on the fifth day after operation the urine was free from bile. On the seventh day no icterus could be detected.

CASE 14.—E. W. The patient was operated on for symptoms of gall-stones. There was no jaundice. A stone was removed from the common duct and the gall-bladder drained. After the operation there was deep jaundice, acholic stools and bile in the urine.

In both of these patients the manipulations during the operation caused a closure of the common duct, and the drainage through the fistula was not sufficiently free to prevent the occurrence of jaundice. There was, therefore, a complete or almost complete obstruction to the passage of bile into the duodenum combined with the escape of a part of the bile through the gall-bladder fistula.

Though clinically so similar, there is, nevertheless, a remarkable contrast in the urobilin figures. The patient A. R. might be cited as an excellent example in support of the purely enterogenous origin of urobilin, while the patient E. W. appears to offer very strong evidence in support of the possibility of the formation of urobilin in the liver under certain conditions.

The subsequent course of urobilin excretion in these two cases appears to be worthy of mention, and for this purpose it is most conveniently divided into periods corresponding with changes in the biliary excretion.

In Case 13, during the days immediately following the operation, when bile was still present in the intestinal tract, considerable amounts of urobilin were found in the urine and some in the bile. (Table 19, first period.) Thereafter, during the time in which there was jaundice, acholic stools and bile in the urine, urine, no urobilin was found either in the bile or urine. (Table 19, second period.)

In the third period, the jaundice diminished, the stools became colored, and with this urobilin reappeared in the bile and urine.

The fourth period seemed to illustrate the effect of recovery of the liver function, for as the patient's condition improved, the urobilin was found to diminish and at last disappear from the urine in spite of the fact that more bile was entering the intestine because of the removel of the gall-bladder drain.

TABLE 19.—UROBILIN IN CASE 13

Period and Date	Bile	Urine	Stools
First	432	1,270	*
Second	0	0	0
Third	492	1,240	5,896
Fourth:—			
April 1	144	704	0
April 2	18	864	1,600
April 3	†	656	7,680
April 4	...	448	8,960
April 5	...	0	7,680
April 6	...	0	6,400
Fifth:—			
May 1	0	0	160
May 2	0	1,224	240
May 3	0	3,900	64
May 4	84	4,480	‡
May 5	36	7,800	19,540
May 6	120	5,040	6,784
May 7	224	1,700	9,230

* No stools.
† Drain removed.
‡ Stool lost.

Three weeks later the patient returned with recurrence of the cholecystitis and at least a partial blocking of the common duct. On May 1 the fistula was reopened and much bile, mucus and pus escaped. Urobilin, present at first in very small amounts in the stools, increased in the next few days and urobilin reappeared in the urine and bile. (Table 19, fifth period.) During the whole of the next month during which the patient was under observation, urobilin was present in considerable amount in the urine, as if during this second attack, the liver had suffered some more lasting damage. Subsequently, though sometimes found in small amounts, it was often absent.

This case, therefore, supports what may be called the orthodox theory of the necessity of the presence of bile in the intestine for the production of urobilin, and also may be taken as illustrating the effect of damage to the liver on the urinary excretion of urobilin.

On the other hand, Case 14 cannot be reconciled with the exclusively enterogenous origin of urobilin and is the clearest instance we have encountered of the necessity of admitting that, at any rate in some cases, urobilin may be produced elsewhere than in the intestine.

During a time when the stools were colorless, the skin jaundiced, and the urine laden with bile, that is to say when everything pointed to the practical absence of bile from the intestine, there was a very large excretion of urobilin in the bile and a not inconsiderable amount in the urine.

TABLE 20.—UROBILIN IN CASE 14

Period	Bile	Urine	Stools
First	5,856	640	160
Second. (average daily excretion)	1,176	592	3,625

During the next eight days, urobilin reappeared in the stools and the jaundice diminished, but the urobilin in the bile and urine, instead of increasing as one might have expected, decreased in amount.

It seems most probable that in this case the urobilin in the urine during the first period was absorbed by the blood-stream from the urobilin in the bile and excreted through the kidneys. The diminution during the second period, in spite of the reentrance of bile into the intestine, may perhaps be ascribed to a decrease in the formation of urobilin by the liver, as it began to recover its normal functions. In any case, the large amount of urobilin in the bile cannot have been absorbed from the intestines.

If, in such cases as these, a marked urobilinuria may be present in spite of the total or almost total absence of urobilin from the intestine, it would seem to be likely that in all cases of urobilinuria in liver disease, some part at least of the urobilin has an extra-intestinal origin.

CASE 15.—A. H. Pernicious anemia. During the period in which estimations were carried out, the red blood-cells varied from 1,300,000 to 1,800,000 and the hemoglobin from 31 to 38 per cent.

CASE 16.—A. T. Pernicious anemia. The red blood-cells numbered 1,000,000 and the hemoglobin was 28 per cent.

Diseases Associated with Changes in Hemoglobin Metabolism

TABLE 21.—SUMMARY OF CASES 15-19

No.	Case	Length of Period of Observation, Days	Diagnosis	Average Daily Urobilin Urine	Stools
15	A. H.	25	Pernicious anemia	470	24,977
16	A. T.	12	Pernicious anemia	1,058	22,014
17	E. P.	4	Secondary anemia from intestinal hemorrhage	0	2,400
18	L. S.	4	Primary polycythemia	1,849	3,240
19	H. L.	7	Secondary polycythemia from asthma and emphysema	806	10,011

CASE 17.—E. P. For eleven years the patient had had bleeding from the bowel. The stools were sometimes tarry, sometimes contained bright red blood. No cause was found even after the abdomen was opened. At the time of observation the red blood-cells varied from 2,100,000 to 2,550,000 and the hemoglobin from 27 to 29 per cent. Hemorrhage ceased after a Whitehead operation and the patient entirely recovered.

TABLE 22.—UROBILIN IN CASE 17.

Date	Urine	Stools
November 23	0	5,120
November 24	0	*
November 25	0	2,560
November 26	0	1,920

* No stool.

CASE 18.—L. S. Noticed a bluish tinge in her face and hands three years ago. During the time of observation the red cells numbered from six and one-half to seven and one-half millions and the hemoglobin was over 110 per cent. No normoblasts were found. There was no splenic enlargement.

TABLE 23.—UROBILIN IN CASE 18

Date	Urine	Stools
September 5	4,455	800
September 6	1,560	8,960
September 7	1,080	3,200
September 8	360	*

* No stool.

CASE 19.—H. L. Asthma and emphysema since childhood. Marked and constant cyanosis. At the time of observation there were 7,350,000 red blood-cells, and 120 per cent. of hemoglobin. There was no fever and the general health of the patient was as good as it ever had been.

TABLE 24.—UROBILIN IN CASE 19

Date	Urine	Stools
September 2	980	8,000
September 3	2,016	14,080
September 4	0	11,520
September 5	864	8,000
September 6	0	9,600
September 7	900	4,480
September 8	880	14,400

Pernicious Anemia.—The amounts of urobilin in the stools of these two patients on whom a careful study was made are much higher than we have found in any other condition, although in neither of them was there any acute blood crisis during the time of observation. The

quantity in the urine was not very great, but in two other patients from whom the stools could not be obtained for examination, very large amounts were found, corresponding to 4,895 and 16,800 dilutions.

That the increase of urobilin in the urine is in the main due to an overburdening of the liver with an excessive amount of urobilin absorbed from the intestinal tract, and not to a primary functional deficiency of the liver, is most probable.

The marked increase of urobilin in the stools is of special interest as evidence of a distinction between those forms of anemia associated with a great increase in hemoglobin metabolism, such as the cases of pernicious anemia we have cited and others, such as the secondary anemias following hemorrhage and carcinoma, in which the low urobilin content of the stools indicates a diminished destruction of hemoglobin.

Secondary Anemia.—It is especially in cases of secondary anemia that combined urobilin estimations in the urine and stools ought to afford interesting and valuable results.

The case of secondary anemia from hemorrhage which is given above affords a striking contrast when compared with the cases of pernicious anemia. Other cases in which secondary anemia was a complication of some other condition such as carcinoma, showed a diminution rather than an increase in total urobilin excretion.

On the other hand, the case of H. D., who had a diplostreptococcal endocarditis, may be cited as an example of an anemia due to increased blood destruction. During the first twenty-six days the average excretion in the stools was 12,841, twice as great as the normal average, and there was a dilution value of 781 in the urine. During the next seventeen days there was a fall in the stool excretion to 7,801 and in the urine to 659, although there was no increase in the percentage of hemoglobin or red blood-cells. The estimation of red blood-cells and hemoglobin can give no adequate guide as to the total turn-over of hemoglobin in the body, but an approximation to this may be obtained by urobilin determinations in the urine and stools. In cases of anemia of unknown origin, the finding of an increased urobilin excretion might be of diagnostic importance in directing attention to the search for some active blood-destroying agent as the cause.

Polycythemia.—The case of primary polycythemia gave unexpected results. The urobilin in the stools was low instead of being increased, and at the same time there was a very considerable amount of urobilin in the urine, although there was no evidence of any liver disturbance. The finding of urobilin in the urine of the patient with secondary polycythemia was also unexpected.

Both these patients were markedly and constantly cyanosed, and when the connection between cyanosis and the presence of urobilin in

the serum is recalled, the question rises as to whether, under normal conditions, a part of the urobilin absorbed from the intestine may not escape the liver and be changed in the blood, except under certain conditions, such as a diminution in the percentage of oxygen, when it might be excreted in the urine.

Unfortunately, we have not had an opportunity of following up this point in other cases with cyanosis.

Miscellaneous Cases

In a large number of patients with various disorders, no pathological increase in the urobilin in the urine was found.

In some, however, who showed no evidence of gross anatomical disease of the liver or of great blood destruction, considerable amounts of urobilin were present in the urine. One of these, a case of alcoholism, is of interest.

The patient was 59 years old and had suffered from attacks of acute gout for forty years. There were large urate deposits round the joints. At the time of observation, he was recovering from a prolonged spell of drinking, and was in a condition of great prostration with complete anorexia and vomiting. There was some edema of the legs. There was no liver or splenic enlargement. A few months later he died suddenly, but the cause of death was not ascertained.

Unfortunately, satisfactory collections of the stools could not be made, but the amount of urobilin appeared to be very low. On one day no urobilin could be detected and on two others amounts of 216 and 1,728 were found.

The urine over a period of nine days showed a daily average of 1,292.

In the absence of any indication of gross liver disease, the most probable interpretation of the appearance of the large amounts of urobilin in the urine in this case is to be found in damage to the functional efficiency of the liver by excessive amounts of alcohol.

In a patient with a huge retroperitoneal sarcoma who was cachectic and feverish, the average urobilin excretion in the urine was 1,545 and 11,893 in the stools. A case of carcinoma of the stomach without any metastases in the liver had an amount in the urine corresponding to 794, and 4,576 in the stools.

These cases are more difficult to interpret than two others in which there were very large masses of cancerous tissue in the liver, in both of which urobilin was found in great excess in the urine.

The patient with cardiac decompensation and swollen liver, whom we mentioned in our first paper, had an average of 2,682 in the urine and 8,619 in the stools.

Three cases of effusion of blood into the tissues were examined, but cannot be said to throw any light on the question as to whether or not

urobilin in the urine may have its origin in the breaking down of hemoglobin outside the vessels. One was a case of lymphatic leukemia with hemorrhages into the pharynx, gums and orbital cavities. There was no urobilin in the urine and only 2,792 in the stools.

In a case of caseous tuberculous peritonitis with a large amount of blood in the peritoneal cavity there was an average urinary excretion of 1,264, and in a large pulmonary hemorrhage in a case of compensated mitral stenosis there was an average of 415 in the urine and 15,106 in the stools. There are too many other possibilities which might account for the urobilin in the urine in the two cases in which it was present. Cases of complete occlusion of the common duct with large hemorrhages are the only ones by which it is possible to decide this point.

A disturbance of liver function is, of course, a possibility which is difficult to exclude in almost any case, but it does not seem justifiable at present to attribute every case of excessive excretion of urobilin in the urine without increase in the stools to this cause alone.

XI. CONCLUSIONS

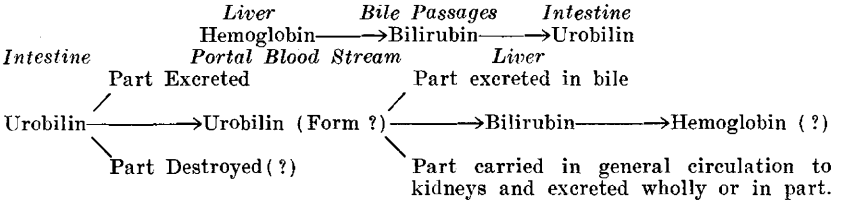
A. General

1. The intestinal formation of urobilin from the decomposition of bile present within the bowel is the usual mode of origin. In complete closure of the common duct due to carcinoma, for instance, there is usually absence of urobilin from the urine, bile and stools.

2. There is evidence that the diseased liver may originate urobilin either directly as a product of its cells, or indirectly from decomposition of bilirubin within the bile passages. Thus, patients in whom no bile is reaching the intestine, may nevertheless show urobilin in large amount in the bile, and to some extent even in the urine and stools if the liver itself is diseased or functionally deficient. The urobilin in the urine and stools of such cases must be due to excretion from the blood of urobilin absorbed from the liver.

The usual sequence in hemoglobin metabolism may be represented as in the accompanying scheme.

SCHEMATIC REPRESENTATION OF USUAL SEQUENCE IN HEMOGLOBIN METABOLISM



On this hypothesis, bilirubin is broken down to urobilin in the intestine and the absorbed urobilin is synthesized in the liver to bilirubin and perhaps by a reverse action again to hemoglobin.

Under abnormal conditions in the liver, formation of bilirubin from the absorbed urobilin from the intestine may fail, and further there may be a reduction of bilirubin to urobilin in the liver itself, a reversal of the normal process.

Under these circumstances, the accumulated urobilin in the liver will be absorbed by the blood-stream and excreted in the urine.

3. An increased quantity of urobilin in the stools indicates increased blood destruction.

In such cases, urobilin may appear in excessive amount in the urine apart from any obvious disease of the liver, because the quantity of urobilin carried to the liver from the intestine being so much increased, the fraction carried past the liver into the general circulation and so to the kidneys is sufficient to lead to an obvious urobilinuria.

4. There is a close association between increase of urobilin in the urine and disturbance of liver function. But even if increased blood destruction is excluded by urobilin estimations in the stools, it is not at present possible to regard the amount of urobilin in the urine as an approximate guide to the functional efficiency of the liver. The occasional occurrence of large amounts of urobilin in the urine of patients in whom there is no reason to suspect damage to the liver, the great and irregular fluctuations of urobilin excretion in cases of liver disease from hour to hour and from day to day, and the want of knowledge as to the fate of urobilin in the blood and tissues make it necessary to recognize that there may be other factors besides the condition of the liver and the amount of blood destruction which influence the excretion of urobilin in the urine.

B. Clinical

Before discussing the clinical importance of the phenomena of urobilin elimination, we should like to draw particular attention, (1) to the insufficiency of our present quantitative methods of urobilin estimation, especially in the urine, and to state that they undoubtedly will be very difficult to overcome because of the marked instability of the group of substances included under this name; and (2) to the value, until now insufficiently appreciated, of estimations of urobilin in the stools as a guide to the amount of blood destruction in the body.

The interpretation of urobilin findings in the urine is interesting, but at present somewhat problematical, whereas the determination of an increased intestinal excretion rests on a much firmer basis, and should yield results of diagnostic importance.

Since this work was undertaken with the primary object of finding out just how much significance should be attached to the presence of abnormal quantities of urobilin and urobilinogen in the excretions in ordinary clinical work, we shall summarize briefly our conclusions, basing

them on our own experimental studies, clinical observations and a fairly exhaustive study of the literature. The difficulty of the problem has been to form a judgment as to how far one is able clinically to make assertions from the data at present obtainable. To choose the essential facts from the maze of non-essential detail and from the numerous observations made to substantiate a previously accepted theory has been difficult. Nevertheless, we feel that we are within safe limits in the following conclusions as to the significance of the presence of abnormal quantities of urobilin in certain pathological conditions.

1. *Hepatic Cirrhosis.*—Urobilinuria is of marked value as evidence of a definite pathological change in the enlarged liver of alcoholic persons and occurs almost constantly in the hypertrophic stage of hepatic cirrhosis. In our cases, we did not find any increase of urobilin in the stools. While in advanced cases, part of the urobilin in the urine may be derived from the formation of urobilin from bilirubin in the liver, it is probable that as a rule it is due to failure on the part of the liver to synthesize to bilirubin, the urobilin brought to it from the intestine.

2. *Hepatic Stasis.*—Estimations of urobilin in the urine are of value in judging the amount of damage done to the liver parenchyma by chronic passive congestion. Hence, a marked increase of it is of ominous prognostic significance in cardiac decompensation.

3. *Jaundice.*—Urobilinuria is absent or insignificant in cases of obstructive jaundice. Its intermittent occurrence points to an incomplete obstruction with concomitant damage to the liver. It is present in the icterus of cases of increased destruction of the red blood-cells. Careful studies for urobilin should be made in suspected cases of chloroform poisoning, acute yellow atrophy and other similar conditions in which the liver is apt to be damaged.

4. *Malaria.*—The great increase of urobilin in the stools and the urobilinuria which occurs in severe cases of malaria is of diagnostic importance in obscure febrile conditions. The persistence of urobilinuria after malarial attacks, when there is no fever and no increase of urobilin in the stools, can be taken as evidence of some complication such as hepatic cirrhosis or abscess.

5. *Anemias.*—By means of urobilin estimations in the stools and urine, those forms of anemia associated with an increased blood destruction may be differentiated. It is probably in this field that the most valuable clinical results will be obtained. The contrast between the very large total urobilin elimination in our cases of pernicious anemia as compared with the small amount found in secondary anemias following hemorrhage or carcinoma was very striking.

6. *Pneumonia.*—A slight increase of urobilin with additional amounts at the time of resolution does not indicate a severe damage of the liver

parenchyma in this disease. It is probably due to a clogging of the liver with the products of resolution and absorbed hemoglobin from the affected lung. A study of the curve of this elimination may be of value in certain pneumonias. Allowances must always be made for other factors such as constipation, diarrhea and the inability of the kidney to eliminate urobilin readily in the presence of a complicating severe nephritis. The early appearance of large quantities of urobilin in the urine in pneumonia, particularly in the presence of jaundice, makes the prognosis grave. The occurrence of urobilin in the serum of pneumonia patients is of the gravest prognostic significance. It is recognized apparently by our present methods only in the presence of a degree of cyanosis markedly unfavorable to the life of the individual.

7. *Carcinoma.*—Except in those cases in which there is practically a complete disappearance of urobilin from the excretions, due to an obstruction of the common duct from carcinoma, we have not found its estimation in carcinomatous cases to be of evident clinical value.

8. *Infections.* — In infectious processes causing parenchymatous changes in the liver or accompanied by hemolysis, urobilin estimations are of definite value in estimating both of these factors in any given case. There is apparently an approximation between the urobilin excretion in the urine and feces and the amount of blood destruction. In such infectious processes as amebic colitis, the appearance and constant presence of urobilin may be of marked value in indicating the presence of inflammatory liver conditions, particularly abscess.

9. *Scarlet Fever, Measles.*—The majority of measles cases present urobilinuria in excess, but, as might be expected, the amount is less and the duration shorter than in scarlet fever with its more definite damage to the hepatic cells.

10. *Decompensation.*—As indicated previously, marked urobilinuria is indicative of pathological change in the liver in the stasis of decompensation. This circulatory stasis calls for the use of increasing amounts of hemoglobin within the body and is associated with a greater production and elimination of bilirubin leading to an inability on the part of the overburdened and congested liver to modify the abnormal amounts of urobilin reaching it. A return of compensation is often indicated by the disappearance of a qualitative urobilinogen or urobilin test. Decompensations in markedly anemic persons are not so apt to be accompanied by increased amounts of urobilin until very profound stasis occurs.

11. *Nephritis.*—Severe nephritis of various types, even in the presence of increased production of urobilin, may prevent definite urobilinuria. Large amounts of urobilin in the urine may be taken as indirect evidence of a certain degree of efficiency in the renal epithelium.

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