

BLOOD PIGMENT METABOLISM AND ITS RELATION TO LIVER FUNCTION *

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The exact mechanism involved in the metabolism of the blood pigments, and the precise relation of the liver to these processes, are still but imperfectly understood. Certain theories, however, concerning blood pigment metabolism are very generally accepted. The pigments of the bile have long been believed to be derivatives, in part at least, of hemoglobin. The work of Eppinger and Charnas,¹ Wilbur and Addis,² Robertson,³ Schneider,⁴ Hansmann and Howard,⁵ Giffin, Sanford and Szlapka,⁶ and others has shown that excessive degrees of red cell destruction are accompanied by an increased elimination of bile pigments. Most observers agree that the liver is the main agent concerned in these metabolic changes. However, the lower bile pigments, principally urobilin and urobilinogen, have been supposed to be formed independently of the liver, by the action of bacteria in the lower intestine, on the bilirubin of the bile. The recent work of Hooper and Whipple⁷ on dogs with biliary fistulae has made necessary a modification of previous theories. These investigators question the intestinal production of urobilinogen and urobilin, and the absorption of these pigments from the portal circulation. They suggest that the liver itself is capable of forming these substances. They also prove that bilirubin can be formed in various parts of the body without the intervention of the liver, and conclude that normally the liver may be only one of several agents in the process of hemoglobin metabolism.⁸ Furthermore, they produce evidence that red cell destruction, with the consequent liberation of hemoglobin, is not the only factor in the production

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1. Eppinger and Charnas: *Arch. f. klin. Med.* **78**:387, 1913.
2. Wilbur and Addis: *Arch. Int. Med.* **13**:325 (March) 1914.
3. Robertson, O. W.: *Arch. Int. Med.* **15**:1072 (June) 1915.
4. Schneider, J. P.: *Arch. Int. Med.* **17**:32 (Jan.) 1916.
5. Hansmann and Howard: *J. A. M. A.* **73**:1262 (Oct. 25) 1919.
6. Giffin, Sanford and Szlapka: *Am. J. M. Sc.* **182**:562, 1918.
7. Hooper and Whipple: *Am. J. Physiol.* **40**:332, 1916.
8. Whipple and Hooper: *J. Exper. M.* **17**:612, 1913.

of bile pigments. Dietary changes⁹ and various drugs¹⁰ are able to cause marked alterations in the elimination of pigments in the bile.

The resynthesis of the bile pigments to hemoglobin is a further property usually attributed to the liver. At present it is generally believed that the liver is able in some way to build up the lower pigment fractions into the more complex molecule of hemoglobin. However, evidence is lacking on this point, as well as to the manner in which hemoglobin becomes incorporated in the red corpuscles.

It is the purpose of this paper to present certain evidence regarding the normal and abnormal physiology of blood pigment metabolism in man, and to demonstrate, if possible, a further relation between the liver and such processes.

METHOD

The introduction of "biliary drainage" by Lyon,¹¹ in 1919, has provided a method by means of which a more systematic clinical study of the duodenum and biliary tract is permitted than was previously possible. Lyon claims that a solution of magnesium sulphate introduced through the duodenal tube relaxes the sphincter of Oddi, and thus a free flow of bile into the duodenum is obtained. Furthermore, Lyon believes, following Meltzer's¹² theory of contrary innervation, that the magnesium sulphate causes a contraction of the gallbladder musculature, with a resulting flow of gallbladder contents into the duodenum. Following the flow of dark gallbladder bile Lyon obtains a flow of lighter colored bile which he believes is derived from the upper biliary radicles and liver. Lyon thus attempts, after the use of magnesium sulphate, to divide the bile drainage into three fractions, "A," "B" and "C," which are supposed to contain respectively bile from the common duct, gallbladder and liver. By a study of the gross color, certain other physical characteristics, the sediment and the bacteriology of these fractions, Lyon believes it possible to diagnose and localize pathology existing in the duodenum and various portions of the biliary tract.

Although Lyon presents clinical data which are quite consistent with his assumptions, nevertheless definite experimental proof that magnesium sulphate, when introduced into the duodenum, causes a contraction of the gallbladder is conspicuously lacking. The exact action of the salt in the duodenum has yet to be determined. There is a certain amount of evidence that the relaxation of the sphincter of the common bile duct may not be accompanied by contraction of the

9. Hooper and Whipple: *J. Exper. M.* **23**:137, 1916.

10. Bauer and Spiegel: *Deutsch. Arch. f. klin. Med.* **1**:129, 1919.

11. Lyon, B. B. V.: *J. A. M. A.* **73**:980 (Sept. 27) 1919.

12. Meltzer, S. J.: *Am. J. M. Sc.* **153**:469, 1917.

gallbladder walls. Chrohn, Reiss and Radin¹³ were unable to prove experimentally the existence of such a contrary innervation, although they apparently believe that the so-called "B" bile contains gallbladder contents. Einhorn¹⁴ claims that various salts produce a flow of "B" bile into the duodenum. This assumption is undoubtedly correct, although magnesium sulphate produces more constant and better results than other salts. Einhorn further concludes from his experiments that the flow of "B" bile is not due to a flow of bile from the gallbladder into the duodenum, but that it is due merely to stimulation of liver cells to increased activity, with a resulting excretion of bile pigment in increased concentration. This conclusion, however, is based on crude quantitative estimations of pigment values, and is probably incorrect. Careful determinations of the bile pigments in a series of fractions taken before and after the use of magnesium sulphate, in a number of cases in which there was known to be no flow of bile possible from the gallbladder, either on account of a previous obstruction of the cystic duct, or on account of a previous cholecystectomy, tend, by comparison with a series of normal cases, to disprove Einhorn's conclusions. Further reference will be made to these determinations in a later portion of this paper.

For practical consideration, in spite of the fact that experimental work is still lacking as to the exact source of the "B" bile, it seems expedient to assume that it is made up, in part, of bile from the gallbladder. It is highly probable that a solution of magnesium sulphate, when instilled into the duodenum, accomplishes two things. First, it relaxes the sphincter of the common bile duct and causes a free flow of bile into the duodenum. Second, it probably causes a slight contraction of the gallbladder musculature, with the result that some bile from that organ is mixed in with the bile proceeding down the common duct. The result is a mixture of duct, liver and gallbladder bile.

Bile pigments in the duodenal contents have received but slight attention. Schneider,⁴ in 1916, and subsequently others, have made quantitative estimates of the bile pigments of the duodenal contents, using a spectroscopic method. These observers concluded that in those cases in which it is generally considered that increased blood destruction is taking place the excretion of bile pigments is also increased. Schneider attempted to show a definite relation between the level of the bile pigments in the duodenum and the actual degree of hemolysis obtaining in any given case. Eppinger, Wilbur and Addis, Robertson, and others had previously obtained high pigment value in similar instances, by making bile pigment determinations of the stools. Hans-

13. Crohn, Reiss and Radin: *J. A. M. A.* **76**:1567 (June 4) 1921.

14. Einhorn, M.: *New York M. J.* **113**:313, 1921.

mann and Howard compared the method of estimating the pigments in the stools with the estimations based on the duodenal contents. Figures obtained by either method gave relatively high pigment values in those cases in which increased hemolysis was apparently taking place. Their findings were confirmatory of results obtained by Wilbur and Addis, but Hansmann and Howard do not believe that estimates based on duodenal contents run exactly parallel to those obtained from stool examination. Hansmann and Howard, however, believe the stool method to be more correct. Examination of duodenal contents seems, nevertheless, the more logical method of study. Such a method allows a study of the bile before the pigments have become diminished or altered by action of the intestinal bacteria. Furthermore, analyses based on estimation of bile pigments in the duodenal contents are performed more easily than similar determinations on the stools and are not subject to errors due to such variable factors as constipation, diarrhea, etc.

Lyon's method of obtaining a continuous flow of bile offers a distinct advantage over the method employed by Schneider and others, in which determinations were based entirely on single specimens. Single specimens, in the present studies, were subject to the greatest variations, on account of the intermittent flow of bile from the common bile duct, and on account of various other factors such as salivary, gastric and pancreatic secretions, which introduced errors by causing a dilution of the pigment content in the duodenum.

The technic used in this series of cases consisted in the introduction of the duodenal tube, and the collection by siphonage of duodenal contents in six fractions. These six fractions were collected over fifteen minute intervals, two fractions being taken from the fasting duodenum prior to the introduction of a 33 per cent. solution of magnesium sulphate, and four immediately following the use of the salt. The entire collection of duodenal contents thus covered a period of about one hour and a half.

The duodenal tube was retained over a period of from two to three hours in the majority of cases, depending on the length of time necessary for the tip to reach the duodenum. The exact location of the tube in the duodenum was determined by fluoroscopic examination in the majority of cases. Atropin sulphate, given before the introduction of the tube, practically eliminated any undue flow of saliva. The use of magnesium sulphate provided a nearly continuous and concentrated flow of bile into the duodenum and minimized the errors caused by the flow of gastric and pancreatic secretions. The objection might be raised that atropin might of itself introduce an error, by causing individual variations in the output of bile. Atropin does cause a slight

diminution in the excretion of bile by the liver cells.¹⁰ This diminution is, however, very slight and in the cases studied the administration of the drug caused no appreciable effect in the flow of bile into the duodenum.

Bile pigments were estimated by Wilbur and Addis' method of spectroscopic examination, for each of the six fractions. This method consists essentially in dissolving the urobilinogen, urobilin and other lower bile pigments in a saturated alcoholic solution of zinc acetate, and then determining the pigment content by the spectroscope. The number of dilutions necessary to cause the disappearance of the characteristic absorption bands of the individual pigment was taken as the reading for any particular fraction, and a curve was plotted from the values obtained. Values of urobilinogen and urobilin were added together, and the total taken as the pigment value of the fraction. An attempt was also made to quantitate the bilirubin values of the duodenal contents, by the method described by Hooper and Whipple in their work on dogs, but it was found impossible to obtain consistent readings on human bile owing to the conversion of bilirubin in some of the fractions into bilicyanin. The color obtained by this method, by treating the bile with acid alcohol, was in some instances the characteristic blue-green desired and could be read against a standard solution of copper sulphate as described by these authors. In the majority of cases, however, the color ranged from a decided green to a dark blue, and occasionally the entire series of fractions was intensely purple owing to the presence of bilicyanin. Similar observations on animals have been made recently by Rous and McMaster.¹⁵ Bilirubin figures, when obtained, ran approximately parallel to those of urobilin and urobilinogen. The actual dilution figures obtained from spectroscopic examination were not multiplied by a constant, as done by Wilbur and Addis in their original work, and later by Schneider, as there seemed no advantage to be gained by this purely artificial procedure. The curves shown on the accompanying charts, therefore, represent actual dilution values of urobilinogen plus urobilin.

The method of fractional analysis, I believe, offers distinct advantages over the method of studying only a single specimen. It provides a free flow of bile into the duodenum over a considerable period of time, and permits the taking of an average figure as well as the value of individual fractions. In this way it is possible to make a comparative study of the different fractions, and to obtain a much more exact picture of the level of bile pigments than can be gained from any single observation. Even such a method, however, is open to error,

15. Rous and McMaster: *J. Exper. M.* **34**:47, 1921.

and I wish only to point out its advantages and to emphasize its relative accuracy.

In addition to an estimation of the bile pigment in the duodenal contents, Blankenhorn's¹⁶ method for studying the bilirubin content of the blood plasma was employed. This method consists essentially in a comparison of the yellow color of oxalated plasma with distilled water. Dilutions of the plasma with water are made until the yellow color has disappeared. The number of dilutions necessary to remove the yellow color of the plasma are taken as the approximate bilirubin content of the specimen. Normally between fifteen and twenty dilutions give the desired end-point.

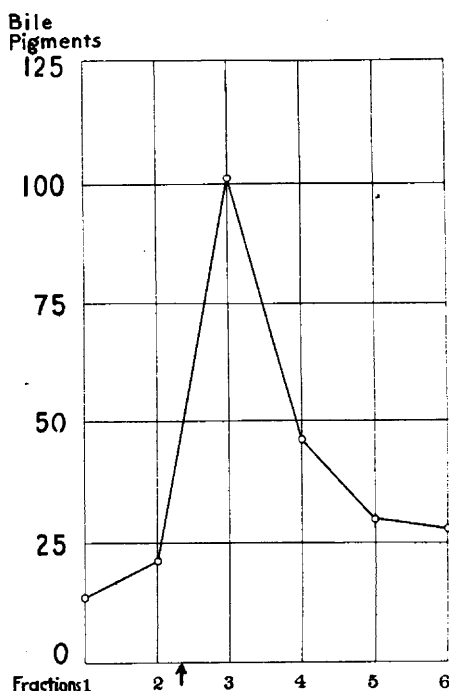


Chart 1.—Duodenal pigments in normal individuals. In this, and subsequent charts, points along the ordinates represent dilution values of the bile pigments, urobilin and urobilinogen. Points on the abscissae represent separate fractions of duodenal contents collected over fifteen minute intervals. The above curve is identical with the "normal" curves given in Charts 2, 3, 4 and 5, although in each case the scale varies. The arrow indicates the administration of 50 c.c. of a 33 per cent. solution of magnesium sulphate.

Bile Pigments in the Duodenal Contents in Normals.—As a basis for comparison with pathologic cases, observations on the pigment values in the duodenal contents of eight normal individuals were made

16. Blankenhorn, M. S.: Arch. Int. Med. **19**:344 (March) 1917.

and an average curve drawn from the results obtained. As shown in Chart 1, the average reading of all the six fractions in this series of normal cases was forty dilution units, with a maximum variation from this figure of ten units. The peak of the curve came shortly after the administration of magnesium sulphate and represents Lyon's "B" bile. The average pigment value of the peak of the curve was one hundred dilution units, with an individual variation up to fifty units. It will be seen that the variation from the average figures is a wide one, both in individual fractions and in the case of the general averages obtained from the total fractional estimations. These variations occurred in spite of the fact that duodenal contents in all cases were taken under similar conditions as regards the fasting state, the time at which the duodenal contents were collected and general freedom from symptoms. This point should be emphasized, inasmuch as previous investigators have inferred that the individual variation among normal persons is only a slight one. Furthermore, it is noticeable that there was a wide variation between individual fractions in the same normal person, even before the administration of magnesium sulphate. Bauer and Spiegel¹⁰ have noticed similar variations in normal individuals in estimating the bilirubin content of the blood plasma. A further discrepancy may be observed between the pigment values in normals as given by Schneider and the values obtained before the use of magnesium sulphate in this series of determinations. My figures for normal individuals are relatively higher than those of Schneider. His average normal figure is about five dilution units. The results obtained from my series of normals, in the fractions that are comparable to his analyses, average about 8.5 dilution units. The difference between the two figures may be explained (1) by individual differences in obtaining end-points by spectroscopic examination, or (2) by the fact that specimens of duodenal contents were taken in this series after waiting a relatively long time following the introduction of the tube. Such a wait would insure a better flow of bile. In either event the differences are purely relative, and conclusions based on examinations of similar cases in both series are in the main identical.

In cases such as gastric ulcer, and so forth, in which there was no apparent cause for abnormal pigment values, there was essentially no deviation from the normal range.

Evidence of a Flow of Gallbladder Bile Following the Use of Magnesium Sulphate.—Following the establishment of the normal figures a series of cases was studied in which there was absolute obstruction of the cystic duct, as proved at operation, or in which the gallbladder had previously been removed. Obviously, there could be no flow of gallbladder or "B" bile in these cases and a comparison of results

obtained in these cases with the normal figures already given showed no characteristic peak after the administration of magnesium sulphate. On the other hand, there was only a moderate rise in pigment values after giving the salt, as shown in Chart 2. This rise can be explained entirely by a relaxation of the sphincter of Oddi, with a resulting free flow of undiluted bile into the duodenum.

In another series of cases in which there was definite gallbladder pathology without obstruction, the fractions taken immediately after the administration of magnesium sulphate, or in other words, those fractions taken at a time corresponding to the peak of the pigment curve, were the only ones to show certain cellular and crystalline ele-

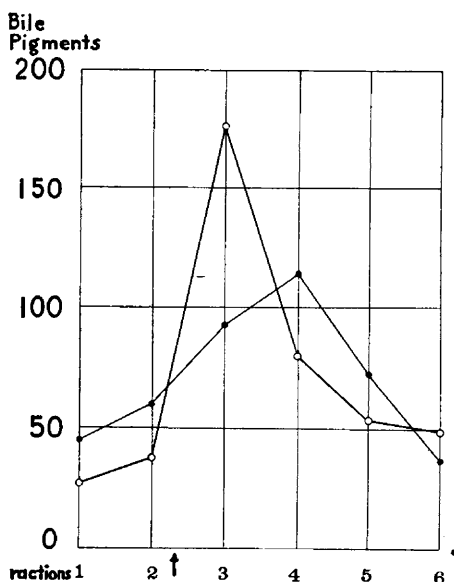


Chart 2.—Duodenal pigments in normal individuals as compared with pigments in patients with no flow of gallbladder bile. Normal pigment values o—o—o—o. Values with no gallbladder flow ●—●—●—●.

ments believed to be characteristic of gallbladder contents. The similarity of the sediments in these fractions to those actually obtained from the gallbladders at the time of operation was striking. The close correspondence between these sediment findings, their occurrence coincident with the peak of the pigment curves, and the absence of a characteristic pigment curve in cases where there was known to be no flow of bile from the gallbladders all confirm the assumption that the so-called "B" bile consists, at least in part, of actual gallbladder contents. The importance of this assumption will be discussed in a later portion of the paper in a study of cases in which there was known gallbladder pathology.

Relation of Blood Destruction to Bile Pigment Elimination.—It was desirable to establish, if possible, further definite evidence that there was a distinct relation between the amount of hemolysis going on in the body, and the level of the bile pigments. Observations were made in two cases of paroxysmal hemoglobinuria which have been reported in a separate paper.¹⁷ In these cases immersion of the extremities in icewater caused immediate and marked intravascular hemolysis. The plasma, duodenal contents and urine were examined for changes in pigment values during the course of the observations, which covered a period of about twenty-two hours. There was no important change in urinary pigments inasmuch as the attacks produced were not severe enough to cause any but the slightest traces of hemoglobin to appear in the urine. The hemoglobinemia was immediate and intense and was accompanied in one case by a drop in the red count of over 800,000 cells per c.mm. Subsequently the hemoglobin content of the plasma rapidly diminished, with an accompanying marked increase in the bilirubin content. This increase in bile pigment in the plasma continued until it reached its height at a point coinciding with the disappearance of hemoglobin from the plasma. It then gradually dropped, reaching normal at the end of about eighteen hours. Coincident with the peak of the bilirubin content of the plasma, the duodenal pigments rose rapidly, reaching a level about six to eight times the normal level in about three hours. These pigments did not return to the normal level until after eighteen to twenty hours. These results rather definitely confirmed the generally accepted theory that increased blood destruction is accompanied by increased elimination of bile pigments. Furthermore, it seemed safe to assume from the above observations that the bulk of the hemoglobin liberated into the circulation as a result of any hemolytic process is rapidly taken care of within the liver and broken down into lower bile pigments. Although other organs and tissues possess a similar property of carrying on the metabolism of blood pigments, under normal conditions the liver probably carries on the greater part of this important chemical process.

Bile Pigments in Various Types of Anemia.—With the clear recognition that increased blood destruction is accompanied by an increased elimination of bile pigments in the plasma and bile, as demonstrated by the above observations on paroxysmal hemoglobinuria, and as brought out by numerous investigators, a series of cases of various types of anemia was studied. This series included cases of anemia due to severe hemorrhage, lowered bone-marrow activity, pernicious anemia, hemolytic jaundice, malaria, and so forth. A somewhat similar series had been studied by Schneider, and later by Giffin, Sanford and Szlapka.

17. Jones, C. M., and Jones, B. B.: Arch. Int. Med. **29**:669 (May) 1922.

I wished, however, to obtain a comparative set of figures by the fractional method of duodenal analysis, and to attempt a more detailed study of the abnormal physiology occurring in these diseases. The cases studied fell roughly into two groups: (1) cases in which increased blood destruction is believed not to be present, or at least is not an important feature, and (2) cases in which it is generally believed that abnormal blood destruction is an important feature of the disease process.

As examples of the first type of cases a group of patients was studied in which the anemia was due entirely to blood loss. The anemia was due in two cases to hemorrhage from duodenal ulcers, in one to renal hemorrhage, in one to a series of attacks of paroxysmal hemoglobinuria, and in one to prolonged menorrhagia. The case of paroxysmal hemoglobinuria had been free from attacks for more than a week, so that there was no complicating factor of recent hemolysis. In none of these cases was there any evidence of abnormal red cell destruction. As was to be expected, the actual pigment values were all under the normal average (Table 1), indicating possibly an attempt

TABLE 1.—BILE PIGMENTS IN ANEMIA FROM BLOOD LOSS

Case	Average Bile Pigments in Duodenal Contents	"Relative" Duodenal Pigments	Plasma Bilirubin Content	Hemo- globin, per Cent.	Red Blood Cells (Millions)
20.....	28	35	12	65	4.0
21.....	7	12	11	32	2.6
22.....	36	72	10	28	2.5
23.....	21	30	9	40	3.7
24.....	13	20	18	50	3.2

on the part of the body to conserve hemoglobin. "Relative" figures, based on the actual pigment readings in the duodenal contents and the percentage of red cells in relation to normal, with one exception (Case 22), were also within or below the normal range. The single case referred to, with high "relative" figures, had a profound anemia, and the explanation for the high figures may lie in the fact that the liver was improperly functioning on account of the anemia itself.

"Relative" figures were obtained on the following assumption: The pigment values in the duodenal contents are in a sense absolute values, in that these values do not take into consideration the amount of circulating hemoglobin. Obviously, even if the pigment values in the duodenal contents are the same, there is greater relative blood destruction in a case with a low red count and hemoglobin than in a case with a normal red count and hemoglobin. It is interesting, therefore, to attempt roughly to correct these figures of pigment values to the same standard of circulating hemoglobin. Thus it is possible to ascertain the relative intensity of the blood destruction. It does not of

course necessarily follow that the same relative intensity of blood destruction would obtain if the red corpuscles and hemoglobin were at the normal level. Because it was simpler to carry out this correction on the basis of the numerical differences of red corpuscles, this procedure was adopted, rather than correction by utilization of hemoglobin variations, which theoretically is more logical. Relative figures were obtained by dividing the pigment values in the duodenal contents by the percentage of normal which the red count of the individual case showed, and then multiplying by 100. Thus, for example, a patient with a count of 3,000,000 red corpuscles per c.mm., and a pigment average of 100 units, other things being equal, would theoretically be destroying one-half the percentage of total red cells destroyed by a patient with a count of 1,500,000 red corpuscles per c.mm., and a pigment average also of 100 dilution units.

TABLE 2.—BILE PIGMENTS IN A CASE OF APLASTIC ANEMIA

Case	Average Bile Pigments in Duodenal Contents	"Relative" Duodenal Pigments	Plasma Bilirubin Content	Hemo- globin, per Cent.	Red Blood Cells (Millions)
25.....	28	108	11	26	1.3

A single case of true aplastic anemia (Table 2) was studied, which also showed actual bile pigment values well below the normal. However, the "relative" figures were high. Inasmuch as in this case also the anemia was extreme, the explanation of the high "relative" figures is possibly the same as that given for Case 22 of the preceding series, namely, the effect on liver function of the profound anemia. A more logical explanation may possibly be that, with an extremely low level of red corpuscles, and with practically no new blood formation, the few cells in the circulation undergo more rapid dissolution than normal on account of the undue work put on them.

TABLE 3.—BILE PIGMENTS IN A CASE OF POLYCYTHEMIA VERA

Case	Average Bile Pigments in Duodenal Contents	"Relative" Duodenal Pigments	Plasma Bilirubin Content	Hemo- globin, per Cent.	Red Blood Cells (Millions)
38.....	54	32	24	155	8.0

One case of polycythemia vera was studied. The patient had a red cell count of 8,000,000 cells per c.mm., and a hemoglobin content of 155 per cent. The actual pigment values averaged only slightly above normal (Table 3), but the "relative" figures were below the normal average. In spite of the enormous increase in the number of red cor-

puscles the process of blood destruction in this case was apparently normal, or even relatively below normal.

In contrast to the above cases, and as an instance of disease in which it is generally conceded that there exists an apparently high degree of blood destruction, a series of nineteen cases of pernicious anemia was studied. Other observers have pointed out that in pernicious anemia there is a marked increase in the bile pigments in the blood, duodenal contents, stools and urine.

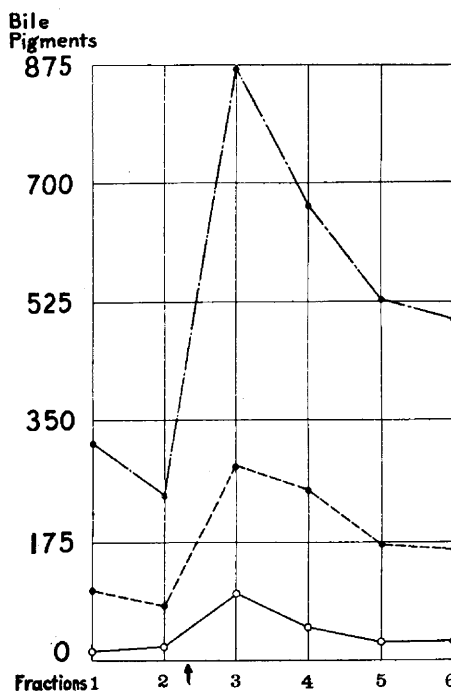


Chart 3.—Duodenal pigments in pernicious anemia. "Relative" pigment values ————·———. Actual pigment values - - - - - . Normal pigment values o-o-o-o-o-o.

Observations made in this series of cases entirely confirmed the results of previous investigators. There was a high level of bile pigments both in the blood plasma and in the duodenal contents. The average increase in duodenal pigments over normal was more than 500 per cent.; the highest averages were nine times, and the lowest twice the normal figures. Plasma bilirubin was, in the average, about four times normal; the highest figure was about eight times the normal, while the lowest was about twice normal. Based on average figures, therefore, the bile pigment content of the blood and duodenal contents ran about parallel, but in individual cases there was marked discrep-

ancy between the two values. This difference, in individual cases, between the pigment content of the plasma and the pigment values of the duodenal contents, is perhaps significant, and suggests some interference with liver function—a point that will be discussed later.

A question of considerable interest to be determined in studying these cases was whether the level of bile pigments in the duodenal contents corresponded with the actual clinical condition of the patients. Weakness, elevation of temperature, icterus, level of hemoglobin and red cells, etc., supply the clinician with evidence for comparison between individual patients. The condition of the individual patient theoretically depends, in large measure, on the relative severity of the hemolytic process and on the relative degree of blood-forming activity. Thus, a patient who is subjectively sick, and who presents the typical features of a relapse, usually gives evidence of a marked predominance of blood destruction over blood formation. A patient, on the other hand, with few subjective symptoms, a high hemoglobin content, usually shows evidence of little blood destruction and, on the contrary, a satisfactory blood formation. In spite of the theory that varying degrees of blood destruction are accompanied by corresponding variations in the level of the bile pigments, it is evident that changes in blood formation may modify the clinical picture to such an extent that the level of the bile pigments in the duodenal contents, although measuring the amount of blood destruction taking place, will not reflect the patient's clinical condition. High pigment values might, therefore, be obtained, even in the presence of a severe degree of blood destruction, without correspondingly severe clinical symptoms. Furthermore, any alteration of liver activity should modify the bile pigment excretion, both in the bile and in the blood plasma.

These theoretical considerations were well sustained by the findings. Examination of the figures obtained in the nineteen cases of pernicious anemia showed that the actual pigment values corresponded only in a very rough way to the clinical condition of the patient. Patients whose duodenal pigments were very high frequently were clinically less sick than those who showed relatively low bile pigment estimations, and vice versa. A second set of figures, however, did correspond closely to the condition of the individual patient, both as regarded his clinical condition and as concerned the actual physiologic processes taking place. These second figures are the "relative" figures already referred to, and were obtained by dividing the actual pigment readings by the percentage of red cells of the particular case. Such a modification of the actual pigment readings gave a close approximation to the clinical state of the patient, and in addition appeared to serve as a much clearer index of the relation of blood destruction to blood formation. Actually,

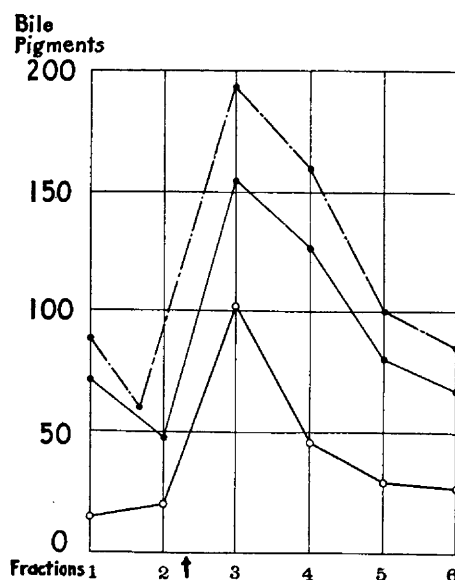
patients with high "relative" figures were sick, and presented the clinical findings of severe blood destruction—elevation of temperature, jaundice, etc., that are typical of a severe relapse. Those patients whose "relative" figures were only moderately high, on the contrary, were free from the more marked symptoms, while patients with "relative" pigment values at a still lower level were in a well marked remission. The average level of the "relative" figures, however, was about three times that of the actual readings, and indicates clearly the severity of the disease process. Furthermore, a comparison of the actual and "relative" values in any individual case provided a fair estimate of the balance between blood destruction and blood formation. When the two sets of figures were not far apart it would appear that the two processes were taking place at about equal rates; when the "relative"

TABLE 4.—BILE PIGMENTS IN NINETEEN CASES OF PERNICIOUS ANEMIA

Case	Average Bile Pigments in Duodenal Contents	"Relative" Duodenal Pigments	Plasma Bilirubin Content	Hemo- globin, per Cent.	Red Blood Cells (Millions)
1.....	185	1,543	96	25	0.6
2.....	331	1,370	36	42	1.6
3.....	252	1,260	56	40	1.0
4.....	209	858	64	37	1.2
5.....	162	845	40	31	1.2
6.....	233	832	160	46	1.4
7.....	193	801	32	35	1.2
8.....	141	748	90	27	0.9
9.....	165	717	64	35	1.2
10.....	99	596	90	27	0.8
11.....	194	571	30	50	1.7
12.....	164	512	90	35	1.7
13.....	360	500	100	94	3.5
14.....	357	496	32	75	3.6
15.....	175	323	65	45	2.7
16.....	133	302	30	70	2.2
17.....	132	500	50	55	2.2
18.....	117	278	32	60	2.1
19.....	83	112	80	85	3.7
Average.....	194	682	66	48	1.8

figure was much higher than the actual reading it would seem that blood destruction was exceedingly active, and vice versa. Table 4 illustrates these points. The actual readings in the first column and the "relative" figures in the second column are both obtained by averaging the total pigment values of the six fractions obtained during duodenal drainage. The cases are arranged in order of magnitude of the "relative" figures and, as noted above, this order closely approximated the severity of the patient's clinical condition. Case 1, for example, was a patient in a very severe relapse, while Case 19 was a patient in a well marked remission with almost complete freedom from symptoms. Chart 3 illustrates the marked increase in big pigment elimination in these cases over the normal level, and further emphasizes the difference between the actual and "relative" findings.

Mention has already been made that in these cases of pernicious anemia the bile pigment in the plasma did not always exactly parallel the pigment content in the bile, although it was always well above normal, as previously shown by Blankenhorn. Such a finding suggests that this failure of the plasma bilirubin to parallel the bile pigments in the duodenal contents may be due to an alteration in hepatic function. It is pertinent, at this point, to call attention to a question only briefly noted in the literature. The average level of bile pigments in the duodenal contents in this series of cases of pernicious anemia is by actual reading 194 dilution units, or about five times the normal values.

[illegible]

Individual cases went as high as eight to nine times the normal level. Such an increase over normal has previously been considered as due entirely to increased blood destruction, with excessive liberation of hemoglobin. Undoubtedly some part of the excess of bile pigments is due to the products of increased red cell destruction, but there is in addition a second factor which must be taken into account. Comparison with the observations on paroxysmal hemoglobinuria already mentioned makes this clear. One of the artificially produced attacks of hemoglobinemia was accompanied by a lowering of the red cell count by as much as 850,000 cells per c.mm. The hemoglobin liberated into the circulation was presumably derived solely from the destruction of

red cells, a destruction which approximated more than one-tenth of the total number of red cells, as measured by a routine red count. This excessive destruction of blood was followed shortly afterward by a rise in the bile pigments in the duodenum to a level of about 300 dilution units, or between six and eight times the normal figures. In paroxysmal hemoglobinuria there is no known evidence of any liver injury. Deranged liver function, therefore, need not be considered in paroxysmal hemoglobinuria, and any increase in bile pigments can safely be attributed essentially to increased blood destruction. In this example of pure hemolysis, uncomplicated by any other factors, a drop in the red cell count of 850,000 was accompanied by a rise in duodenal pigments to a level of about 300 dilution units. This level of bile pigments was well above the average of the entire series, and was but little under the level found in the most severe cases of pernicious anemia. It is difficult to conceive, even in the most severe cases of pernicious anemia, or in any other so-called hemolytic disease, that there is a constant rate

TABLE 5.—BILE PIGMENTS IN OTHER "HEMOLYTIC" DISEASES

Case	Average Bile Pigments in Duodenal Contents	"Relative" Duodenal Pigments	Plasma Bilirubin Content	Hemo- globin, per Cent.	Red Blood Cells (Millions)
Malaria:					
27.....	95	120	25	45	3.9
28.....	182	284	128	38	3.2
Hemolytic jaundice:					
29.....	228	251	120	70	4.6
30.....	129	430	100	30	1.5
31.....	152	625	65	40	1.2
32.....	108	99	45	70	5.5

of blood destruction going on so rapidly as to cause in a few minutes the dissolution of more than one-tenth of the total blood corpuscles in the body. A process causing such a degree of blood destruction, in the absence of a correspondingly rapid degree of blood formation, ought to result in complete exsanguination in a very short space of time. A second factor seems necessary to help explain the high level of bile pigments found in pernicious anemia. Ashby¹⁸ has recently reached a somewhat similar conclusion. This second factor, I believe, lies in a marked impairment of liver function. Such an assumption, although suggested by Hooper and Whipple as a result of their work on dogs, has not been made as a result of observations in man.

In addition to the above cases of pernicious anemia, a number of cases were studied in which it is also usually agreed that there exists an abnormally high degree of blood destruction. This series contained two cases of malaria and four cases of acquired hemolytic jaundice. One of the latter had previously had his spleen removed. All of these

18. Ashby, W.: J. Exper. M. **34**:147, 1921.

cases showed high pigment values (Table 5) entirely comparable with those observed in pernicious anemia. As in the former cases, the "relative" figures gave the more accurate picture, and closely paralleled the clinical condition of the patients. In these cases, also, as in pernicious anemia, it seems reasonable to assume that there must be a second factor to account for the extremely high bile pigment values obtained. Blood destruction alone could hardly account for the increased bile pigment elimination. In two of the cases of acquired hemolytic jaundice in this series (Table 5, Cases 30 and 31) the liver enlargement was so marked indeed as to dominate the entire clinical

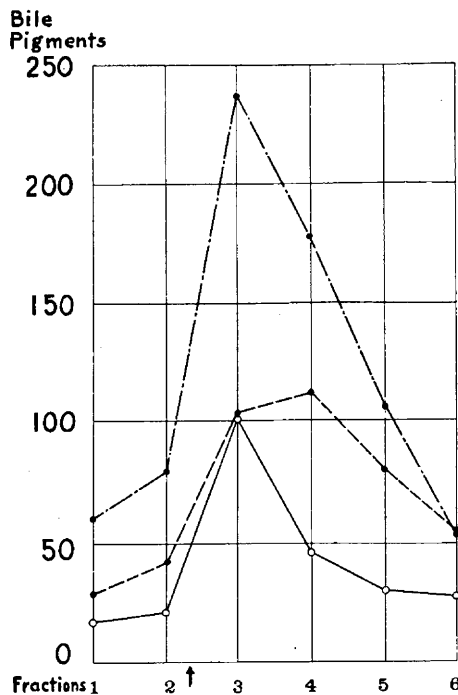


Chart 5.—Duodenal pigments in gallbladder disease. Pigment values in cholecystitis ————, Pigment values in cholelithiasis -----, Normal pigment values o-o-o-o-o-o-o.

picture and to suggest definitely that a hemolytic process was not responsible for the entire condition. Impairment of liver function in these cases would also seem to be the logical additional factor to be considered, and such an assumption would seem to be even more logical in the cases of hemolytic jaundice than in the cases of pernicious anemia.

Bile Pigments and Impaired Liver Function.—In the type of cases already studied, in which the process of abnormal blood destruction

has been of long duration, it is well recognized that clinically, and at post-mortem examination, it is commonly possible to demonstrate liver pathology. Forty per cent of cases of pernicious anemia have during life palpable livers,¹⁹ which at autopsy show a certain amount of fatty infiltration and deposits of iron containing pigment. Cases of hemolytic jaundice usually, and malaria not infrequently, present the clinical evidence of liver enlargement. The suggestion has already been made that in the above type of disease the abnormally high elimination of bile pigments may be due in part to an alteration of liver function. It is furthermore reasonable to assume, even in the absence of any clinical signs of liver derangement, that there exists a marked alteration in liver function, due merely to the presence of a severe anemia. Such an assumption finds support in the well-recognized fact that in cases of severe anemia the kidneys may show evidence of definite alteration of function by renal function tests and by the presence of albumin in the urine. With improvement in the anemia, the renal function also improves. It is, therefore, appropriate to turn from a consideration of the so-called hemolytic diseases, in which abnormal blood destruction and liver damage may be accompanying factors, to the study of a series of cases in which it is evident that the liver is the primary seat of disease, and in which there is considered to be little or no question of abnormal hemolysis.

A series of eighteen cases was examined, all of which presented clinical evidence of moderate to severe liver damage. This group included cases of carcinoma of the liver, cirrhosis, either alcoholic or syphilitic, hepatitis or cirrhosis due to a local or general infectious process, and Banti's disease. These types of cases are not usually considered to have any important degree of increased blood destruction, with the possible exception of Banti's disease. These cases, therefore, were of interest as a basis for studying the functional capacity of the liver, in terms of hemoglobin and bile pigment metabolism. The findings are tabulated and shown in Table 6 and Chart 4.

Six of the eighteen patients were clinically jaundiced, and nine more showed a "potential" jaundice. By the term "potential" jaundice is meant a condition in which the bilirubin content of the blood plasma is abnormally high but not sufficiently high to cause tissue icterus. As has been shown by Blankenhorn,¹⁶ in certain chronic diseases in which there is a continual abnormal elimination of bilirubin, the concentration of this pigment in the blood plasma may be many times normal without causing tissue icterus. In spite of the presence of jaundice, either actual or "potential," in fifteen out of these eighteen cases the output of bile pigments into the duodenum was above normal. In the

19. Minot, G. R.: Oxford Medicine, 2:623.

remaining three cases, the concentration of the bile pigments in the bile was within normal limits. Although some clinical abnormality in the liver was demonstrable, the abnormality may not have progressed far enough to overstep the large factor of safety present in this organ.

In fifteen out of eighteen cases, therefore, the liver, even when almost entirely invaded by foreign tissue, tended not to eliminate a decreased amount of bile pigment into the duodenum, but on the contrary to put out highly concentrated bile, as measured by pigment content. The actual pigment readings in the series of eighteen cases of liver disease showed an average curve (Chart 4), the values of which were over twice the normal level. From these facts alone, therefore, it is evident that the jaundice present in these cases was not at all obstructive in nature, at least in the usual interpretation, namely a diminished output of bile into the duodenum with accumulation of the residue in the blood and tissues.

Neither was the jaundice strictly hematogenous. The abnormal elimination of bile pigments, both in the bile and plasma, can not be said to be due to an increased amount of blood destruction. The average red cell count of the entire series was 3,900,000 corpuscles per c.mm., and one of the more severe of the cases, one of advanced carcinoma of the liver, showed a red count as high as 6,000,000 per c.mm. The red cells were markedly achromic, and the average hemoglobin content of the blood was approximately 60 per cent. The average color index of the cases was only 0.75, in contrast to the average index of 1.5 observed in the cases of pernicious anemia. In the stained smear the red cells showed only achromia, with slight variations in size and shape. There was lacking microscopic evidence of increased blood destruction; namely, the presence of microcytes or fragmentation of the red cells. Active blood formation, commonly present in the face of active blood destruction, was not seen, at least as represented by noteworthy changes in the number of young red cells. The usual conception of these types of liver disturbance, furthermore, does not associate them with increased blood destruction. Banti's disease, by some clinicians, is occasionally associated with abnormal destruction of the red corpuscles. The commonly accepted view, however, is that expressed by Krumbhaar,²⁰ who states that blood destruction is not the important element in this disease. It seems fair to assume, therefore, from the generally accepted views, that in these cases under discussion blood destruction was not importantly increased, and that the jaundice is not hematogenous in nature.

In the literature scant reference has been made to the bile pigment excretion in the above type of case. The general statement has usually

20. Krumbhaar, E. B.: Nelson's Loose Leaf Living Medicine, 4:37.

been made that secondary anemias, in contrast to primary anemias, are accompanied by a diminished elimination of bile pigments. It is clearly evident, however, from the results charted in Table 6, that cases of anemia associated with liver disturbance are usually accompanied by increased bile pigment excretion. The so-called secondary anemias, if uncomplicated by liver disturbance, undoubtedly yield low pigment readings. When, however, in the course of such a secondary anemia the underlying cause affects the liver the entire picture of bile pigment excretion is changed, and instead of a low pigment elimination in the bile and plasma, there follows a complete reversion of the physiological processes involved, and the bile pigments reach a new and abnormally high level.

TABLE 6.—BILE PIGMENTS IN CASES OF LIVER DISEASE

Case	Average Bile Pigments in Duodenal Contents	"Relative" Duodenal Pigments	Plasma Bilirubin Content	Hemo- globin, per Cent.	Red Blood Cells (Millions)
Cancer:					
50.....	173	144	40	85	6.0
51.....	144	200	80	68	3.6
52.....	98	213	15	25	2.3
53.....	57	54	40	80	5.3
Cirrhosis (alcoholic):					
54*.....	151	189	30	65	4.0
55.....	64	60	30	80	5.3
56.....	36	...	50	75	
Cirrhosis (syphilitic):					
57*.....	77	96	140	65	3.9
58.....	41	51	66	40	1.2
Hepatitis (infectious):					
60.....	98	...	28	75	
61.....	115	174	25	45	3.3
62*.....	78	87	100	70	4.5
63 (typhoid).....	81	96	10	...	4.2
64.....	30	35	15	65	4.3
65*.....	30	35	250	67	4.2
70*.....	248	248	72	72	4.8
Hepatitis (toxic):					
66.....	58	55	30	80	5.3
Banti's disease:					
67*.....	230	280	150	65	4.1
Average.....	101	114	59	66	4.1

* Clinically jaundiced.

It has already been shown that the jaundice occurring in such cases is neither strictly obstructive nor hematogenous in nature, in spite of the fact that in all the cases the liver parenchyma was severely damaged. It is well known that the liver, like the other organs, has a large factor of safety, as regards all of its functions. Exact information as to the extent of this measure of safety has never been determined in man. McMaster and Rous²¹ have recently shown that the bile ducts from three-quarters of the liver substance can be obstructed in dogs and monkeys without the development of any clinical evidence of pigment or cholate accumulation in the organism. They also

21. McMaster and Rous: J. Exper. M. **33**:731, 1921.

showed that in the dog nineteen-twentieths of the liver substance can be placed in a condition of stasis, without the occurrence of tissue icterus such as regularly follows total obstruction in this animal. In their experiments, they found that invariably a local obstruction resulted sooner or later in atrophy of the affected tissue, with compensatory hypertrophy elsewhere. Their conclusions are of particular interest in the present discussion: "the clinical jaundice encountered in association with local liver lesions should be viewed, not as the result of local bile absorption, but as due to a general injury to the hepatic parenchyma or ducts, or to blood destruction." Such injury with its resulting hypertrophy, would accordingly result in functional changes, and bile pigment excretion would accordingly be modified. The nature of the cause of this functional disturbance is apparently not specific. An examination of the accompanying table will show that in no particular group of liver conditions was there any predominance of high pigment values. Bile pigment excretion was apparently influenced neither by the nature of the process, nor by the amount of the anemia. The mechanism is probably similar in all the cases, and the degree of derangement of hepatic function is solely dependent on the extent and rapidity of the disease process.

There remains, then, to discuss the actual nature of this alteration in liver function. As already noted, there was a marked increase in the actual amount of bile pigments eliminated by the liver. In the absence of any abnormal process of blood destruction the source of the excessive amounts of bile pigments is still to be determined. Changes in diet, according to Hooper and Whipple,⁹ can cause marked alterations in bile pigment elimination in animals. Such a factor, however, can readily be excluded in the present series. The most logical explanation seems to be the following: Under normal conditions the liver is the principal agent in the metabolism of hemoglobin set free during the normal processes of red cell destruction. This pigment metabolism involves the breakdown into less complex molecules, through bilirubin and biliverdin, to the lower derivatives, urobilinogen and urobilin. The formation of bilirubin from hemoglobin may take place in the blood vessels and tissues without any intervention on the part of the liver, and similarly, urobilin is undoubtedly formed in the intestine by the action of bacteria on bilirubin. It is highly probable, however, that the liver itself is capable of breaking down the bilirubin into urobilin, without the intervention of the intestine. The observations already mentioned made on cases of paroxysmal hemoglobinuria suggest such a possibility. Furthermore, the liver has long been thought capable of resynthesizing hemoglobin from the lower bile pigments by building them up to more complex molecules and combining them with the iron

known to be retained by the liver. Such a process of resynthesis is entirely analagous to the general physiologic properties of all human cells and is not necessarily much more complicated than the formation of urea or glycogen from lower chemical constituents. The process of breaking down hemoglobin into its lower derivatives is, however, probably a less difficult matter than the subsequent resynthesis of hemoglobin from bile pigments. The latter function would perhaps logically be the first to be altered or lost. With the failure of the normal resynthesis of hemoglobin from bile pigments the unaltered bile pigments would then form an excess and would be eliminated as such in the bile.

The findings in this group of cases seem to confirm this supposition. The loss of resynthesizing power in a damaged liver would, of course, be only partial. The lowered formation of hemoglobin ought eventually to be reflected in a diminished hemoglobin content of the red cells with resulting low color index and achromia. In all these cases marked achromia of the red cells and a low color index occurred. The hemoglobin averaged 66 per cent., and the red count averaged 4,100,000 per c.mm. This slight diminution was possibly the result of a gradual slowing up of bone marrow activity. Such findings may be regarded as probable evidence of a diminished production of hemoglobin. That portion of the bile pigments not resynthesized into hemoglobin would be excreted as such, and would account for the increased elimination of bile pigments, even in the face of normal blood destruction. That such a theory further corresponds with the actual findings in the individual cases is attested by the fact that in the majority of cases showing the greatest reduction of hemoglobin content there was a proportionally high level of bile pigments in the duodenum. One case, for example, with a hemoglobin content of 40 per cent. and a color index of 0.35, showed a bile pigment elimination in the bile of over four times the normal.

The above theory would satisfactorily account for the appearance of jaundice and lowered hemoglobin content so frequently noticed in the course of acute infections such as pneumonia, typhoid, scarlet fever, septicemias, etc. In such conditions the infection, or the accompanying toxemia, may be assumed to cause a temporary alteration of the liver function, with resulting alterations in hemoglobin metabolism. The icterus frequently accompanying severely decompensated heart disease may also be explained on the basis of altered liver function.

In a severely damaged liver not only should there be an increase in the actual amount of bile pigments eliminated, but the relation of the various pigment elements in the bile should be distinctly altered. Those pigments most easily formed ought to be excreted at once instead of

being completely broken down to the lower forms. In confirmation is the frequent occurrence of excessive amounts of intermediate bile pigments—cholecyanin and urobilinogen—found in the duodenal contents in this series of cases of excessive red cell destruction, and those with liver disease. Schneider and others have already noted the presence of large amounts of urobilinogen in the duodenal contents in severe cases of blood destruction. This excess of urobilinogen was noted, not only in the present series of cases with liver disease, but also in those cases in which there was pathologic blood destruction. In addition, in those cases of severe anemia, the presence of cholecyanin was observed, frequently in large amounts. This latter pigment, as well as urobilinogen, is intermediate between bilirubin and urobilin, and its presence would seem to indicate very rapid and incomplete metabolism of hemoglobin derivatives by the liver. The presence, therefore, of these intermediate pigments in excess in cases of pernicious anemia would seem further evidence of liver damage in this disease.

It is therefore, tempting to assume that a disturbed function of the liver in the disease pernicious anemia is a considerable factor in creating abnormal pigment values in the plasma and in the bile. It is not as easy, however, to apply this explanation to pernicious anemia as to the other anemias in which abnormal pigment values are found. In pernicious anemia there is a relative increase in hemoglobin and iron pigment is found in various organs. The irregular and bizarre course of pernicious anemia, the attractive assumption that the red corpuscles in pernicious anemia are not only abnormal but different from the red corpuscles in other conditions, may account in part for the seeming discrepancy. The existence of difference between the red corpuscles in pernicious anemia and in other conditions has been indicated by work recently done on various types of anemia by Buckman²² at the Boston City Hospital. In any event, while a part of the increase in bile pigment elimination in pernicious anemia may be attributed to excessive blood destruction, the remainder may perhaps be laid to a damaged liver. Inasmuch as continued attacks of hemolysis per se cause liver damage, the two factors are really related.

Furthermore, as pointed out by Brulé,²³ the proper conception of such a disease as catarrhal jaundice should locate the primary pathology not in the biliary passages but in the hepatic cell itself. Such a disease is primarily an infection of the liver parenchyma, and the pathology and abnormal physiology should be centered in the degree of actual parenchymal damage and disturbance of liver function. Such a conception of catarrhal jaundice offers the logical explanation of the

22. Buckman, T. E.: Personal communication.

23. Brulé, M.: *Bull. méd.* **8**:279, 1920.

diminished hemoglobin frequently found after an attack of even moderate severity, and accounts for the increased amounts of bile pigment eliminated in the bile after the flow is reestablished.

Findings in Cases of Gallbladder Disease.—In view of the frequent association of liver disturbance with chronic disease of the gallbladder it is pertinent at this point to examine briefly the results obtained in a series of cases of cholelithiasis and cholecystitis. Observations were made on ten patients suffering from typical gallstone attacks and on six patients with typical symptoms of chronic cholecystitis. In a majority of the cases the preliminary diagnosis was confirmed by subsequent operation. The pigment curves, as shown in Chart 5, are easily explained. They did not vary from the normal curve in their general contour. The actual level of the bile pigments, however, was distinctly higher than normal. (This series, of course, did not include cases of cystic or common duct obstruction.) The point of interest in these cases is that there was a distinct difference between the pigment values obtained from patients with stones and those obtained from patients with only cholecystitis. Those patients with cholelithiasis gave an average pigment curve approximately 75 per cent. above the normal level, although in individual cases the average figure was as high as three times normal. The cases of cholecystitis, on the contrary, gave a distinctly higher average. The average pigment values in this group were nearly twice those observed in cases with stone formation, and and were three times the normal figure. Individual cases of this group went as high as eight times normal. Furthermore, the peak of the pigment curve representing the greatest concentration of gallbladder bile was on the average more than twice that found in the group of cases with stones. According to the present conception of gallbladder disease, blood destruction does not play an important part in the disease process. The high pigment values, therefore, were due either to abnormal stasis or to liver pathology. The work of Rous and of McMaster,²⁴ recently published, indicates that in stasis the gallbladder has a great power of concentration, with the result that any bile contained in it, even for short periods of time, becomes abnormally high in pigment and other constituents. They show that this power of concentration diminishes in the face of a pathological process such as the presence of stone formation with partial or complete obstruction to the normal entrance of bile into the gallbladder. The high pigment content in cases of cholecystitis may thus be partially explained as well as the difference between those cases with stone formation and those with only low grade gallbladder inflammation leading merely to stasis. It is of especial interest in this consideration to note the findings reported

24. Rous and McMaster: J. Exper. M. **34**:47, 75, 1921.

in a personal communication from Fitz²⁵ from the Mayo Clinic. He reports finding highly pigmented bile in most cases in which operation was performed for cholecystitis, whereas the bile in those cases showing calculus formation was also dark but less highly pigmented. Furthermore, he was able to demonstrate that the specific gravity and the nitrogenous content of those cases with only cholecystitis tended to be much higher than in cases of cholelithiasis. Such findings are strongly confirmatory of the concentrating ability of the gallbladder in cholecystitis and help to explain the pigment values found in this group of cases.

While the high pigment values in these cases are undoubtedly due in part to gallbladder concentration, it is also highly probable that they may be due in part to an accompanying cholangitis and hepatitis. The recent paper by Judd,²⁶ emphasizing the common association of gallbladder and liver infection, is also confirmatory. Such a conception would also explain the low hemoglobin content frequently found in connection with long standing cases of cholecystitis, and occasionally persisting even after cholecystectomy. The high pigment values in such cases are probably due both to abnormal gallbladder concentration and to an alteration in liver function.

CONCLUSIONS

1. Increased blood destruction is accompanied by an increase of the bile pigments in the blood plasma and bile.
2. Alterations in liver function, due to infection, new growth, cirrhosis, or even a profound anemia per se, are also accompanied by marked increases in bile pigment, both in the bile and plasma.
3. Jaundice, in cases with liver damage, may be entirely due to an alteration in bile pigment metabolism, without the necessity of any accompanying obstructive process or increase in the normal process of blood destruction.
4. The high level of the bile pigment in pernicious anemia can not be due solely to a process of increased blood destruction. A second factor is necessary to explain the increased pigment elimination. This second factor may well be an alteration in hepatic function.
5. In gallbladder disease the bile pigments in the duodenal contents are abnormally high, especially in those fractions containing the greatest concentration of bile from the gallbladder.
6. Cases of uncomplicated cholecystitis show a greater concentration of bile pigments than cases of cholelithiasis.

25. Fitz, R.: Personal communication.

26. Judd, E. S.: J. A. M. A. **77**:197 (July 16) 1921.

7. A presumable functional incapacity of the liver properly to metabolize hemoglobin, due to any cause resulting in liver damage, is accompanied by a lowered hemoglobin content of the blood. Such cases also show high bile pigment values in the plasma and bile.

8. Owing to the frequent association of hepatitis with cholecystitis it is probable that the frequent accompaniment of a low hemoglobin content and an apparent anemia in chronic gallbladder diseases is due to an alteration in liver function.

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