A WORKING HYPOTHESIS OF HEMOGLOBIN PIGMENT METABOLISM *

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In the following pages arguments in favor of the view that hemoglobin pigment is produced and decomposed in the liver are presented.

This view was suggested by work which has already been published from this laboratory, and to some extent the evidence in favor of it is drawn from that source. New work has been started on the basis of this hypothesis along several lines, but there are so many questions raised, which circumstances prevent us from attempting to answer, that it seemed better to present what evidence we have at present.

It is claimed that those data which we already possess are harmonized and rendered intelligible by the adoption of this view, and that there is sufficient evidence to justify its acceptance as a working hypothesis.

As it was in the hope that applications to clinical medicine might be found that the subject was approached, special stress has been laid on the possibilities opened up along these lines.

THE DECOMPOSITION OF HEMOGLOBIN PIGMENT

One of the outstanding features of blood metabolism is its intensity, the rapidity with which it is continually being broken down and rebuilt. We have no means of measuring exactly the rate of hemoglobin decomposition and regeneration, but from the data which we possess it has been calculated that not less than 7 per cent. of the total red blood-cells are daily disintegrated in the body. Each individual red blood-cell, therefore, lives for about fourteen days. What is then its fate?

It would seem that there are special cells distributed throughout the body whose function it is to remove decrepit red blood-cells from the blood-stream. These are endothelial phagocytic cells which may differ considerably in morphology, but are united in possessing certain functional capacities. Thus they take up finely suspended "vital" stains, or colloidal metallic solutions, when these are injected into the blood-stream, and they have some connection with cholesterin metabolism,

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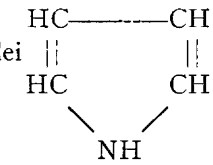
for the doubly refractile droplets characteristic for this substance may be demonstrated within them.

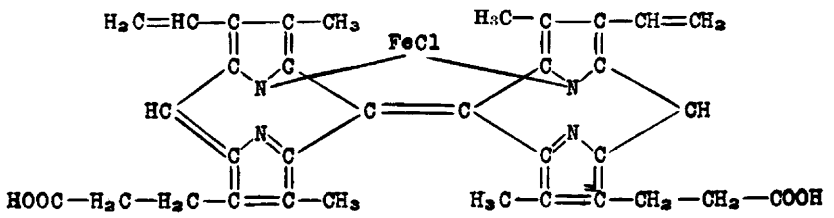
They are situated in the sinuses of the spleen especially, to a less extent within the capillaries of the lymphatic glands and bone-marrow, and stretching across the lumen of the capillaries they constitute in the liver, the Kuppfer or stellate cells.

The cells of this type may be seen to engulf the red blood-cells, and, as the work of Bain[1] and of Paton and Goodall[2] indicates, it is the old and damaged corpuscles which are thus taken up. Within or near the endothelial cell the corpuscle breaks down. There is evidence that in the spleen at least the hemoglobin which is thus set free is excreted into the blood plasma. And the special function of the Kuppfer cells is not only to phagocytose red blood-cells, but also to take up the free hemoglobin which is brought to it in the form of a fine emulsion from the splenic vein. The droplets of hemoglobin have been seen to pass from the Kuppfer cells into the liver cells.

This liberation of hemoglobin from the corpuscles is the first step in the process of hemoglobin pigment decomposition. The next is probably the detachment of the pigment moiety hemochromogen—from the protein globin. Hemochromogen can be readily separated from globin by hydrolysis outside the body, and by the action of hydrochloric acid, a stable substance—hemin—is produced with altering its essential constitution, in which four pyrrol nuclei



are linked to an atom of iron.

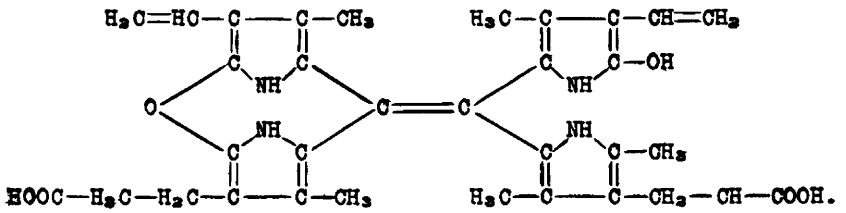


Hemin

The hemoglobin pigment which is thus set free is converted into bilirubin. This is the most important change which it undergoes, but it is the one about which we know least. No one has succeeded in reproducing the necessary reactions outside the body, either in per-

1 Bain: Jour. Physiol., 1903, xxix, 352.
2 Paton and Goodall: Jour. Physiol., 1903, xxix, 411.

fusion, autolytic or digestion experiments, and there is consequently a gap in our knowledge of the chemical processes which are involved. The proof that hemoglobin pigment is the source of the formation of bilirubin rests on experiments which show that when hemoglobin pigment is injected into the blood-stream it produces an almost quantitative increase in the bilirubin excreted in the bile. But although we do not understand the manner in which the conversion is accomplished, we can see the extent and nature of the change by comparing the formulae of hemin and of bilirubin.



Bilirubin

The four pyrrol nuclei are still present, but the iron has gone, and there is more oxygen present.

Where does the change of hemoglobin pigment into bilirubin take place?

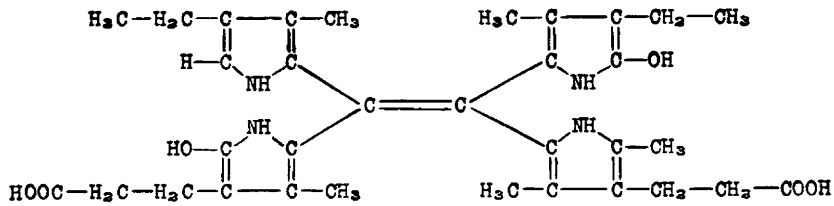
A short time ago there would have been few who would have had any hesitation in maintaining that it was in the liver cell alone that this transformation occurs, although it was admitted that in old blood extravasations a substance indistinguishable from bilirubin was occasionally found.

There is still no doubt that the liver cell under normal conditions is the place where bilirubin is formed. But since the work of Whipple and Hooper[3] and of McNee[4] has appeared, it must be admitted that in all probability, bilirubin can be formed elsewhere, for they have succeeded in producing icterus in the absence of practically the entire liver. This extrahepatic icterus of Whipple and of McNee's experiments finds its clinical counter-part in the cases of hemolytic jaundice in which with no diminution or hindrance to the excretion of bilirubin in the bile, there is, nevertheless, a more or less marked degree of icterus. Widal, some years ago, pointed out that the discarded theory of hematogenous jaundice was the only one which could account for such cases. And, as in Banti's disease, we may reasonably postulate a hyperactivity of the hemolytic function of the endothelial phagocytic cells, so also there is some evidence for the hypothesis that in "hemolytic jaundice" con-

3. Whipple and Hooper: Jour. Exper. Med., 1913, xvii, 593 and 612.
4. McNee: Jour. Path. and Bacteriol., 1914, xviii, 325.

ditions are present which allow this group of cells to assume in part the function of bilirubin formation.

The final stage in the decomposition of hemoglobin is the conversion of bilirubin into urobilinogen. This is an entirely extracellular process which takes place in the intestine. There the bilirubin begins to undergo a change which is the reverse of the process by which it was formed from hemoglobin pigment, in that it is essentially a reduction. Urobilinogen can be made from bilirubin outside the body by the use of strong reducing agents, and its formation in the intestine is no doubt favored by the relative absence of oxidation reactions there, but as yet we know little about the conditions under which it is produced. Hans Fischer and Röse[5] have succeeded recently in isolating it and give the following formula:



Urobilinogen

Urobilinogen contains a hydrogen atom attached directly to a carbon atom of one of the pyrrol rings and on account of this grouping, it reacts with paradimethylaminobenzaldehyd to form a red pigment which has a characteristic absorption spectrum.

By making use of this reaction, changes in the amount of urobilinogen in a solution under different conditions can be readily followed. Such studies immediately show that urobilinogen is not an end-product of hemoglobin metabolism. It is an extremely unstable body which readily takes up oxygen, and loses the capacity to form the red pigment with paradimethylaminobenzaldehyd. In its place there appears the pigment urobilin which has certain spectroscopic characteristics which allow of rough estimation.

The studies of Hans Fischer and Meyer-Betz[6] indicate that the formation of urobilin depends on the union of two molecules of urobilinogen under the influence of oxygen, a reaction analagous to the production of indican by the oxidative polymerization of indoxyl sulphates. The reaction may be expressed schematically thus:

Urobilinogen + oxygen + urobilinogen = urobilin.

The reduction of bilirubin to urobilinogen in the intestine is complete. No evidence could be obtained that the conversion stops in man

5. Fischer and Röse: Ztschr. f. physiol. Chem., 1914, lxxxix, 255.
6. Meyer-Betz: Ergebnd. inn. Med. u. Kinderkr., 1913, xii, 733.

at substances intermediary between bilirubin and urobilinogen, although in the dog it would seem probable that this occurs. But the process does not stop with urobilinogen, so that human stools practically always contain a varying amount of urobilin before they are passed. As soon as the stool is exposed to the action of the oxygen of the air, the formation of urobilin goes on more rapidly, especially under the influence of light. This explains the familiar fact that after a stool has stood for some time, it is darker on the surface than in the central unexposed parts. The colorless urobilinogen has been turned into the brown pigment urobilin.

Should we then look on urobilin as the end-product of hemoglobin metabolism? We cannot do so if we define an end-product as a final cleavage product which cannot be made use of or further changed in the body.

Urobilin is not a fixed substance. Its instability has, so far, not allowed of the determination of a constitutional formula, and we do not know with certainty the products of its change. But we do know that the same conditions which favor the conversion of urobilinogen into urobilin also bring about the disappearance of the urobilin itself. I believe that this is a very important fact and that it is the key to the understanding of the whole subject. It will be returned to in considering the formation of hemoglobin.

In summing up our knowledge of the decomposition of hemoglobin pigment we may conclude that, under normal conditions, hemoglobin is liberated from the old red blood-cells by phagocytic endothelial cells; that the hemoglobin thus set free passes into the blood plasma and is taken up by the Kuppfer cells which pass it on to the liver cells. Here the pigment is separated from globin, and after removal of iron and undergoing intramolecular changes which involve the addition of oxygen, is converted into bilirubin. The bilirubin passes into the intestine, and is reduced to urobilinogen, which again in part is polymerized to urobilin.

THE FORMATION OF HEMOGLOBIN

Is the animal organism capable of forming for itself the keystone in the structure of hemoglobin pigment — the pyrrol nucleus — or is it dependent on the ingestion of preformed pyrrol nuclei in the food?

A new formation of pyrrol within the body has never been demonstrated. That does not mean much, but on the other hand there is nothing inherently improbable in the conception that in the last resort the body has to rely on sources outside of itself for the formation of hemoglobin. We have the analogy of the benzene ring, which can be evolved only by processes peculiar to the vegetable organism, although

it forms an essential part of many of the structures of the animal body (Baumann[7]).

Apart from blood and chlorophyll, the only food constituent which is known to contain the pyrrol nucleus is the amino-acid, tryptophane, a body which is found in considerable amounts in nearly all proteins.

Thanks to the work of Osborne and Mendel,[9] we are in possession of exact and detailed data on the effect of a diet free from blood, chlorophyll and tryptophan on the weight and growth of rats. They show conclusively that under these conditions the body tissues cannot be maintained. There is a progressive loss of weight, which can be at once arrested by the addition of small quantities of tryptophan to the food. They make no mention of anemia, but even if there were no anemia, the possibility of hemoglobin formation by pyrrol synthesis within the body would not be proved, for the total quantity of tryptophan in the body is large, and it is conceivable that with a reduction of the rate of hemoglobin metabolism to a minimum, enough hemoglobin might be formed at the expense of pyrrol liberated from the wasting tissues. This seems to be a rather strained interpretation, however, and it is true that the absence of any note as to pronounced anemia in these observations and in the numerous records of complete starvation experiments, is a point which speaks for the capacity of the body to produce pyrrol for itself, even though it does not by any means prove it. We are planning work in connection with this point, but in the meanwhile it may be left on one side.

So far as the question at issue is concerned, it is enough to know that even if the organism can synthesize pyrrol, this product must of necessity pass through many complicated changes before it is finally welded into the intricate molecule of hemoglobin pigment.

On the other hand, in the urobilinogen in the intestine we have a substance in which the pyrrol nuclei are already linked together in their proper relationships to one another. All that we have learned of the economy of natural processes would tend to make us expect to find that urobilinogen would be used in the synthesis of hemoglobin pigment.

We have proof that a considerable part of the urobilinogen daily formed is not lost to the body by being excreted in the stools, but is absorbed from the intestine in the portal blood-stream. This was proved in Müller's classic experiment where he showed that the urobilinogen which appears in the urine has its origin in the intestine. This has been amply confirmed most recently and most directly by Fischer and Meyer-Betz.[10] With the absence of bilirubin from the

7. Baumann: Ztschr. f. physiol. Chem., 1886, x, 123.
9. Osborne and Mendel: Jour. Biol. Chem., 1914, xvii, 325.
10. Fischer and Meyer-Betz: Ztschr. f. physiol. Chem., 1911, lxxv, 232.

intestine, no urobilinogen is formed, and none can be detected in the urine until bile or urobilinogen are given by mouth. The quantity of urobilinogen and urobilin in the stools is less than the theoretical quantity which should be obtained from the amount of bilirubin entering the intestine. The deficit is absorbed into the blood.

With the recognition of this fact, however, we come to the end of what is generally accepted as established. The suggestion has of course been made that the absorbed urobilinogen is utilized in the formation of hemoglobin, but no hypothesis has been advanced as to the manner of its ultilization or as to its effects on urobilinogen excretion.

Wilbur and Addis[11] not long ago published a paper on "Urobilin" which contained the results of quantitative estimations of urobilinogen and urobilin under physiological, pathological and experimental conditions. Since that paper was written some important work of a purely chemical nature has appeared which throws light on some obscurities and difficulties which were encountered in presenting a theory of urobilinogen excretion.

It is, therefore, now possible to put forward a working hypothesis of hemoglobin metabolism which brings these observations into line, which formulates the relationship between hemoglobin decomposition and formation, and which has a practical application, in that it serves as a theoretical support of a formerly more or less empirical method of recognizing disturbances of liver action.

Urobilinogen is found only in traces in normal urine. But in disease there are two groups of conditions in which large amounts of urobilinogen are excreted in the urine.

In one there is an increased destruction of red blood-cells within the body. Malaria is the typical example. In such cases the proof of an unusual degree of blood destruction is found in the greatly augmented quantity of urobilinogen and urobilin in the stools.

In the other group the amount of urobilinogen and of urobilin in the stools is within the normal limits of variation or is decreased, and there is no evidence of excessive blood destruction. The majority of these cases are outspoken examples of disease of the liver — the initial stages of catarrhal jaundice, the toxemic stage of advanced cirrhosis, suppurative cholangitis, etc. — especially conditions in which the liver as a whole is involved, whereas in such local disease as carcinomatous metastases no very marked urobilinuria may be found. Great increase in urobilinogen excretion is also evident in some cases of acute infections such as lobar pneumonia, where a diffuse liver involvement is probable.

11. Wilbur and Addis: THE ARCHIVES INT. MED., 1914, xiii, 235.

On the whole, the clinical evidence is very strong that the presence of considerable amounts of urobilinogen in the urine is associated with liver disturbance, if marked increase in hemoglobin disintegration can be excluded.

Since the urobilinogen in the urine of these cases of liver disease is known to have its origin in the intestine, the current theory is that the normal liver absorbs the urobilinogen brought to it in the portal vein, whereas the diseased liver allows it to pass into the general circulation from which it is excreted by the kidneys. But in what form does the normal liver absorb urobilinogen? Not as such, for none can be extracted from the liver tissue. Not as urobilin, for this substance also is absent. It does not simply pass through the liver into the bile, for only traces of urobilinogen and urobilin are found there under normal conditions.

It is not even brought to the liver as urobilinogen, for none can be found in the blood, not even when large amounts of urobilinogen are being excreted in the urine.

Obviously, therefore, urobilinogen when it is absorbed is changed into some substance which has not been identified.

It will be remembered that urobilin is formed under the influence of oxygen by the union of two molecules of urobilinogen. But if a solution of urobilin is left exposed to air and light it will steadily decrease in quantity, just as the amount of urobilinogen diminishes when its solutions are left under the same conditions. The parallelism between both phenomena is so marked as strongly to suggest that both are the result of the same process, and that the disappearance of urobilin is simply a continuation of the reaction which is known to underlie the formation of urobilin from urobilinogen, namely, a polymerization. The unidentified substance formed might be called urobilin-complex, and the process might be expressed thus:

Urobilinogen + oxygen + urobilinogen = urobilin.
Urobilin + oxygen + urobilin = urobilin-complex.

The following experiment is an example of many which have been carried out in connection with this point.

An acid alcohol extract of a stool after the addition of zinc acetate in absolute alcohol was found to contain urobilinogen to the dilution value of 17 and urobilin to 20 in each 10 c.c. volume. It was left exposed to the air under the action of diffuse room light. After seven hours samples were removed and separate estimations of urobilinogen and of urobilin were made. The urobilinogen had entirely disappeared, but the urobilin had increased to a value of 40; that is to say, the urobilinogen had been converted into urobilin. But the increase in urobilin would have been still greater if another process had not been

proceeding coincidentally, i. e., the polymerization of the urobilin molecules to form the urobilin-complex. This process we have no means of measuring except by the disappearance of urobilin which is shown in the chart to be progressive, though not regular, since the decrease was more rapid during the day-time under the action of light, than at night.

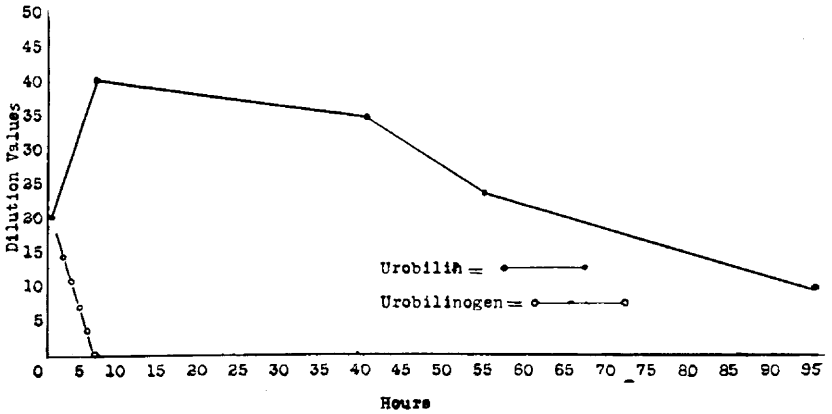


Chart 1.—The dilution values for urobilinogen and for urobilin depend on their respective light-absorbing capacities. Since urobilin has not been isolated its power in this respect cannot be determined with exactitude, and its dilution value must not be assumed to be directly comparable with that of urobilinogen. As a matter of fact, if equal weights of the two substances could be compared, it would be found that the dilution value of urobilin was higher than that of urobilinogen.

In the numerous efforts which have been made to find urobilinogen or urobilin in the blood, nothing has been learned which is in opposition to this hypothesis, and there are some points which indirectly support it.

The blood of patients in whom there is a large excretion of urobilinogen in the urine has never been found to contain any urobilinogen. Urobilin has been found in a few cases. But it is noteworthy that a common factor in such cases is that the patients have been cyanosed. Almost all of them have been cases of lobar pneumonia in the terminal stages with dilated right hearts and marked cyanosis. Now it was shown some years ago by Roth and Herzfeld[12] that when urobilin is added to the blood in vitro it disappears. If it is added to serum alone, however, or even to blood through which carbon dioxid has been passed, it can be recovered. It would seem that both the clinical and the experimental findings may be explained on the assumption that the formation of the urobilin-complex is greatly hastened by oxyhemoglobin, so that it is only when the oxygen of the blood is diminished that urobilin can exist in the blood-stream.

12. Roth and Herzfeld: Deutsch. med. Wchnschr., 1911, xxxvii, 2129.

We may therefore amplify the theory of urobilinogen excretion in the urine of cases of hepatic disease by explaining it as being due to the incapacity of the liver to absorb the urobilin-complex brought to it from the intestine, so that part of this substance escapes past it into the general circulation and reaches the kidneys.

The kidneys excrete only urobilinogen, never urobilin, or urobilin-complex. Probably because of their size, these molecules have to be reduced to urobilinogen before they can pass through the kidney cells. To judge from the prompt appearance of urobilinogen in the urine after injury to the liver, this is a function which the kidneys accomplish without much difficulty, and it would seem reasonable to argue from the fact that only very small traces of urobilinogen are found in the normal urine that the healthy liver is capable of holding almost all the urobilin-complex which is brought to it. The large amounts which may be excreted when the liver is diseased is some indication that the quantity thus absorbed may be not inconsiderable, although it cannot, as we shall see, be taken as a measure of the normal capacity of the liver.

One of the observations of Wilbur and Addis which could not at the time be fully understood was the extraordinary variations in the quantity of urobilinogen in the urine at different periods of the day. Charts 2 and 3 show the quantities of urobilinogen excreted every two hours in a case of bronzed diabetes.

This case was observed over a long period, and at no time was there any relation between the excretion of urobilinogen and of water, urea, chlorids or sugar in the urine. Every night the amount of urobilinogen was greatly decreased as compared with the amount which was passed during the day, but when the patient's habits were reversed and he slept during the day and remained awake and took his meals through the night, the quantitative relationship between the day and night urobilinogen was also reversed. This cannot be satisfactorily explained simply by variations in the absorption of urobilinogen derived from bilirubin, for this is a process which occurs mainly in the large intestine, and there is no reason to believe that there are any sudden changes either in the formation or in the absorption of this urobilinogen.

The study of the urobilinogen and urobilin content of the bile also gave results whose signifience was obscure. In post-mortem bile wide variations were found, from traces too small to estimate to very large quantities, and there was apparently no relation between the amount in the feces and in the bile. In following the daily variations in the urobilinogen and urobilin in the bile discharged from gall-bladder fistulas in patients in whom the gall-bladder had been drained for gall-stones or cholecystitis, it was demonstrated clearly that during the periods

when the patient was feverish and toxemic the quantity of urobilinogen and urobilin was greatly increased, even though little bile was reaching the intestine, and there was consequently little urobilinogen in the stools.

These anomalies in the urobilinogen excretion by way of the urine and bile were not to be explained by any of the current views, and they were simply recorded as facts which any future theory would have to reckon with. The following hypothesis does elucidate not only these facts, but also puts the other data which have been accumulated in their proper place as parts of an ordered sequence of events.

The liver is the central and regulating organ in the metabolism of hemoglobin, not only of its decomposition, but also of its formation. Hemoglobin is converted by the liver into bilirubin. Bilirubin is excreted in the bile into the intestine and is changed to urobilinogen, part of which is excreted in the stools and part absorbed into the portal blood-stream. The urobilinogen in the blood is polymerized to the urobilin-complex. The urobilin-complex is practically all taken up by the liver, but a small amount gets past into the general circulation and is decomposed in the kidneys to urobilinogen and excreted.

But what happens to the greater part of the urobilin-complex which under normal conditions does not get past the liver?

The idea that urobilinogen may be stored in the liver in the polymerized form of urobilin-complex, just as sugar is stored as glycogen, comes at once to the mind as a possibility. Now we know something about the capacity of the liver in storing glycogen, and something also about its power to contain fat, so that it would not be surprising to find that the liver exercised a similar function in connection with hemoglobin metabolism. On the other hand, we also know that the storage capacity of the liver is one of the last of its functions to be disturbed in disease. No matter how disorganized a liver may be, so long as life continues it retains the power to hold glycogen. The relative freedom of the liver from glycogen which is frequently seen post mortem, is the result of diminished intake and increased utilization of sugar, rather than of any inability of the liver to store glycogen, for it is not relatively passive, but active functions which are the first to fail.

But it is preeminently the failure to hold the urobilin-complex which is the first indication of hepatic disturbance. For instance, any toxemic condition, such as that which accompanies an acute tonsillitis, is usually followed by a marked increase in the excretion of urobilinogen in the urine. The liver as a whole is no doubt adversely affected in such conditions, but not as a rule sufficiently to disorganize any of its other functions to an extent which permits us to recognize any abnormality.

Again, it is a well-known fact that if there is need for it the liver can make room for enormous quantities of glycogen, as much as 18 per cent. of the liver weight may be pure glycogen, after large quantities of carbohydrates have been taken.

But even apart from disease of the liver, the limits of its capacity to hold the urobilin-complex are relatively narrow. Since so large a proportion of the total hemoglobin of the body is daily decomposed, it is surprising to find what a relatively small increase in the amount of hemoglobin destroyed is sufficient to lead to the escape of part of the urobilin-complex past the liver.

The conception of a simply passive storage of urobilin-complex in the liver seems to me to be entirely inadequate to account for these facts. It would rather seem that the liver had some active function to perform in connection with the urobilin-complex — a function in which many factors were concerned, which therefore could not be made to work overtime simply by an increase in the urobilin-complex alone, and so intricate that unfavorable circumstances acting on the liver as a whole might readily slow or stop it.

A function which certainly answers this description is the syntheses of hemoglobin-pigment, and the suggestion is put forward that the liver rearranges the atoms in the urobilin-complex to some nearly complete stage in hemoglobin pigment formation in which it is ready for assimulation by the erythroblasts of the bone-marrow.

Such an idea does not find ready acceptance, because one is accustomed to look on the bone-marrow as the site of hemoglobin formation. But so far as I have been able to find the only reason for this belief is the fact that it is possible to trace there all the stages between the early erythroblast, with apparently no hemoglobin, up to the fully formed erythrocyte full of hemoglobin. But that does not prove that the pigment is actually formed within these cells. When hemoglobin is in a coagulated form (Miura[13]) it no longer has its characteristic color or staining reactions, and it may very well be that the erythroblast simply has the capacity to absorb hemoglobin or hemoglobin pigment in some physically altered form, and that the apparent growth of hemoglobin which can be observed within this cell as it develops, is simply a gradual reduction of colorless hemoglobin to the form with which we are familiar.

Looking at the matter simply from the point of view of probability, one would be more ready to believe that such an intricate matter as the synthesis of hemoglobin would be carried out in highly specialized cells such as the liver cells rather than in the undeveloped protoplasm of the

13. Miura: Biochem. Ztschr., 1913, xlix, 137.

early erythroblasts. In this connection it will be remembered that Goldmann's[14] work shows that the early embryo is incapable of producing hemoglobin for itself and has to receive it fully formed from the mother through the placenta, and that in the hen's egg Hugonneng and Morel[15] found a substance which they termed hématovine, which is simply hemoglobin in a slightly modified form. Until the liver is functionating the organism is incapable of synthesizing hemoglobin from pyrrol nuclei.

This conception of the liver as the site of hemoglobin formation is the only one which can account for the unexplained facts which have been mentioned in regard to urobilinogen excretion in the bile and urine.

When this function of the liver is in abeyance the urobilin-complex brought to it cannot be used. It is therefore excreted again into the bile in the form of urobilin and urobilinogen. Thus in cases of gall-bladder fistula in which through bacterial infection the liver was damaged, a large amount of these substances was found in the bile, an amount which bore no relation to the small amounts of urobilinogen found in the stools. On the other hand, when the infection was overcome and the liver recovered, only traces of urobilinogen and urobilin were found, even when enough bilirubin passed into the intestine to lead to the excretion of considerable amounts of urobilinogen and urobilin in the stools. The urobilin and urobilinogen content of the bile depends, therefore, rather on the capacity of the liver to use the urobilin-complex than on the amount which is brought to the liver from the intestine.

There is another point, however, which has to be borne in mind. Urobilinogen is, relatively to urobilin, a readily diffusible substance, and therefore the urobilinogen which is excreted in the bile is in great part rapidly absorbed from the small intestine. In this way the same urobilinogen may continue to circulate between the liver and intestine so long as the liver is incapable of using the urobilin-complex. If in addition urobilinogen derived from the intestinal decomposition of bilirubin is also being absorbed from the large intestine, it can be seen that the quantity of urobilinogen and urobilin in the bile may become very large, as in fact we have found in such cases. Under these circumstances the portal blood-stream will soon become saturated with urobilin-complex and the excretory capacity of the liver be overtaxed, so that part of the urobilin-complex escapes past the liver into the systemic circulation from which it is removed by the kidneys. It is well

14. Goldmann: Aussere und innere Sekretion des gesunden und kranke Organismus., 1912, p. 30.

15. Hugonneng and Morel: Jour. physiol. et de path. gén., 1906, viii, 391.

known that the excretion of bile into the intestine is not constant, but is much augmented at the times when pancreatic secretion occurs. Immediately after the entrance into the duodenum of a bile rich in urobilinogen, there is a rapid absorption of urobilinogen in the form of

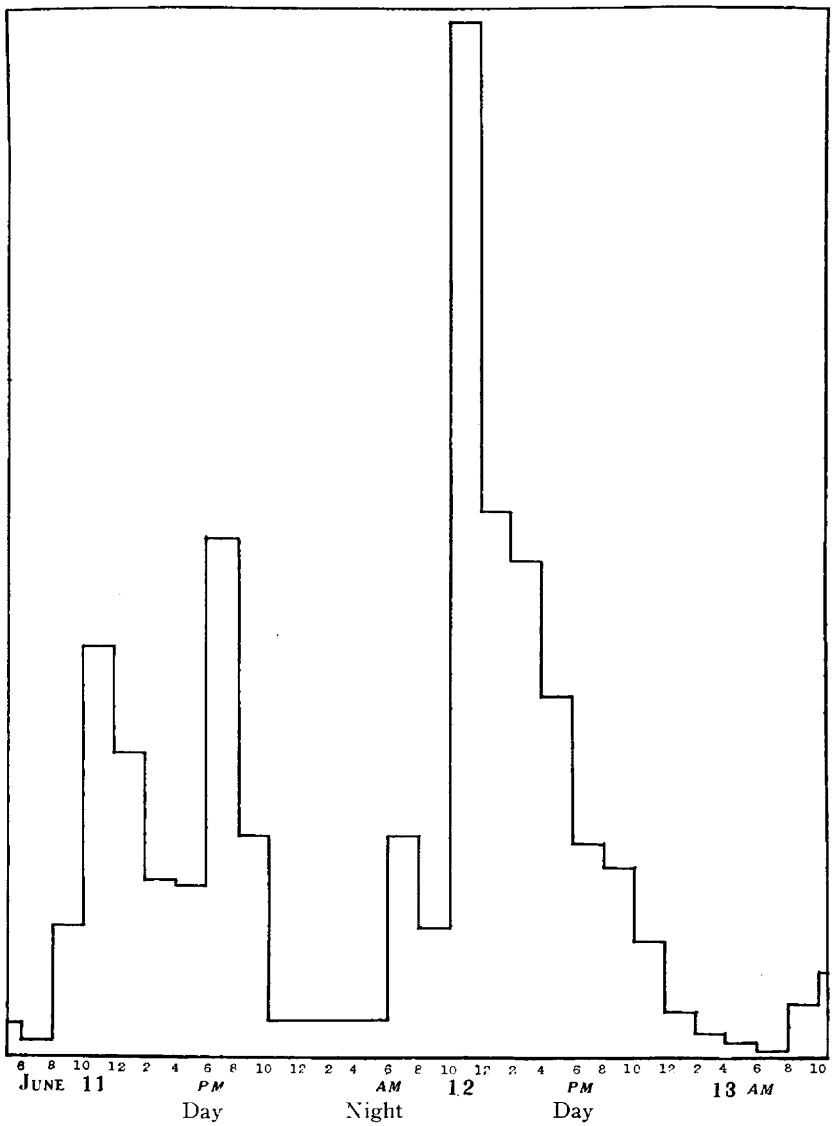


Chart 2.—Excretion of urobilinogen June 11 and 12. Food taken during day time.

urobilin-complex, the liver is overflooded, and as a consequence there is a sharp rise in the urobilinogen excretion in the urine. That the marked excess of urobilinogen excreted during the day is in fact to be

associated with the processes of digestion, is shown by the inversion of this relationship when the food is taken during the night, as is shown in the curves of two-hourly urobilinogen amounts in the case of bronzed diabetes.

The constancy with which any generalized interference with the liver is followed by urobilinogenuria, even though it may be a com-

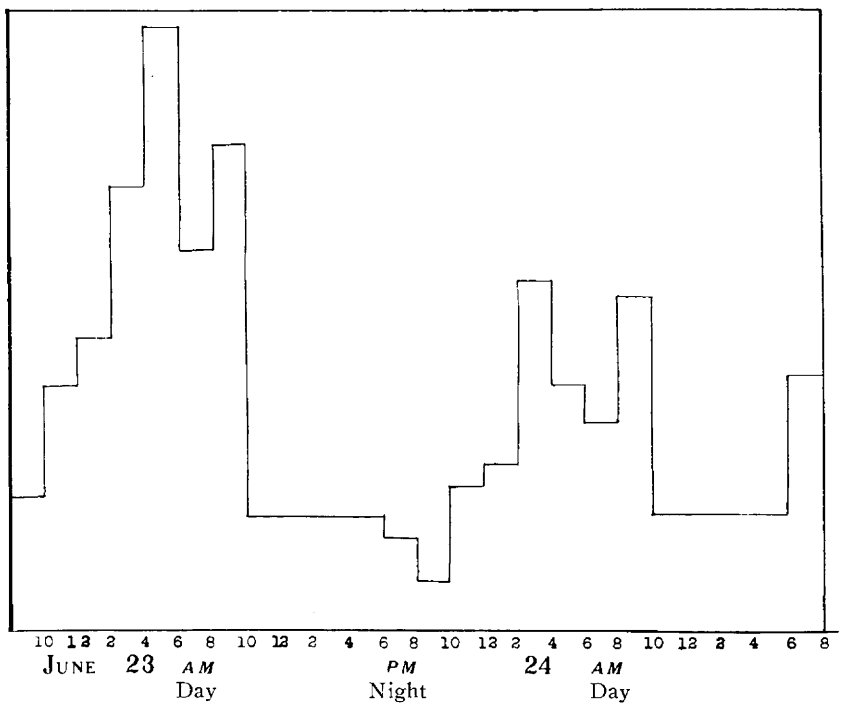


Chart 3.—Excretion of urobilinogen June 23 and 24. Food taken during night. Shows that the largest excretion of urobilinogen occurred during the night instead of during the day, as in the preceding chart.

paratively mild and transient disturbance, and the similar appearance of urobilinogen in excessive amounts in the urine whenever there is even a moderate increase in hemoglobin destruction, is explicable on the theory of a synthesis of hemoglobin from urobilin-complex. It has its analogy in the relative instability of the processes involved in the synthesis of the nitrogen-containing as compared with those concerned with the non-nitrogenous substances in general tissue metabolism. There is a fundamental distinction between protein metabolism and carbohydrate and fat metabolism, which is shown in the fact that when an excessive amount of protein food is taken, the excess is promptly excreted, whereas whatever quantity of carbohydrate or fat is assimilated, is stored in the body. The presence of an excessive amount

of amino-acids does not determine any increase in the reactions involved in the synthesis of new tissue. These reactions require an exact balancing of different constituents, and so any excess of amino-acids above what are required by the tissues is decomposed and excreted as urea in the urine. This parallels the conditions in hemoglobin pigment metabolism, when there has been an excessive formation of bilirubin and as a consequence more of the urobilin-complex is carried to the liver than is necessary for hemoglobin formation. The excess is at once broken down into urobilin and urobilinogen and excreted in the bile.

On the other hand, the flooding of the portal circulation with the urobilin complex, which occurs when the formation of hemoglobin in the liver is in abeyance, has no parallel in the protein metabolism of the tissues. The urobilin and urobilinogen into which the unused urobilin-complex is decomposed as it is excreted through the liver cells, is quickly reabsorbed from the small intestine. With it comes an added increment of urobilinogen from the decomposition of bilirubin. So long, then, as the liver ceases to make use of the urobilin-complex it will heap itself up in the portal circulation, until the blood becomes so saturated with it that the liver can no longer absorb all of it and part escapes into the general circulation to be decomposed in the kidney and excreted as urobilinogen in the urine. In the general protein metabolism there is no such reabsorption of the products of decomposition in order that they may again be used in the building of the tissues, and therefore no accumulation in the blood-stream when anabolism is hindered.

The fact that the hemoglobin-forming function of the liver may show signs of failure, while its other functions, such as, for instance, the capacity to convert sugar into glycogen, are still carried out without difficulty, is explicable when the sensitiveness to any unfavorable change in environment of the comparable synthetic processes concerned in the growth and maintenance of the body tissues, as compared with the relative stability of the general carbohydrate and fat metabolism, is remembered. Bacterial infections, many gastro-intestinal conditions, sometimes even merely psychic disturbances, may lead to a cessation of the maintenance and growth of the protoplasm of the body, even though there are no signs of interference with the power of the tissues to store and utilize carbohydrates and fat. Just as the delicate and intricate reactions on which the building of the nitrogen-containing structures of which the body is composed are the first to be disorganized of all the processes in the general metabolism of the tissues, so in the special metabolism which is carried on in the liver, the rearrangements of the pyrrol nuclei required in the synthesis of

hemoglobin, may be interfered with before the other hepatic functions are involved.

If hemoglobin pigment formation is carried on in the liver and is so easily disturbed, it would follow that any continued interference with this function should be followed by anemia.

It must be borne in mind that here, as in all other important functions of the body, a wide margin of safety may be expected. No doubt the liver is capable, if need be, of producing a much larger amount of hemoglobin than is usually required of it, so that only a fraction of the total liver substance may be sufficient to carry on the work of the whole. In Eck-fistula dogs anemia is not a constant finding, so that it would seem that a relatively small part of the liver may be sufficient if it is functionating normally. It is to be remembered, however, that Enderlen and Magnus-Alsleben[16] have shown recently that the amount of normal liver in an Eck-fistula dog is much greater than is usually assumed, for the hepatic artery rapidly acquires the capacity to give sufficient blood to a great part of the liver. Nevertheless, we know that purely local liver lesions are not necessarily accompanied by urobilinuria and we may assume that so long as a part remains active — how large a part is necessary we do not know — no anemia from decrease in the formation of hemoglobin will result.

The cases in which anemia may be expected are those in which the liver as a whole is involved.

What then are the clinical conditions in which a generalized lesion of the liver exists?

Acute yellow atrophy is not a diffuse lesion. Patches of liver tissue remain and when the patient does not die at once, are sufficiently healthy to grow in size and to divide with great rapidity. Nor is there always any general involvement of the liver cells in conditions grouped under the term cirrhosis, except in the terminal stages.

It is in the cloudy swelling which accompanies severe general infections, such as pneumonia, that one comes nearest to a lesion affecting the liver universally. It is in such cases that one finds large amounts of urobilinogen in the urine, amounts which rise and fall with the course of the disease. It is in such cases, too, that an anemia develops, which becomes more evident the more protracted and severe the infection.

As it is generally understood, the term "anemia" signifies either a decrease in the number of red blood-cells per cubic millimeter of blood, or a fall in hemoglobin percentage, or, as is usual, both combined in varying degree.

16. Enderlen and Magnus-Alsleben: Ztschr. f. d. ges. exper. Med., 1914, iii, 223.

The word, then, covers changes in two substances which under the hypothesis which has been put forward, are genetically distinct, the red blood-cell stroma which is derived from the bone-marrow and its content, the hemoglobin pigment which comes from the liver. Under widely varying physiological and even pathological conditions there is a considerable degree of constancy in any individual in the number of red blood-cells and in the percentage of hemoglobin. Whenever a constant is found we must have a regulating mechanism by which it is maintained. But our methods are as yet too crude to carry us very far in the study of the method of its control. The clinical blood-count and hemoglobin estimation are quite inadequate to give us any idea of the number of red blood-cells and amount of hemoglobin formed and destroyed in a given time, since both are products of two different factors — a balance between production and decomposition. Thus an increase in the number of red blood-cells may mean either that more are being formed or less are being broken up — we cannot tell which. Obviously, we must seek to determine separately the quantity formed and the amount destroyed.

We have no way of directly estimating even approximately the number of red blood-cells or the amounts of hemoglobin which are produced. We can only assume, when young forms of red blood-cells are found, that the bone-marrow is probably active and that the formation of red cells is proceeding at a greater rate than usual.

But we are beginning to get a little insight into one part of the other side of the problem, i. e., the rate of destruction of hemoglobin. We know that the quantity of bilirubin formed is directly dependent on the amount of hemoglobin which is decomposed, and we find that when an excess of bilirubin is excreted a larger amount of urobilin and urobilinogen appears in the stools and vice versa. The proportion of urobilinogen absorbed from the intestine introduces a variable factor, but in spite of this the urobilinogen and urobilin content of the stools gives us a reliable indication of the amount of hemoglobin destruction.

As yet we have not had an opportunity to study more than a few cases of anemia by this method, but the results seem to indicate that there is a reciprocal relation between the destruction and formation of hemoglobin, so that when there is a large amount of urobilinogen and urobilin in the stools, evidences of increased rapidity of production are found, while on the other hand in those cases in which the anemia is due to a deficient formation of hemoglobin, the stool examinations show a decrease below the normal in the urobilinogen and urobilin content, as if the body were endeavoring to bring about a return to the normal by slowing the rate of blood destruction.

This point will be best illustrated by citing three cases in which the hemoglobin percentage was less than normal from three different causes: In Case 1 from increased hemoglobin destruction, in Case 2 from decreased hemoglobin formation because of loss of pyrrol nuclei from the body, and in Case 3 from defective formation because of failure of the hemoglobin-producing function of the liver.

In the following table the normal averages as well as those obtained in these cases are given. The figures for urobilinogen refer to spectroscopic dilution values obtained under the same conditions.

	Blood			Urobilinogen Avg. Daily Excretion	
	Red Blood Cells	Hemoglobin Per Cent.	Color Index	Stools	Urine
Normal ...	5,000,000	100	1.00	6,475	0
Case 1.....	1,600,000	34	1.07	24,977	470
Case 2.....	2,375,000	28	0.58	2,400	0
Case 3.....	4,100,000	65	0.79	2,095	580

Patient 1 had pernicious anemia. The figures given above are an average of daily observations for twenty-four days. This time may be subdivided into two periods of fifteen and of nine days, respectively.

	Blood			Urobilinogen Avg. Daily Excretion	
	Red Blood Cells	Hemoglobin Per Cent.	Color Index	Stools	Urine
Period 1...	1,430,000	31-32	1.10	26,276	604
Period 2...	1,800,000	37-38	1.04	9,546	199

During the first period the rate of hemoglobin destruction as judged from the excretion of urobilinogen and urobilin in the stools, was more than four times as great as normal. Throughout this period normoblasts and megaloblasts were present in considerable numbers.

During the second period the rate of hemoglobin destruction was only moderately increased. No normoblasts or megaloblasts were found in the smears.

Apparently, therefore, when blood destruction was proceeding rapidly, the rate of blood formation was also increased. That it, however, did not succeed in fully compensating for the increased loss is suggested by the lower hemoglobin percentage and red blood-cell count during this period.

In this case in spite of the marked anemia the corpuscles contained slightly more than the normal quantity of hemoglobin. The reason is not far to seek. There was no failure of hemoglobin-forming power, and the liver was supplied with such an excess of urobilin-complex, that part escaped past it into the general circulation. This, then, is an example of a decrease in hemoglobin percentage due to a preponderance of destruction over formation.

Case 2 was an example of anemia from long continued small losses of blood from internal hemorrhoids. The rate of hemoglobin destruction here was much slower than normal. No evidence of rapid blood regeneration was found. In this case each red blood-cell contained only about half as much hemoglobin as the normal. The production of hemoglobin had lagged behind that of the red blood-cell stromata. Yet there was nothing in the case to support the idea that the hemoglobin-forming power of the liver was failing — no advanced anatomical lesion of the liver, no general infection to cause diffuse liver cell degeneration. That this function was, in fact, intact was shown by the fact that the anemia disappeared after a Whitehead operation had been performed, and the loss of blood had been stopped. Why then, since there was less than the normal amount of blood destruction going on within the body, and since the liver function was potentially, at least, unimpaired, was there any anemia at all? Why did not the body compensate at once for the small losses of blood?

The reason is to be found in the distinction which must be drawn between destruction of hemoglobin within the body, and loss of hemoglobin from the body. In Case 1, although the hemoglobin was destroyed, a considerable part of the urobilinogen into which it was disintegrated was reabsorbed as urobilin-complex and supplied the liver with material for the building of new hemoglobin, whereas when blood is lost from the body as in Case 2 the pyrrol nuclei it contains have gone for good and all. There is no possibility of saving any. If such losses are repeated frequently, even though individually they are small, an impoverishment of the body in pyrrol nuclei may ultimately arise, and even though the liver is fully efficient, hemoglobin formation will fail for lack of building material. Even in those cases in which the loss of blood from the body has not been so great as to lead to a decisive decrease in the pyrrol nuclei available for hemoglobin formation, there may be a relative diminution in the amount of hemoglobin which is produced. For the most powerful stimulus to hemoglobin formation would seem to be the product of hemoglobin disintegration — urobilin-complex. Possibly this substance has some specific excitatory influence on the liver cells apart from its use as raw material in the manufacture of hemoglobin.

Case 3 was in a patient with marked cachexia in whom large, irregular abdominal masses were palpable. An exploratory incision revealed general carcinomatosis.

In this case urobilinogen was found in excess in the urine. There was no increased hemoglobin destruction to account for it as in Case 1; in fact, the rate of hemoglobin disintegration was shown by the stool estimations to be much less than normal. A defect in the capacity to

utilize urobilin-complex because of liver degeneration arising from the poisons which had produced the cachectic state found in the patient, was assumed. As in Case 2, each red blood-cell contained less than its usual quota of hemoglobin. The type of anemia was the same in both cases. They were only to be distinguished so far as the study of the blood and excreta went, by the fact that urobilinogen was present in the urine of the patient in Case 3, and absent in that of the patient in Case 2. But this difference is important, for it points to a difference in causation. The decreased hemoglobin production which was common to both cases was produced in Case 2 by pyrrol starvation, whereas in Case 3 it was derived from an inability on the part of the liver to make use of the pyrrol nuclei with which it was supplied.

The success which has attended the introduction of methods of functional diagnosis in connection with the kidneys, stomach, pancreas, etc., has stimulated many efforts to find a satisfactory test for the liver also.

The functional efficiency of the kidneys can be estimated by the study of the amount and rate of excretion of water, nitrogen, chlorids and phenolsulphonephthalein. By means of the stomach-tube and the Roentgen rays we can arrive at conclusions as to the secretory and motor power of the stomach. Test diets and stool examinations reveal any marked abnormality in the absorptive capacity of the intestine, or in the secretion of the pancreas. But we have no well established and widely used test of liver function. We still have to depend in the diagnosis of most hepatic disorders on anatomical changes which are frequently absent, and on symptoms which are often misleading.

A consideration of the methods of functional diagnosis, which have proved useful in other organs, would indicate that the basis of any hepatic test must be some work performed by the liver alone. Now the liver has many functions, but what are the specific functions peculiar to the liver?

Ten, or even five years ago, the list of such probably exclusive functions was longer and there was less doubt and hesitation about most of them than there is now. Yet it is within these years that great efforts have been made to answer this question. Workers in many different fields have joined in a general attack on the problem from all sides. Clinical pathology has acted as a sort of skirmishing force in the front of this attack, and has often temporarily occupied positions which later have had to be abandoned, as the accumulation of well established knowledge has shown them to be untenable. History, especially recent history, is full of this, and a review of the work of the last decade shows that the advance has been, in the main, a retreat and a retrenchment.

Thus, we used to hold that the synthesis of urea from ammonia, water and carbon dioxid could be carried out only by the liver, but now we know that urea may be produced elsewhere. The more recently advanced test of liver function based on the capacity of the liver to remove ammonia from amino-acids has also been shown, by the fine work of Van Slyke and Meyer[17] and others, to rest on an insecure foundation.

The knowledge that creatin appears in the urine in conditions of disturbed carbohydrate metabolism, and not only in cases in which liver function is particularly disorganized, has thrown doubt on the hypothesis that the transformation of creatin into creatinin is a special function of the liver alone. The work of Fischler[18] with Eck-fistula dogs has shown that Strauss' contention that levulose can be converted into glycogen only by the liver has no foundation in fact, at least as regards warm-blooded animals.

The capacity of the liver to form sugar from certain amino-acids, which may be one of its specific functions, cannot be utilized as a test of liver efficiency except in phloridzinized dogs or the severest forms of diabetes.

The tests founded on the so-called detoxicating functions of the liver have not proved clinically useful and Rothberger and Wintersberg[19] have come to the conclusion that there is no sufficient evidence that the liver has any capacity in this respect which other tissues do not also possess.

In general, it seems that the hopes that have been entertained of finding some distinctive and necessary part played by the liver in the chemical reactions involved in the intermediary protein, fat and carbohydrate metabolism of the tissues, on which to found a functional test, do not at present seem likely to be fulfilled.

The more we learn about the intermediary metabolism of the tissues, the more does it seem that these processes are too generalized to form a basis for testing the function of any one organ.

But there is a group of chemical changes in the body which to a great extent may be regarded as shut off from the general tissue metabolism, namely, the processes concerned with the formation, decomposition, and excretion of the substances which go to form the blood.

It is sometimes said that the blood is a tissue, as much a tissue as muscle or connective tissue. The analogy may be correct in many particulars, but it remains true that the blood is widely separated both

17. Van Slyke and Meyer: Jour. Biol. Chem., 1913-1914, xvi, 213.
18. Fischler: Verhandl. Cong. f. inn. Med., Wiesbaden, 1913.
19. Rothberger and Wintersberg: Arch. Pharmacod., 1905, xv, 339.

in its physical characters and in its functions from any other tissue of the body; and this separation of the blood becomes clearer the more we learn about the chemical changes involved in its metabolism.

Thus the other tissues grow from within themselves by assimilating and incorporating substances abstracted from the blood. But the essential components of the blood are not built within itself, but are given to it ready formed from the other tissues, exactly the reverse process.

The products of the breaking down of the other tissues — urea, uric acid, creatinin, etc. — are removed from them and carried by the blood to the kidneys for excretion, but many, at least, of the special and peculiar end-products of the components of the blood itself are eliminated not through the kidneys, but through the liver.

It is just because of this very separation of the blood metabolism from general tissue metabolism that it is possible to recognize defects in the hemoglobin-forming capacity of the liver. It is this distinction that makes urobilinogen estimations a valuable and practical test of hepatic function. But here the excretion of urobilinogen in the urine alone is not decisive, for a large increase over the usual amount may be determined by augmented hemoglobin destruction quite apart from any liver disturbance. Only when the hemoglobin disintegration has been shown by quantitative estimations of urobilinogen and urobilin in the stools to be within or below the normal limits, does urobilinogenuria acquire any significance as an indication of liver insufficiency.

It may be found that the hypothesis which has been advanced as to the rôle played by the liver in the metabolism of hemoglobin pigment is applicable in some degree to other constituents of the blood. It is significant that the bile acids, taurocholic and glycocholic acid, are most readily accounted for as derivatives of the decomposition of the red blood-cell stromata and that they follow the urobilinogen in its absorption from the intestine and its passage to the liver. The part played by the liver in the formation of fibrinogen has long been known, and it is possible that the serum albumin, serum globulin and the protein constituent of hemoglobin have the same origin.

These are interesting possibilities, but it must be admitted that the data as yet are too meager to allow of any constructive theorizing. It is facts rather that are needed.

It is otherwise with hemoglobin pigment metabolism. Here we have a mass of chemical, experimental and clinical findings which have never been satisfactorily coordinated. In particular, I believe that in urobilinogenuria we have a valuable method of recognizing hepatic disturbances which has been misused and misinterpreted since the time of Jaffé, because the relations between this phenomenon and hemoglobin formation and destruction have never been formulated.

Finally, and this is a point of great importance, if this hypothesis is well founded, we have in urobilinogen and urobilin estimations in the excreta a method which will give us valuable information in the investigation into the cause of various types of anemia.

SUMMARY

The hemoglobin liberated from outworn red blood-corpuscles is separated into pigment and protein. Within the liver cells the pigment is converted into bilirubin. In the large intestine bilirubin is reduced to urobilinogen.

The change from hemoglobin pigment to urobilinogen is accomplished by intramolecular rearrangements. The four pyrrol nuclei retain their characteristic grouping. Only the side chains are altered.

But in the form of urobilinogen the pigment molecules have acquired two new properties. They have become diffusible, so that they can be absorbed into the portal blood-stream, and they have a tendency to take up oxygen and become linked in pairs, a process of oxidative polymerization which results in the formation of the body known as urobilin.

In the formation of hemoglobin the urobilinogen absorbed from the intestine, since it retains the essential structure of the blood pigment, would form a readily available building material. A difficulty in accepting this as a possibility lies in the fact that urobilinogen has never been found within the body. But here the capacity for polymerization under the influence of oxygen which urobilinogen possesses suggests the reason. The first product of this reaction — urobilin — disappears into some unknown substance, under the same conditions which favor its own formation from urobilinogen. This is assumed to be a continuation of the same process of polymerization, so that a body is formed which consists of an unknown number of urobilinogen molecules linked together. This hypothetical substance has been termed urobilin-complex. It is in this form that urobilinogen exists in the blood and in the tissues.

The kidneys decompose urobilin-complex into urobilinogen. But under normal conditions, although urobilinogen is constantly being absorbed and converted into urobilin-complex in the portal blood-stream, there is practically no urobilinogen in the urine because the urobilin-complex is removed by the liver.

The data we possess on the varying capacity of the liver to absorb and hold urobilin-complex under various experimental and clinical conditions cannot be explained on the assumption that the liver simply stores up the urobilin-complex, but they are in accordance with the conception that the liver has some active work to perform in connection

with this substance. It is maintained that this work consists in the restitution of the original side chains to the pyrrol nuclei of the urobilinogen molecule, so that hemoglobin pigment is formed again from its own decomposition product.

The surest indication of the presence and of the degree of a generalized disturbance of the liver function is based on the determination of the extent of failure of this synthesis of hemoglobin pigment from urobilin-complex.

There is another and more complicated synthesis of hemoglobin pigment from those pyrrol nuclei which are not, as in urobilinogen, prearranged in their proper relationships to one another. For there is a daily loss of pyrrol nuclei from the body in the stools, and the urobilinogen which is absorbed from the intestine cannot replace the total amount of hemoglobin pigment which is constantly being disintegrated. But of this synthesis, of the site, and of the manner of its building, we, as yet, know nothing.

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