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C. Robert Hankins
University of Nebraska Medical Center

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C. Robert Hankins

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PART I

Normal Bilirubin Metabolism

PART II

Abnormal Bilirubin Metabolism

PREFACE

The term bilirubin immediately calls forth in the mind of the physiologist the normal processes of biliary pigment formation and the normal hepatic functions. Bilirubin to the pathologist usually brings up pictures either of liver damage or of some hemolytic process with deposition of this pigment in the tissues of the body. In the mind of the chemist the extensive chemical analyses of hemoglobin, bilirubin and the related pyrroles done by Fischer are recalled. To the mind of the medical clinician, the term bilirubin integrates the above pictures as a single working unit portrayed to him by his patient.

It is the purpose of this paper to present the normal and abnormal aspects of bilirubin metabolism giving some of the major contributions that have taken this subject out of the realm of conjecture and have placed it upon a scientific basis. Some points are still not explained, and these points of dispute will be pointed out. It is not the purpose of this paper to discuss the various pathological entities that may cause disruption in the normal bilirubin metabolism, but there will be an attempt to show that these entities may be classed into groups fundamentally the same as far as the principle involving the disrupted bilirubin metabolism is concerned; and, that through simplified classification of these processes, bilirubin disorders are more readily understood. Finally, an attempt will be made to show the various practical

applications that can be made upon the basis of the known facts

of bilirubin metabolism.

C. Robert Hankins

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PART I

NORMAL BILIRUBIN METABOLISM

Berzelius is credited with the first scientific studies of the biliary constituents, or bile. He studied bile as a whole, and gave a fair analysis of the various constituents as to their percentage composition. To an orange-red pigment found in the bile he attached the term cholepyrrhin. This work was done in 1840. (21)

Virchow in 1847, (13,39) made the first observations of extrahepatic formation of bile pigment. He found a substance which he called hematoidin occurring in tissues around old hemorrhages discovered at autopsy. In his report he stressed the close relation of hemoglobin to this substance, and also stated its close relation to the biliary pigment termed cholepyrrhin by Berzelius. However, Virchow could not prove conclusively that the pigment formed under such circumstances is identical with cholepyrrhin (or bilirubin) and he therefore gave it the name hematoidin.

Frerichs and Stadler, 1850, (39) worked with bile and renamed the orange-red pigment bilirubin. The name is derived from "bilis" meaning bile, and "ruber" meaning red. They found that a pigment resembling bilirubin could be produced in vitro by the action of sulphuric acid upon bile salts, and more important, that the injection of bile acids into the blood stream of an animal would be followed by the appearance of undoubted bilirubin in the urine. Their conclusion was that the body could transform bile acids into bile pigment.

Kuhne, 1858, (39) repeated and confirmed a forgotten or unnoticed observation of von Dusch that bile acids are powerful hemolytic agents; he insisted that the experiments of Frerichs and Stadler did not prove the origin of bilirubin from bile acids, for those investigators had not taken into account the fact that a large amount of hemoglobin is set free in the plasma by the injection of bile acids. Kuhne was unable to satisfy himself that the injection of hemoglobin alone, in the absence of bile acids, would be followed by bilirubinuria, and he was forced to hold to the idea that the bile acids were necessary in some way for the formation of bile pigment.

Herrmann, 1859, (39) was able to produce bilirubinurea at will by inducing intravascular hemolysis with injections of distilled water. This was the first clear demonstration that simple liberation of hemoglobin into the blood stream may be followed by an increased output of bile pigment in the urine. Neither Naunyn, 1868, nor Steiner, 1873, could confirm Herrman's results, a fact that is now easily explained, since the absence of bilirubinuria is by no means a proof that there has been no increased formation of the pigment. Tarchanoff, 1874, on the other hand not only confirmed Herrman's work, but, with the use of bile fistula animals, carried the proof of the relation of hemoglobin to bile pigment still further by demonstrating, for the first time, that the introduction of pure hemoglobin into the circulation is followed by a marked increase in the amount of

bile pigment excreted by the liver.

As early as 1769, Morgagni taught that the liver excreted the bile which was brought to it, preformed, by the blood. An altogether different view was held for many years following the experiments of Minkowski and Naunyn, 1886, (13) who found that jaundice was produced in geese by poisoning with arseniuretted hydrogen, whereas jaundice could not be so produced if the liver was removed immediately after the administration of the substance. From this they concluded that the liver was necessary for the production of jaundice, and the belief arose that bile pigment was formed by the hepatic cell.

McNee, 1913-14, (30) repeated the experiments of Minkowski and Naunyn, and found small traces of bile in the urine and the tissues of hepatectomized geese following the administration of the arseniuretted hydrogen. McNee observed, in histologic studies of the normal goose liver, that the von Kupffer cells contained erythrocytes and gave a marked iron reaction. In the liver of jaundiced geese, the Kupffer cells were seen to contain, besides many erythrocytes, large quantities of yellowish green pigment, which resembled biliverdin, although chemical proof was lacking. McNee concluded that in geese bile pigment was formed by the endothelial (Kupffer) cells of the liver and by the small number of endothelial cells found elsewhere (spleen and bone-marrow). In geese, hepatectomy accomplishes removal of the greater part of the reticulo-endothelial system, but in mammals it does not. Thus

the conception of bile pigment formation returns to the concept
of Morgagni, 1769, that bile constituents are produced in the
body and carried to the liver via the blood stream, at least
in relation to bilirubin.

Before reviewing further physiological experimentation, one
should consider the type of cell of which the Kupffer cell is
an example. During the last half century, certain fixed cells
and certain wandering cells having phagocytic properties have
been studied by many workers. Ribbert, 1904, (13) showed the
interrelation of all these different cells in their common a-
bility to engulf particles of lithium carmine injected into the
circulation. To Aschoff belongs the credit for the conception
that these reticular and endothelial cells of the various tissues
possess common properties and functions, and may be designated
together as the reticulo-endothelial system.

As these physiological principles were being studied, the
biochemists were doing much work in determining the composition
of the bile pigments. The discoveries of Virchow and Herrman
were the guiding lights in their studies. It was up to them to
show the chemical similarity of bilirubin to hemoglobin, or if
possible definitely prove the derivation of bilirubin from
hemoglobin. Fischer has probably been the greatest student of
this subject, and to him is attributed most of the advancement
made in this field. Without going through the long processes
involved in determining this technically difficult problem, it

is the purpose of this paper to present the modern concept of
this problem.

As stated before, the chemists started working with hemo-
globin and attempted to prove the derivation of bilirubin from
this source. Hemoglobin is classified as a hematoprotein.
The hematoproteins are present in all protoplasm, but are found
more accessable in the blood. Their purpose is to carry oxygen,
and they are associated with the processes of oxidation in every
tissue of the body. Hemoglobin is insoluble in itself, and so
must be carried about physically in the red blood cells rather than
in solution. Hemoglobin is a sub-group of the conjugated proteins.
Conjugated proteins are made up of two radicals, one being the
protein radical and the other being termed the prosthetic group.
In the case of hemoglobin, the protein fraction is made up of a
globin and the prosthetic group is a porphyrin. The prosthetic
group is a relatively small part of the molecule, roughly making
up about 4% of the molecule. Porphyrins, chemically, are con-
structed on the porphine nucleus; this nucleus is made up of
4 pyrrole rings arranged in rosettes and called tetra-pyrroles.
The porphyrins are found widely distributed throughout nature
in both animal and plant kingdoms, their main characteristic
being their ability to produce color in reflected light, or,
in other words, they are pigments.

When hemoglboin is treated, under appropriate conditions,
with glacial acetic acid and sodium chloride, and the mixture

warmed gently, a substance is obtained which crystallizes readily
as brown cyrstals--this substance has been called hemin. (See
the plates at the end of this paper for formula and reactions
mentioned in this chemical discussion of bilirubin) The other
product of this cleavage is globin. Hemin has been synthesized
by Fischer. (6) It contains four methyl-pyrrol radicals and an
atom of iron, and may be represented as shown in the aforementioned
plates. If the crystals of hemin are treated with sodium
hydroxide, the corresponding base, heme, is liberated; this is
the original prosthetic radical of hemoglobin.

The porphyrin nucleus related to hemin is called protopor-
phyrin. (see plate) With the introduction of an iron atom into
the molecule, neutral heme is formed, which may be isolated as
hemin, indistinguishable from the product derived from hemoglobin.
This is indirect evidence to the fact that hemoglobin is built up
from preformed protoporphyrin. It is assummed by the chemist
that the body is capable of forming this nucleus as a necessity
to the making of hemoglobin. (Evidence will be given later to
substantiate this fact from the physiological viewpoint.)

Bilirubin has been studied extensively by Fischer and he
has given the substance a definite formula. (See plate) As
yet, he has not been able to synthesize this substance, so the
formula rests merely upon analysis of the substance. As shown
by his formula, Fischer believes that bilirubin arises by oxi-
dative scission of the porphyrin nucleus of hemoglobin, the iron

being lost in the process. The method by which the iron is lost, and the nature of the compound in which it is lost is still a vague subject. This iron containing radical is called hemosiderin; the material can be demonstrated to contain iron; and beyond that, there is little information. Lemberg (22,23) believes that the process leads to biliverdin first, and then to bilirubin. 'Biliverdin is an oxidized product of bilirubin, containing two more oxygen atoms, but otherwise the same emperically. In his experiments, Lemberg has been able to obtain bilirubin from hemin as outlined in the plates previously mentioned, and has identified the intermediary products. He suggests that the formation of bile pigments in the animal body proceeds in a similar fashion yielding biliverdin which is afterwards reduced to bilirubin. He admits that from a physiological point of view much remains to be done, but he believes that this model will provide a good guide to further research.

Andrews (1) and Rich (41) have proven that the hematoidin found by Virchow is the same substance as bilirubin.

In summarizing the modern chemical viewpoint, we must admit that definite proof of the formation of bilirubin from hemoglobin is still lacking. An important step in clearing up this matter would be the synthesis of bilirubin, and the demonstration that the formula as given is correct. But in all fairness, we must say, the only chemical evidence of derivation of bilirubin from hemoglobin is the chemical similarity of the bilirubin to the porphine

nucleus. Lemberg's work seems to be the closest approach to definite proof of this derivation.

Knowing these chemical relationships of bilirubin to hemoglobin, we can now proceed more intelligently to follow the physiological developments that have been made in the last few years.

Interesting observations were made by Peabody and Brown (13) on the phagocytosis of erythrocytes in the bone marrow. These workers employing the Berlin blue iron reaction, observed in the bone marrow the presence of erythrocytes and granules of iron in the same phagocytes. They concluded that the phagocytes of the bone marrow ingest erythrocytes and that the hemosiderin (an iron containing substance derived from the breaking down of hemoglobin) is formed within the phagocytes from hemoglobin.

P. Corr (8) recently studied material from 71 necropsies with respect to the distribution of hemosiderin and bilirubin. Sections of liver and spleen and occasionally of bone-marrow, kidney, lymph node, lung, suprarenal gland and pancreas were stained for iron by the Berlin Blue method. Hemosiderin was found in varying amounts in the splenic phagocytes, in the hepatic and Kupffer cells of the liver and in the reticulo-endothelial cells of the bone marrow. Corr concluded that the reticulo-endothelial cells of the spleen and the bone marrow were most active in breaking down the hemoglobin. He believed that the Kupffer cells and large wandering phagocytes served as a

reserve of the reticulo-endothelial system.

Haldeman (13) anesthatized a group of dogs and removed pieces of liver, spleen, bone marrow, lymph nodes and various other tissues. The hemolyzed corpuscles of 100 ccs of dog's blood were injected into the juglar vein. At intervals of 15 minutes to 3 hours thereafter, additional pieces of the same organs were excised. Samples of blood from the juglar vein, corresponding in time to the various sets of tissues removed were studied by spectrophotometric methods to determine the quantity of bilirubin circulating in the blood at the various stages of the experiment. Since it is known that iron is liberated in the breaking down of hemoglobin into bilirubin, it is reasonable to conclude that the cells that contain iron are those concerned in the conversion of hemoglobin into bilirubin. Furthermore, when samples of various tissues are removed before the intravenous injection of hemoglobin and at regular intervals of time thereafter, it may be justifiably assumed that the tissues showing the striking increase in iron content are those actively concerned in the process of converting hemoglobin to bilirubin, while those tissues containing a constant amount of iron (as the spleen) probably have to do with the storage of the iron until it is again built up into hemoglobin. Haldeman concludes that the bone marrow plays the major role in the formation of bilirubin, with the liver and spleen and possibly the lymph nodes having lesser parts in the process.

Mann and Bollman (31) to afford conclusive proof of this extrahepatic formation of bile pigment made a series of studies on hepatectomized dogs. These demonstrated clearly that bilirubin formation continued in the absence of the liver. By measuring the arterial and venous flow to the various organs and then comparing the bilirubin content indicated that the spleen and the bone marrow, particularly the latter, are the chief sites of formation of bilirubin. They also found bilirubin in the splenic vein and in the veins returning from the bone marrow after injections of hemoglobin solution into the arteries afferent to these structures.

Rich (40) stated it was obvious that if red blood cells could be kept under continuous observation after being ingested by clasmatocytes, or while disintegrating in the tissue fluids outside of living cells, a much clearer idea of the process might be obtained. This Rich has tried to do by the use of the Lewis tissue culture. Fresh red blood cells were added to cover glass cultures containing wandering phagocytic cells of mesodermal origin, and the fate of the red cells was followed under the microscope. It was found that the phagocytes wandered among and ingested the red cells in large numbers, and that, as the hemoglobin was broken down within the phagocytic cells, beautiful rhomboid or needle-shaped crystals typical of bilirubin, and often diffuse, bright green biliverdin appeared within the cytoplasm of the living phagocytes. These pigments gave a perfect Gmelin test for

bilirubin under the microscope and possessed every other chemical and physical property of bile pigment for which they were tested. The Prussian blue reaction often showed the presence of an iron-containing residue within the phagocytic cells by the side of the bile pigment. Up to the stage in which crystals of bile pigment first appeared within the phagocytes, no crystals were ever found outside of these cells, although there were often great numbers of red cells disintegrating extracellularly. Later, as the clasmatocytes themselves began to die and to disintegrate, the intracellular crystals and soluble pigment were, of course, set free; so that in older cultures there might be numerous crystals lying outside of cells some of which apparently formed extra-cellularly from soluble bile pigment. It was certain, however, from the study of hundreds of such cultures that the hematoidin crystals can make their first appearance within phagocytic cells, and that they were not phagocytized preformed. The experiments are not yet entirely free from the possibility that bile pigment may have been present invisibly in soluble foam in the tissue fluids, and until this possibility of phagocytosis of the pigment in soluble form from the surrounding media can be controlled, it cannot be stated with any certainty that hemoglobin can be converted into bilirubin within phagocytic cells of the reticulo-endothelial variety. However, this is one of the best proofs we have of the extrahepatic formation of bilirubin in the reticulo-endothelial system.

Despite the extensive evidence of the formation of bilirubin extrahepatically, some men still cling to the idea that the hepatic cell in itself is able to produce bilirubin. The main evidence to date that the hepatic cell makes bilirubin is that the jaundice produced by certain substances, notably toluylene-diamine, is prevented by the removal of the liver. Toluylene-diamine produces both increase in formation of bilirubin and retardation of its excretion, together with demonstrable injury to some of the hepatic cells. In order to explain the jaundice produced by this substance, on the basis that the retained bilirubin is formed by the hepatic cells, it is necessary to postulate that toluylenediamine injures the hepatic cells in such a manner that they form more bilirubin than normal and ex-crete less than normal. Mann (33) believes that this consideration must remain as a possibility until the mechanism of the jaundice caused by the drug is conclusively determined, although it appears rather unique that injury to a cell should cause it to increase its activity in one respect and decrease it in another.

Among numerous data which have been assembled both in support of and against hepatocellular bilirubin formation, one observation of Lubarsch, 1921, (13) deserves mention. This is the appearance of bilirubin in distant metastases from primary carcinomas of the liver. Lubarsch states that since these lesions do not contain von Kupffer cells, the presense of bile pigment is evidence of formation by hepatic cells. On the other hand, Aschoff, 1922,

has pointed out that these cells might be serving simply in an excretory capacity, removing bilirubin from the blood, the only function which he attributes to the normal liver cell insofar as bilirubin metabolism is concerned.

Lepehne (9) felt that the question of bile pigment formation could best be explained as a dualistic role, one in which both hepatic and reticulo-endothelial cells participate. This view of Lepehne's is difficult to accept since it seems unlikely that glandular epithelial cells, (the hepatic cells) and cells of mesenchymal origin (the v. Kupffer cells) would both posses the rather specific function of converting hemoglobin into bile pigment. In the following it will be seen that epithelial cells of ectodermal or entodermal origin have not been shown to have this ability, while the evidence already discussed makes it clear that the reticulo-endothelial cells generally are able to make this conversion. In connection with this argument, van den Bergh and Papendieck (9) have seen marked variation in the relative amount of bilirubin taken from hemorrhagic fluids of different sources. In an extensive series of tests, it was found that bilirubin was regularly present in those fluids within mesothelial-lined cavities, but was present only twice in epithelial-lined cysts. It also showed that epithelial specialization of cells which were originally mesodermal is associated with a diminished production of the ferment necessary to bilirubin formation. In this connection it is noteworthy that bleeding into the gastro-intestinal canal does not result in bilirubin formation. Cases of gastro-intestinal

cancer are not infrequently seen, having blood in the feces and at the same time exclusion of bile from the duodenum because of neoplastic disease. Hemoglobin or hematin is readily demonstrable in the feces of these patients, but bilirubin or its derivatives are absent.

The evidence for the extracellular formation of bilirubin is rather convincing; in fact, Leschke's study of bilirubin formation in the spinal fluid has proved that bilirubin may be formed extracellularly, probably by means of a ferment. (9) This work was based upon Ober-Mayer and Popper's demonstration, 1908, that the yellow color of spinal fluid, as in the Froin syndrome and in other blood containing fluids, was due to the presence of bilirubin. Leschke injected washed erythrocytes in the human lumbar canal and noted bilirubin formation which did not occur with control erythrocytes kept sterile in the incubator. After centrifuging, the supernatant fluid was placed in the incubator with more washed erythrocytes while the other half was incubated without addition of cells. An increase of bilirubin in the half containing erythrocytes was observed. This pointed strongly to a fermentative mechanism as the basis for the transition of hemoglobin to bile pigment, which was also suggested by the studies of van Czike, the results of which indicated that small amounts of bilirubin may form in vitro, when blood alone is allowed to stand.

Besides the possibilities of bilirubin being formed intra- and extracellularly from hemoglobin, the possibility of the body

making this pigment independently of hemoglobin must be considered. As mentioned under the chemical discussion of this subject, it has been suggested that the body is capable of making the pyrrole nucleus, and thus might be capable of manufacturing bilirubin. Hawkins (20) found that anemic dogs formed new hemoglobin from the entire amount of foreign hemoglobin administered, but at the same time formed additional bilirubin equivalent to the entire amount of hemoglobin given. These observations indicated that new hemoglobin molecules cannot be utilized in the formation of new hemoglobin, although other portions of the hemoglobin molecule may be incorporated in the new hemoglobin. Whipple (49) also agrees that the body is capable of producing pyrrole rings. Whether the body produces some of the pyrrole rings directly for bile pigment formation is a question, but it does produce them for the formation of hemoglobin, using the old globin and iron fractions in the process.

Rich (39) in summarizing the evidence that hemoglobin furnishes a source of bile pigment, states: "1. that hematin and bilirubin are closely related chemically; 2. that hemoglobin spilled into the tissues almost anywhere in the body may be transformed, locally into bile pigment; and 3. that the presence of an excess of hemoglobin in the circulation, whether introduced in pure form experimentally or liberated during hemolysis in experimental or pathological conditions, is regularly followed by an increased formation of bile pigment." Mann and Bollman (32)

have shown that bile pigment continues to be formed in animals after complete removal of the liver and there can be no doubt that hemoglobin is the material from which it is derived. Further summary to this point on the evidence presented, one may conclude that the major site of bilirubin formation is located within the reticulo-endothelial system, particularly within the bone marrow and spleen. It is also reasonable to conclude that the bilirubin that is formed within the liver is formed within the von Kupffer cells and not the hepatic cells.

It is of some interest at this point to consider briefly the methods used to determine bilirubin concentrations in the blood. These methods can be grouped into four main groups: oxidation method; icteric index; methods based on Ehrlich's reaction; and spectrophotometric methods.

The first method, that of oxidation of bilirubin has proven to be rather inaccurate and has been replaced by other methods. The principle involved was that the bilirubin of the sera was oxidized to biliverdin, the resulting color being compared with previously made color charts. The results were obtained directly from the color chart with which it compared.

The icteric index is a method of direct comparison of the blood sera with a known standard. Assuming the yellow color of blood sera is principally due to bilirubin, methods have been

evolved to determine the amount of bilirubin by comparing the
color of the serum with artificial standards of approximately
the same shade of yellow color--usually a solution of potassium
dichromate. If in crystalloid state, a given value of bilirubin
would impart a greater color intensity to a given volume of sera
than that same amount of bilirubin if it were in a colloidal
state. The icterus index, then, may be regarded as a function
as well of the physical state of bilirubin as of its total
quantity. (Barron, 2) Elton (10) in correlations from over 1700
sera examined found; "1. The icterus index fails to conform
consistently with any constant proportion of total bilirubin;
2. For a given icterus index the bilirubin content of the serum
is higher when it exists in the colloidal state than when it is
crystalloidal; 3. Colloidal bilirubin accumulates in the blood
stream at the icterus index 16.6, but fails to impart a higher
color intensity to the serum in which it is suspended until it
undergoes a physical change, expressed by the development of a
direct type of van den Bergh." In further criticism of this
method, Barron (2) states, "As the chemistry, the amount and the
daily variation of the lypochromes (lutein, carotin, etc.) of the
blood is unknown, the writer is of the opinion that these methods
in which bilirubin as well as all other yellow substances in the
blood serum are estimated, ought to be discarded. It is well known
that the yellow color of sera increases with a diet rich in veget-
ables, producing a pseudo-jaundice." In summary of the icteric index

we may say that it is useful in showing an increased bilirubin content in the blood, but it must not be relied upon to give a quantitative estimation of the bilirubin present.

The van den Bergh reaction is probably the best known method of testing for bilirubin. Erhlich, 1883, first made the observation that when a dizaonium salt is added to a solution of bilirubin, a new compound, azo-bilirubin, is formed, the solution becomes colored, and the color depends upon the reaction of the solution--red when neutral and blue when the solution is acid. Van den Bergh applied it first to clinical work in 1913, thus transferring the outlook on jaundice from the color of the skin to quantitative changes in the blood. (McNee,29)

The van den Bergh test consists in overlaying a small amount of clear serum with the diazo reagent and observing for a purplish or bluish red ring at the line of contact. The diazo reagent consists of two parts, A and B, mixed together just before making the test. Solution A is a 0.5% solution of sulphanilic acid in distilled water. Solution B is a 0.5% solution of sodium nitrite in distilled water. If a reddish violet color reaction occurs immediately on adding the reagent to the serum and reaches a maximum within about 30 seconds, the reaction is known as "immediate direct." When with the same technique, a reddish color is observed to develop only after some time has elapsed (1-10 minutes) and deepens to a violet hue, the reaction is known as "delayed direct." When a reddish color appears immediately and, only after some time deepens to a violet hue, the reaction is known

as a "biphasic direct." When no color reaction whatever occurs
after addition of the reagent to the blood directly, but does
occur after the blood proteins have been precipitated with alcohol
and the reagent has been applied to the clear alcoholic filtrate,
the reaction is known as the "indirect." The van den Bergh is
delicate enough to determine the presence of bilirubin in dil-
utions as great as 1:1,500,000 in alcohol. The significance
of the various reactions obtained in this test will be pointed
out later.

The spectrophotometric method for determination of bilirubin
calls for a costly instrument and careful technique, and is not
suitable for most laboratories. There is no doubt that it is by
far the most accurate and sensitive way of measuring bilirubin.
It is possible to determine the character and the shape of the
spectrophotometric curve of bilirubin for a dilution as low as
one-fiftieth of the smallest amount measurable by the van den
Bergh technique.

By use of the above proceedures a number of facts have been
elicited. Normal blood serum contains between 0.1 and 0.25 mg
of bilirubin per 100 cc; or in other words, it is present in
dilutions of 1:1,000,000 to 1:400,000. (37) As the reaction
is frequently spoken of in "units" of bilirubin, it may be here
observed that one unit corresponds to a dilution of 1:200,000.
In other words, normally, blood serum contains 0.2 to 0.5 units.

Barron (2) believes the normal bilirubin content to vary

between 0.1 and 0.5 mg%. It is possible some of the higher normal figures include some individuals classified among the group of latent familial jaundice or those affected with slight liver insufficiency. However, daily variations occur. The highest reading is found in the morning when fasting; it falls after breakfast, the lowest figure being obtained generally 3 to 6 hours after meals. A prolonged fast seems to increase the bilirubin content of the blood. Sex seemingly plays no difference in the bilirubin level.

Van Berghman (10) estimates that in the average 70 kilogram human adult the daily bilirubin production is about 500 mg, which, in terms of 100 cc units of blood serum volume, may be computed as approximately 14.3 mg. The normal bilirubin level does not exceed 0.2 mg%. Visible jaundice may appear in the white race when this level rises to 1.5 or 2.0 mg and may often be detectable in the sclerae at much lower levels. So, the body must have mechanisms for adequate excretion of bilirubin.

It is almost unnecessary to state the fact that the liver is the main organ of excretion of bilirubin. The bilirubin is removed from the blood in the liver sinusoids and is excreted in the bile. The normally functioning liver keeps the bilirubin level down to figures just previously mentioned. The exact threshold value of the liver in relation to bilirubin has not been

shown; but if it does exist, it is very low.

It appears that, as for sugar and a number of other blood constituents, there is a renal threshold for bilirubin; the normal threshold is about 2.0 mg% or 4 units. It is regularly found in the urine when the concentration is 3.5 to 4 units. (Rabinowitch, 37) A curious exception must be made to this statement; bilirubin giving the indirect van den Bergh reaction is not excreted by the normal kidney regardless of how high the bilirubin sera concentration is. This fact will be discussed later. So we can say that the kidney plays a part in the excretion of bilirubin only under pathological conditions.

The fate of bilirubin in the intestinal tract after leaving the common duct brings up some rather interesting factors. Sackey (43) has proven that bilirubin is not reabsorbed in the small intestine. He placed bilirubin in isolated sterile jejunal loops for a period of two hours and was able to quantitatively obtain the bilirubin back from the loop contents. He has also shown that no change takes place in the nature of the bilirubin while in the small intestine. He has placed bilirubin in intestinal juice and incubated it for 2 hours with no change in the bilirubin.

Blankenhorn(3) devised an ingenious means of obtaining blood from the portal vein of a dog without opening the abdomen. He made corresponding studies of the juglar and portal blood, and the lymph from the thoracic duct. He found that "bilirubin is

not reabsorbed from the intestine by way of the portal vein in healthy animals. Bilirubin may be absorbed from the intestine by the lymphatics but only in minute amounts."

With this evidence, it is reasonable to say that the bilirubin passes through the small bowel with little quantitative loss due to absorption, and with no chemical alteration. But in the large bowel a large proportion of the bilirubin is changed into another substance called urobilin, or into urobilinogen. These substances are reduced bilirubin. Their relation to bilirubin may be seen on the plate showing chemical properties and formulae of the bile pigments. This change in the bilirubin is supposedly due to bacterial action in the colon. The urobilin formed in the intestines is in part excreted in the stools, in part destroyed, and in part resorbed by the portal blood stream and carried back to the liver. Normally the liver removes this urobilin and excretes it in the bile. If the liver does not excrete this urobilin, it is then excreted by the kidney, the renal threshold being practically nil for urobilin. Normally the liver removes this urobilin from the blood to such an extent that urobilin does not appear in the urine in readily demonstrable quantities.

To prove this transformation of bilirubin, Friedrich Muller (48) obtained urobilinogen by bringing bilirubin in contact with peptone solution and putrefactive bacteria obtained from fecal material. (1892)

Methods of estimating urobilinogen and urobilin are:

1. Fluorescence in the presence of zinc salts; 2. Spectroscopic absorption bands; 3. Production of red color with Ehrlich's aldehyde.

The fluorescence in solutions of zinc salts was one of the first methods of determining the quantity of urobilin present. Acriflavine is used as a standard, being standardized against a zinc acetate filtrate from a standard known solution of urobilin with regulated pH (equivalent to a 0.05 N HCl solution). By determining standards in this way, a measurement of the urobilin content is effected by comparing its fluorescence at a great dilution with a standard containing acriflavine, calibrated in turn against pure urobilin. (24)

The spectroscopic method is the most accurate, but again requires expensive apparatus and special technique.

For a simple test, we are again indebted to Ehrlich (1886). The reagent in the original form consisted of a 3 per cent paradimethylamidobenzaldehyde in a 50% solution of HCl. Neubauer, 1903, was the first to use it clinically. When added to urobilinogen solutions a red color develops. The test is not specific for urobilinogen, being common to a variety of chemically related substances, but, for practical purposes as far as urine is concerned, it may be assumed to be so. (37)

Wallace and Diamond, 1925, (47) devised a quantitative test. The test is, essentially, one of serial dilution of urine and the addition of a definite amount of reagent to a definite quantity

of diluted urine. The reagent for this purpose consists of a 2% solution of the aldehyde compound in 20% HCl. The amount of pigment is judged by the greatest dilution of the urine in which the faintest pink color is discernable on the addition of the reagent. The normal figure obtained in this manner ranges from a 1:10 to 1:20 dilution.

Urobilinogen is a normal constituent of urine in man. It is present in smallest amount in the morning urine, or may be absent altogether--rising somewhat in the afternoon and evening. In constipated individuals with increased putrefactive changes in the large bowel, the quantity of urobilogen rises above normal. (47) Urobilin never appears in freshly voided urine, but appears upon oxidation of the urobilogen.

McMaster (24) has shown that the normal presence of urobilin in the bile and feces of dogs depends upon the passage of bile pigment to the intestine, either through the normal channels, or by abnormal ones, as when it is fed by mouth. He devised a method wherby animals can be totally, partially or intermittently deprived of their bile, without infection of this secretion or of the duct system. By this means he was able to study urobilin physiology and to make the above observations. He has shown that complete loss of bile from the body resulted in the total disappearance of urobilin and urobilinogen from the bile, feces and urine. Partial loss of the bile resulted in a corresponding reduction in the urobilin of the dejecta.

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

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

McMaster (24) also employed intermittent diversion of the bile stream of animals from the intestine to a collecting apparatus, and showed that while bile pigment still reached the intestine urobilin was present in the bile secreted by the liver, but that almost at once after the bile had been diverted from the gut urobilin disappeared from it. He has shown that the bacillus putrificus and other intestinal flora were capable of changing bilirubin to urobilin.

So in conclusion we can say that urobilin is an end product of the action of bacterial flora of the large intestine on bilirubin; that bilirubin in itself is not reabsorbed from the gut in any quantitative manner, but this end-product is rather readily absorbed; that for the production of urobilin, bilirubin must reach

the large intestine; that final excretion of the urobilin
is via the feces in the normal individual, only a small
proportion being eliminated by the kidneys.

The following is a graphic summary of the changes
from hemoglobin to urobilin with the intermediary steps;
reading downward:

<pre>
            Hemoglobin
                '
            Hematin
                '
            Bilirubin----(oxidation)----Biliverdin
               '(reduction)
            Urobilinogen
                '
            Urobilin
</pre>

The following chart was taken from Hewlett (15)
after Willis and Addis. This gives in summary the fate of
the bile pigments in the various organs of the body.

<pre>
Reticulo-endo. System ------ Bile Passages ------- Intestine
     Hemoglobin   Bilirubin   Urobilinogen

                  Intestine   Liver
Urobilinogen---Part excreted   (Part excreted in bile
               (Part absorbed----------Part returned to form
               (Part destroyed   (Hemoglobin?
                                 (Part enters general cir-
                                     culation and excreted
                                     by kidney.
</pre>

PART II

ABNORMAL BILIRUBIN METABOLISM

Having considered the normal physiology of bilirubin, one can now view the pathological disturbances of its metabolism with clearer insight. Knowing the normal physiology, one can point to the process of the bilirubin metabolism in which the fault lies in the pathological process.

The most objective sign of abnormal or pathological bilirubin metabolism is jaundice or icterus. This phenomenon is defined by Dorland as "a syndrome characterized by hyperbilirubinemia and deposition of bile pigment in the skin and mucous membranes with resulting yellow appearance of the patient." Jaundice comes from the French word "jaune", meaning yellow.

Jaundice, being such an evident thing, has been described in connection with disease from the times of the early Greeks. The Greeks called the disease icterus. This word is derived from the Greek word applied to an animal resembling our cat. The eyes of this animal were said to be of the yellow color characteristic of jaundice. Others believe that the word is derived from the name of a bird called icterus; the sight of this bird was a cure to the jaundiced patient, but death to the bird, as the belief goes. (21)

The Latins called jaundice "morbus regius", from the yellow color of gold, the "Rex metallorum". For the same reason, another Latin synonym is "aurigo" from "aurum, meaning gold.

Jaundice could hardly fail to be noticed early in the history of mankind, as the change of complexion and color would be a striking appearance. Accordingly there is abundant mention of it in the genuine Hippocratic writings, which most often speak of it as a complication of other diseases rather than as a disease in itself. In the "Aphorisms" it is said to be a bad sign if the liver becomes hard during jaundice; and in the third book of the "Epidemics", that an habitual pain in the neighborhood of the liver, with other symptoms, preceeded a jaundice. It is disputed if Hippocrates conceived any relation between the bile secreted by the liver and the symptom of jaundice. (21)

Galen asserts that "the yellow bile, when it is carried all over the body, still keeping its own nature, causes a disease called jaundice". He believed that "weakness of the gall bladder, which does not draw to itself the bile out of the liver as it is accustomed, leaving the blood impure, is the cause for jaundice". Galen also states that jaundice may be found without any associated liver damage. (21) In the light of our present day knowledge, it is remarkable that such accurate conclusions could be drawn from pure observation.

Returning to the present-day conception of jaundice, Rice (38) in view of the known physiology of bilirubin, states that jaundice will occur if any of the four following qualifications are met:

1. "If the threshold of the liver for bilirubin excretion became greatly raised."

2. "If for any reason, bilirubin were produced faster than the normal liver cells could excrete it."

3. "If the mechanism of the liver were so disturbed that the amount of bilirubin normally produced could not be satisfactorily removed from the blood."

4. "If there occurred any combination of the above."

According to Rice, the first is hypothetical, with no evidence of its existence, and which it is unnecessary to involve in order to explain any form of jaundice.

The second classification, an uncomplicated excessive production of bilirubin, is rarely a cause of jaundice for two reasons, according to Rice: "First, because the normal liver is able to excrete much more bilirubin than is ordinarily delivered to it; and second, because an excessive production of bilirubin is, as we shall see, practically always associated with other conditions which tend to impair the excretory power of the liver." There is a tremendous reserve power in the liver in excreting bilirubin. The excretory power of more than 95% of the liver substance of the dog or monkey can be abolished, and yet the remaining 5% of the liver tissue will suffice to prevent the development of jaundice. In the human, atrophy of over half of the liver has been shown not to produce any noticeable jaundice. The fact that the liver possesses a much greater capacity for excreting bilirubin than it is called upon to use normally makes it clear that up to a certain limit an over-production of bilirubin will be taken care of without

the development of cutaneous jaundice. Just what this limit is
in the human being is not accurately known, but the numerous
pathological conditions in which the amount of pigment in the
stools is greatly increased above the normal in absence of jaundice
stand as evidence that marked increases in the amount of bilirubin
produced can be excreted by the normal liver well enough to
prevent visible jaundice. It is imaginable that with excessive
production over a long period of time the excretory power of the
liver may be impaired due to the strain put upon it. There is
no evidence that this occurs; "on the contrary one would believe
that an uncomplicated demand for increased work would, through the
process of work hypertrophy, increase the liver's efficiency,
as in the case of the other excretory organs, rather than depress
it. Overproduction of the pigment is ordinarily associated with
conditions other than mere overstrain which tend to depress the
excretory power of the liver (e.g. anoxemia, febrile disease,
immaturity of liver cells); thus it is believed that jaundice
rarely, if ever, results from simple overproduction of bilirubin."
(38)

The third possibility (liver damage to the extent that the
normal bilirubin production can not be excreted) undoubtedly
causes certain cases of jaundice. No jaundice is known to result
from a functional depression of the excretory power of the liver
without actual loss of cells through necrosis. There is no patho-
logical condition which is known to impair the function of the

living hepatic cells to such a degree that they are unable to prevent jaundice by excreting the amount of bilirubin normally found. If, however, the total amount of functioning liver substance is reduced by necrosis and cellular damage, jaundice will result wherever the loss of liver tissue reaches a point at which the activity of the cells remaining alive is insufficient for the proper removal of the pigment from the blood (e.g. acute yellow atrophy, yellow fever, chloroform poisoning). (38)

The fourth possibility (a combination of any of the preceeding possibilities) is the most common. A liver with reduced capacity for excreting bilirubin may be able to rid the blood of the amount of pigment formed normally; if bilirubin is produced in excess, such a liver may be quite unable to excrete it at all, and jaundice will result from the retention of the excess pigment. (38)

In line with the preceeding discussion, the clinician classifies jaundice as being hepatogenous or hemotogenous in origin. The hepatogenous jaundice is caused by the inability of the liver to excrete via the bile any or all of the bilirubin brought to it by the blood stream. The pathology of this process is found entirely within the liver. Rice (38) has termed this regurgitation jaundice. The cause of this jaundice is blockage of the bile passages so that bile cannot be excreted (calculus, inflammatory processes, or neoplastic disease). The commonly accepted view on the manner of origin of the jaundice is that after occlusion of the common bile duct the bile continues to be excreted by the liver

parenchyma, and since it is unable to follow its normal course to the duodenum, it is damned back into the delicate bile capillaries. The increase in tension in these very delicate structures causes them to rupture; the extravasated bile is absorbed either by the blood capillaries or by the lymphatics and is then distributed throughout the body, being in part deposited in the tissues and in part excreted by the urine. Bloom (4) has proven that the removal of bilirubin early in obstructive jaundice is via the lymphatics, then later by the blood. That is, before the bile canaliculi rupture, the bilirubin is resorbed by the lymph in the space immediately about the canaliculi and returned to the systemic system before rupture has taken place.

The hemotogenous jaundice has been termed retention jaundice by Rice (38). This implies merely an abnormal retention of the bilirubin within the systemic circulation. Basically it is concerned with a minimal amount of liver damage and a pronounced tendency for peripheral blood destruction (e.g. hemolytic icterus, any poison causing intravascular hemolysis).

Besides hepatic dysfunction due to hepatic disease, there is a type of constitutional nature. The constitutional type shows a light yellow or sallow tint to the skin, however, the conjunctiva is not colored. There is no demonstrable hepatic or splenic damage. The urine contains appreciable amounts of bilirubin and considerable amounts of urobilin. This condition has not been adequately explained, but it has been found in the

people falling within this group a tendency to hepatic disease, especially cholecystitis. (42)

Before continuing further in the field of jaundice it is necessary to reconsider some of the factors in the van den Bergh test for bilirubin. As will be remembered, van den Bergh got four different types of reactions with his reagent, namely the immediate direct reaction, the delayed direct reaction, the biphasic direct reaction, and lastly the indirect reaction. Van den Bergh was unable to discover the factor which underlies these different reactions, nor has this problem been solved up to the present time. A voluminous literature has accumulated which relates to this subject.

Van den Bergh (45), in 1918, found a direct reaction occurred with serum of a patient having obstructive jaundice (regurgitation type). He found that an indirect reaction resulted in a patient having hemolytic icterus (retention type of jaundice). Because of this and other work, van den Bergh suggested that there was some chemical difference between the two forms of pigment, or perhaps the pigment was modified by its attachment to the protein of the serum; the form present in the serum of the obstructive jaundice was probably circulating in a free state, while that giving the indirect reaction was bound with serum protein and, in order to detect its presence, had to be liberated from the proteins by the alcohol. Thus, van den Bergh believed that in the passage through the hepatic cells the bilirubin was changed chemically, thus

accounting for the difference in reaction in the obstructive and hemolytic types of jaundice.

Blankenhorn (10) found that there was present in bile and in many icteric sera a form of bilirubin which would pass through an animal membrane, showing that since it acted as a diffusate on dialysis, it must be in the crystalloid state. He found, however, that this was a relatively unstable form, for on repeated dialysis of the diffusates less and less would pass through the membrane, indicating that it readily reverted to a non-dialyzable or colloidal form. Collinson and Fowweather (11) applied this observation to the van den Bergh reaction, and found that the crystalloid form alone gave the direct positive reaction, while the non-dialyzable, or colloidal, form was direct negative, reacting with the diazonium salt only after the addition of alcohol and giving the indirect reaction. They conclude that the difference is largely in physical state; the direct negative being free bilirubin, a suspensoid colloid; the direct positive being a pure crystalloid.

Vaughan and Hubbard (46) after studying xanthochromic spinal fluid, concluded that the type of reaction depends upon the quantitative relationship between the pigment and protein. They found with material of low protein concentration that when the bilirubin exceeded 0.3 mg% the van den Bergh reaction became direct. They believe, therefore, it is not necessary to consider that passage through the liver cells is the determining factor that changes the indirect to the direct type of bilirubin. Gregory

and Andersch (12) have shown through clinical studies that as
high as 26 mg% of bilirubin can be found in spinal fluid with an
indirect van den Bergh; therefore they do not believe that the
quantity of bilirubin present in the sera determines whether the
van den Bergh will be direct or indirect in contradistinction to
Vaughan and Hubbard's work.

Fowweather (11) has reviewed the literature well on the van
den Bergh reaction, and the following are only a few of the many
theories put forward.

Davies and Dodds, 1926, believed that bilirubin was
responsible for the direct reaction, whereas an oxidation product
of bilirubin was responsible for the indirect reaction.

Roberts, 1928, put forward the view that bilirubin in
the free colloidal condition was the pigment of indirect sera, while
in direct sera the bilirubin was in combination with some substance
the nature of which is as yet undetermined.

Newman, 1928, opposed the view that the pigment of
indirect sera was free bilirubin and claimed that the pigment of
direct sera was a sodium hydrogen bilirubinate.

McGowan, 1930, considered that the pigments of direct
and indirect sera were identical, the types of van den Bergh
reaction being dependent on the alkali reserve of the blood;
indirect reactions were obtained when the alkali reserve was
diminished.

Snider and Reinhold, 1930, claimed that the type of

van den Bergh reaction was dependent on the concentration of the pigment which is present, direct reactions being associated with high concentrations of bilirubin, indirect reactions with low concentrations.

Kuster believes a keto-enol modification of bilirubin exists explaining the varied reaction.

Fowweather, 1926 and 1931, put forth the idea that the pigment of indirect sera was free bilirubin as an acid, while the pigment of direct sera was a salt of bilirubin, probably the ammonium salt.

Mann and Bollman (32) have shown that the van den Bergh reaction for serum bilirubin is indirect in animals that have become jaundiced following complete removal of the liver. The amount of bile pigment that accumulates in the blood for the first 24 hours after the removal of the liver is comparable in amount to that which is found after an equal interval of time following ligation of the common bile duct and extirpation of the gall bladder. In the latter case the bilirubin of the blood gives a direct van den Bergh reaction. Removal of the liver when obstructive jaundice is present produces serum bilirubin with a true biphasic reaction obtained by the van den Bergh method. If the liver is removed several hours after ligation of the common duct and removal of the gall bladder, at a time when a definite direct van den Bergh reaction is present in the serum, the amount of bilirubin reacting directly will be subsequently unchanged.

Additional bilirubin will accumulate in the blood after removal of the liver, so that the total amount of bile pigment in the blood will progressively increase at the same rate that it was increasing following ligation of the common bile duct, but the bilirubin that is added to the blood after removal of the liver gives only the indirect reaction. Thus Mann and Bollman's experiments show the relative importance of liver passage of bilirubin in relation to the way it will react to the van den Bergh reaction, but it also shows a possible mechanism for explaining the delayed direct and the biphasic reactions. McNee, 1922, (29) believes blood from obstructive jaundice would yield an immediate direct reaction, hemolytic jaundice would account for the indirect reaction, and jaundice due to a toxic agent and which, furthermore, may be due to a combination of obstructive and hemolytic types would account for a biphasic reaction.

Probably one of the most ingenious of the theories put out thus far is that of Barron's. (2) Because of its apparent rationality and the usefulness in explaining the various van den Bergh phenomena it is given in more detail than the other theories. It must be remembered, however, that this theory is not accepted by all. Barron, in contradistinction to van den Bergh, does not believe that the passage of bilirubin through the hepatic cells changes the bilirubin in any manner; but to him it has seemed more probable that there is some substance present in the bile which is responsible for the difference in the reaction. In other words, he believes

that the type of reaction depends upon the medium in which the
pigment is contained rather than upon any alteration of the pigment
itself. Barron has shown that, while pure bilirubin at the pH
of blood gives the direct or prompt action, if it be added to normal
plasma it then gives the indirect reaction. This Barron believes
to be the result of the adsorption of bilirubin by the plasma
proteins, the pigment being in that way prevented from reacting
promptly with the reagent. If, however, substances which have
the property of lowering surface tension are first added to the
plasma and then bilirubin is introduced, or if such substances
are added to the plasma at the same time as the bilirubin, the
reaction of the pigment remains direct. This occurs apparently
for the reason that substances which lower surface tension seem
to be adsorbed by proteins more readily than is bilirubin by
the plasma proteins, and it is significant that these substances
are present in whole bile and occur in increased amounts in the
plasma in various forms of jaundice in which the blood bilirubin
gives the direct reaction. Bile salts and acids have the faculty
of lowering surface tension and are the active principles in this
role in the case of bile. In obstructive or regurgitation jaundice,
whole bile, containing both bilirubin and bile salts, is regurgitated
into the systemic circulation. The bile salts collect at the
protein interface leaving the bilirubin free to react with the
diazo compound, giving a direct reaction.

When sodium bilirubinate solution giving a direct reaction is

added to normal serum in increasing amounts up to 12 mg%, a
typical indirect reaction takes place. When the concentration
is increased to 16 mg%, the reaction becomes of the biphasic type.
When the concentration is higher than 16 mg%, a direct reaction
is obtained. Barron believes that up to 12 mg% the bilirubin is
being adsorbed by the protein; from 12 to 16 mg%, small enough
amounts of bilirubin are left free from the protein interface, or
relatively free of this interface so that a biphasic reaction may
take place; from 16 mg% on upwards the bilirubin remains free in
the plasma and thus gives the direct reaction. The direct reaction
can be obtained from the first by adding bile acids which have
the surface tension lowering effect in the case of bile.

Whatever be the ultimate explanation of the different forms
of the reaction, a great amount of experimental and clinical in-
vestigation has made it perfectly clear that when the plasma
bilirubin in a case of jaundice is subjected to the van den Bergh
test, the direct reaction indicates that whole bile, containing
bile acids and cholestrin as well as bilirubin, has been regurgi-
tated into the blood stream, and therefore that one is dealing
either with obstruction of the ducts or with necrosis of the liver
cells, for these are the two conditions which permit bile to
escaped from the canaliculi into the blood; the indirect reaction,
on the other hand, informs us that the bilirubin in the plasma
under examination has not been regurgitated into the blood from
the caniliculi, but that it represents pigment which the liver has

not yet been able to remove from the blood stream. Bloom (4)
brings out a little recognized fact concerning obstructive jaundice
that gives very good proof of the above summarizing statement.
He found that bilirubin in early obstructive jaundice is removed
via the lymphatics surrounding the canaliculi before rupture of
these canaliculi ever takes place. During this period when the
bile pigment is thus being resorbed, the van den Bergh shows an
indirect reaction. He has shown histologically that ruptured
bile canaliculi are not found in the early stages of obstructive
jaundice. But as soon as the bile canaliculi have ruptured,
pouring whole bile into the lymphatic and blood streams, the van
den Bergh becomes direct positive.

The biphasic reaction is due to a small amount of direct
reacting pigment in plasma containing a large amount of indirect
reacting pigment. According to Barron's view, a biphasic reaction
would occur, for example, if bilirubin continued to be added to
the plasma, the proteins of which had already adsorbed all of the
pigment they were capable of holding. This form of reaction does
not yield the clear cut information which the pure direct or
indirect reaction yields, for it can occur as a result of a high
degree of pigment retention in the abscence of regurgitation, or
it may occur in certain stages of biliary regurgitation caused
by obstruction or by necrosis of liver cells. However considered
in relation to findings in the stools and urine in a given case,
it is frequently possible to determine whether the biphasic

reaction results from the retention or from the regurgitation
of bilirubin.

In contradistinction to the condition of jaundice, there
may exist a condition of hypobilirubinemia. This condition is
found in all secondary anemias where blood lost by hemorrhage is
not promptly replaced. It can also be associated with alterations
in the hematopoietic system (diminution of the number of red blood
cells thrown into the general circulation) which have as a conse-
quence a diminished production of bilirubin. Hypobilirubinemia
is found in chronic nephritis with cardiac complication, secondary
anemias, aplastic anemias, and chlorosis. Murphy, in 190 cases of
secondary anemia due to different conditions finds a persistant
hypobilirubinemia. This condition is due, then, to a general
lack of blood destruction in the body. This fact may be taken
as further proof of the derivation of bilirubin from hemoglobin.

The classical work on urobilin has been done by McMasters
and his co-workers already mentioned before. Besides studying
the normal physiology involved in urobilin, they have also included
pathological study that explains very nicely the abnormal physiology
occurring in the human patient.

He has shown (24) that urobilin is not formed normally unless
bilirubin reaches the large intestine. In further experimentation,
(26) urobilinuria has never found after liver damage except when
bile pigment was present in the intestine, Thus, for example,

it appeared during the first days after ligation of the common
duct, but disappeared as the stools became acholic. When this
had happened, a small amount of urobilin-free bile, given by mouth,
precipitated a prompt urobilinurea. After obstruction of the duct
from one-third of the liver, mild urobilinurea was found, but no
bilirubinurea. In animals intubated for the collection of a part
of the bile only, while the rest flowed to the duodenum through the
ordinary channels, liver injury caused urobilinurea, unless indeed
it was so severe as to lead to bile suppression, when almost at
once the urobilinurea ceased, though the organism became jaundiced.
He concludes that "urobilinurea is an expression of the inability
of the liver cells to remove from the circulation the urobilin
brought by the portal stream with the result that the pigment
passes on to the kidney and urine. Urobilinurea occurs with far
less degree of liver injury than does bilirubinurea."

McMasters (27) also found that animals rendered urobilin-free
by the collection of all the bile from the intubated, uninfected
common duct, remain urobilin free during and after extensive blood
destruction due to intravenous injections of hemolyzing agents
and also reinjections of the animals own hemolyzed blood. On the
other hand, when bile flow into the intestine is uninterrupted,
urobilinurea occurs during blood destruction caused in any of the
ways mentioned, and it parallels, both in severity and duration,
the destructive process. He concludes, "Urobilinurea, occurring
during blood destruction, is primarily the result of an increased

excretion of bilirubin from which, in turn, an unusually large
quantity of urobilin is formed within the intestine. The liver
fails to remove from the portal blood all of the latter pigment
which is resorbed and consequently some of it reaches the kidneys
and urine."

And again, McMasters (28) shows that experimental infection
of the intubated and previously sterile biliary tract of the dog
with particles of the stools leads to a formation of urobilin from
the bilirubin of the bile as it flows through the ducts. No
urobilinurea occurs, however, unless temporary biliary obstruction
is produced, or the liver parenchyma is injured. Then urobilinurea
develops, despite the fact that no bile is reaching the intestine
and, by corollary, no urobilin being formed there. "Cholangitic
urobilinurea, as one may term the phenomenon just described, to
distinguish it from the urobilinurea having origin in the pigment
absorbed from within the intestine is far more pronounced in animals
possessing a healthy gall bladder than in those with a pathological
gall bladder or with one prevented from functioning by severance
of the cystic duct. These facts suggest that there may be an
active absorption of urobilin from the normal gall bladder. There
can be no doubt that the pigment is absorbed from within the
bile ducts." He further states that there is no justification
for the belief that urobilin is ever formed through the action of
the liver parenchyma.

In summarizing this work on urobilinurea, we may say;

urobilinurea occurs in excessive hemolysis and whenever there is
an excessive excretion of bile pigments by the liver (as when a
stone suddenly becomes dislodged from the common duct allowing a
large amount of bile to empty into the gut at once); no urobilinurea
occurs with obstruction of the common bile duct since the bilirubin
must reach the intestine to be changed into urobilin, but when
hepatic cells are affected by bacteria or toxins, urobilin may be
formed in the bile ducts themselves resulting in urobilin being
formed within the liver itself. The excretion of urobilin from
the portal blood stream is one of the most delicate functions of
the liver, and is one of the first functions to become impaired
in a pathological process; whereas, the liver may still be function-
ing well in regards to bilirubin excretion, yet some increased
quantity of urobilin may be escaping the liver and be showing
in the urine. Thus, the test for urobilin in the urine is a good
functional test of the liver and rather delicate in nature, too,
and may be one of the first signs of liver dysfunction.

An attempt will now be made to correlate those factors brought
out in the normal and abnormal metabolism of bilirubin and show
their application in clinical medicine.

Being presented with a clinical case of jaundice, one may
usually come to an accurate diagnosis by studying the type and
amount of the biliary pigments within the blood, the urine, and
the stools. By studying these factors, one can determine whether

or not the jaundice is of the regurgitation type or of the retention type. A summary of the characteristics of these two types of jaundice will be given at this point.

Retention jaundice, in its pure form, whatever the etiology, is recognizable clinically by the following characteristics: 1) The bilirubin of the plasma, never having passed through the liver cells and being, therefore, unmixed with the constituents of the bile, gives the indirect van den Bergh reaction; 2) The stools contain an increased amount of urobilin because, in the face of the increased production of bilirubin the liver is excreting more of the pigment than normally, even though it cannot excrete enough of the excess to prevent jaundice; and 3) The urine contains an increased amount of urobilin but no bilirubin; (it is not yet clearly understood why only the direct reacting bilirubin can escape readily from the blood to the urine; but if it be true that the indirect reacting bilirubin is bound to plasma protein while the direct reacting bilirubin is not, it is clear that the latter form could be excreted more readily by the kidneys than the former); and again, the urine will be free from bile salts because bile salts are formed in the liver and are absent from the urine unless they have been poured into the blood by the escape of whole bile from the caniliculi into the blood stream. Pathologically, the liver may show cloudy swelling, or atrophy, according to the etiological reason for their mal-function, but there is no widespread necrosis; and the bile ducts are patent.

Regurgitation jaundice, on the other hand has the following characteristics: 1) Plasma bilirubin gives the direct van den Bergh because the bile canaliculi are altered in such a way that whole bile escapes into the blood stream; 2) The stools contain less urobilin than normally since no bile or very little bile is getting into the intestine--the acholic stool; and 3) The urine contains bilirubin, because the direct plasma reacting bilirubin escapes into the urine. Many clinicians have noted a bradycardia and a pruritis accompanying regurgitation jaundice. At first these symptoms were attributed to the hyperbilirubinurea. But recent work of Horrall and Carlson and of Still (19), in which pure bilirubin spectro-photometrically identical with circulating serum bilirubin was injected intravenously, has found bilirubin to have no toxic effects. The toxic effects of jaundice were found to be due to bile salts mainly. The bradycardia (according to Stewart and King) is due to the bile salts and acids which act by increasing the tonus of the vagus nerve.

The following chart was taken from Rich (38) and summarizes well the differentiating points of the two types of jaundice:

TYPE	(VAN DEN	URINE			(FECES
	(BERGH	(Bilirubin	(Bile Salts	(Urobilin	(Urobilin
Retention jaundice	Indirect	0	0	≠	Increased
Regurgitation jaundice	Direct	≠	≠	0 or ≠	Decreased

"Into these two main groups all various forms of jaundice can be rationally and helpfully placed from the standpoint of pathogenesis, for the results of laboratory findings, as listed above, will enable one to determine the fundamentle mechanism responsible for the jaundice in a given case and will permit one to form a judgement regarding the general type of anatomical alteration present in the liver." (Rice, 38)

We may now mention some of the other clinical applications that are concerned with bilirubin metabolism.

Perkin (36) believes that a routine check of the van den Bergh of patients taking arsenicals affords the earliest possible indication of damage to the liver. If a record of the blood bilirubin is made at the commencement of the treatment and repeated at regular intervals during the course, one may proceed with safety as long as the reading remains normal. A rise of a few mgs. is significant and the patient should be treated accordingly. Any considerable rise calls for immediate cessation of treatment. Once an arsenical hepatitis is established, determination of the bilirubin offers a reliable evidence concerning the severity and progress of the lesion.

Bochner-Mortensen (5), from his results and comparison with the results of other liver function tests, concludes that in its limited field of application, the bilirubin tolerance test is a sensitive test of the liver function. While inapplicable in hepatic disorders with bilirubinurea, it is useful in the diagnosis

of cirrhosis and in evaluation of the condition of the liver in chronic hepatitis, cholangeitis, and in examination of possible injury of the liver in infectious diseases and different disorders such as diabetis, exophthalmic goiter and certain skin and brain disturbances. It may prove to be especially suitable for the investigation of possible lasting functional disorders of the liver following acute hepatitis.

Rozendal (42) states that the mechanism for secretion of bilirubin is a sensitive one. It is influenced by emotion and by disturbances of the sympathetic nervous system, as well as by very minor degrees of hepatic injury. An increased concentration of bilirubin may be the one laboratory evidence of dysfunction and it should be used more widely. It will in all probability disclose many unsuspected cases of mild functional and organic disturbances of the liver as well as a group of individuals of a constitutional type especially susceptable to disease of the gall bladder.

The diagnostic value of hypobilirubinemia in the differentiation of pernicious anemia from aplastic or secondary anemia is now a well known procedure. (2) The increase of urobilogen in pernicious anemia associated with an icteric tint of the sclera and skin and often a palpable liver presents difficulty at times in the differentiation between hepatic disease and primary anemia. Aside from blood morphology, the examination of the feces indicates an increased pigmentation to a dark brown color in contradistinction to the light

or acholic feces accompanying liver disease. In the anemias, bile is never found in the urine, while in hepatic diseases associated with icterus, bile is always present.
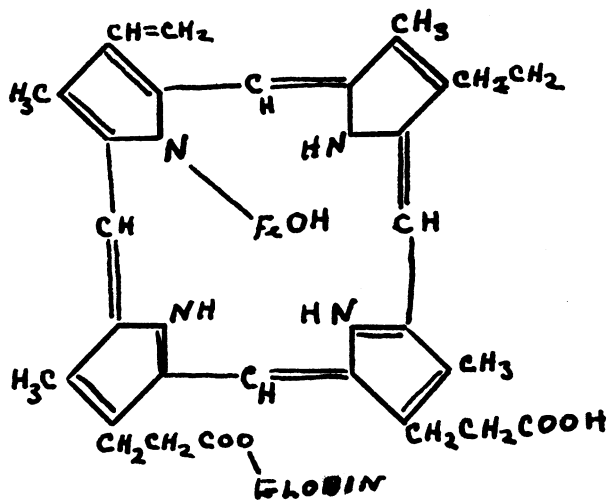
The constant presence of urobilogen in catarrhal jaundice, and its total absence in mechanical icterus, such as encountered in carcinoma of the head of the pancreas and of the biliary tract, forms a marked distinguishing diagnostic feature between this benign form of jaundice and those due to malignant causes. The presence of urobilogen, even in traces, during the state of jaundice promptly rules out an obstruction or mechanical icterus.

Bilirubin metabolism has been studied more carefully than any other function concerned with the liver to the present date. Knowledge of this has been a great aid in diagnosis of liver dysfunction, but can not be relied upon too much. Quite a bit of confusion is present regardless of the nice theories that have been concocted to explain the biphasic and delayed direct van den Bergh tests. These results occur quite frequently in cases of jaundice and are of little diagnostic value in single tests, but when repeated over some time, they often change to a direct or true indirect reaction. (47) However, great aid is obtained by studying the constituents of the feces and urine. The tendency has been to shy away from the use of the van den Bergh test and to rely upon the lactose tolerance tests and such related tests. These newer tests certainly have their place since they test an altogether different function of the liver. But, in the light
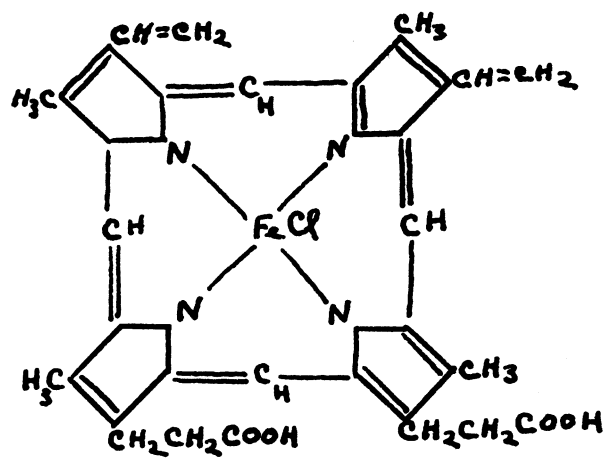
of our present knowledge, the tests for bilirubin and urobilin in the blood, urine and feces are still important steps in diagnosing a given case of jaundice.

Recent experiments with bilirubin have offered what may be a truely great advance in our knowledge of the purpose of bilirubin. To date it has been considered only as a waste product of hemoglobin metabolism. Najib-Farah (35) working on pneumococcal infections in rabbits noted that the rabbit sera contained no bilirubin. Knowing the lytic action of bile on the pneumococcus, he added bilirubin to the rabbit sera. Whereas before the pneumococci grew rapidly in the sera, the growth of the virulent pneumococci was inhibited by the addition of bilirubin. The organisms undergo agglutination and disintegration (partial and complete lysis), the capsules being denuded of their contents. The possibility of bilirubin being an aid to body defense in certain infections is indeed a new concept--the possibility that bilirubin may be an agglutinin and a lysin in vivo is interesting, and certainly is a line of thought that is worthy of further study.
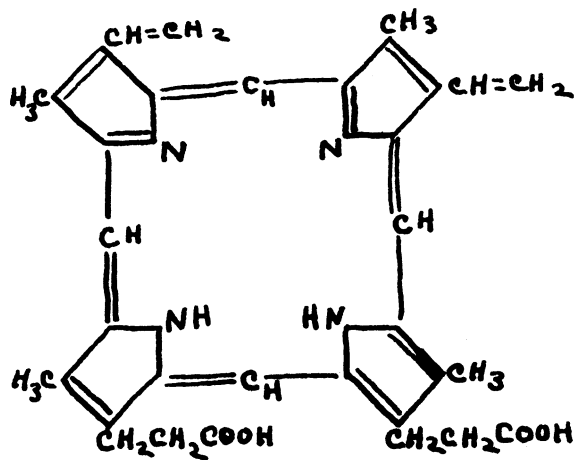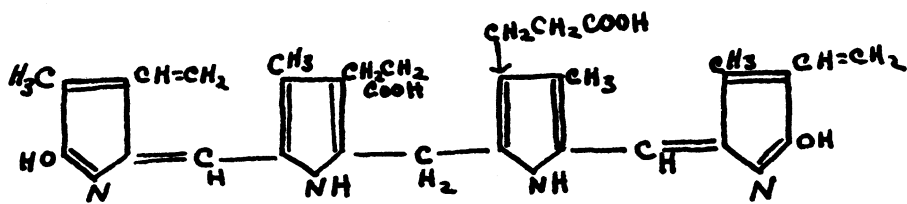
* * * * * * * * * * *

HEMOGLOBIN

HEMIN

PROTOPORPHYRIN

Labels: $CH=CH_2$, CH_3, H_3C, $CH=CH_2$, CH, N, N, CH, CH, NH, HN, CH, H_3C, CH_3, CH, CH_2CH_2COOH, CH_2CH_2COOH

BILIRUBIN

Labels: H_3C, $CH=CH_2$, CH_3, CH_2CH_2COOH, CH_2CH_2COOH, CH_3, CH_3, $CH=CH_2$, HO, CH, N, NH, CH_2, NH, CH, OH, N

UROBILINOGEN

Labels: H_3C, C_2H_5, CH_3, CH_2CH_2COOH, CH_2CH_2COOH, CH_3, CH_3, C_2H_5, HO, NH, CH_2, NH, CH_2, NH, CH_2, OH, NH
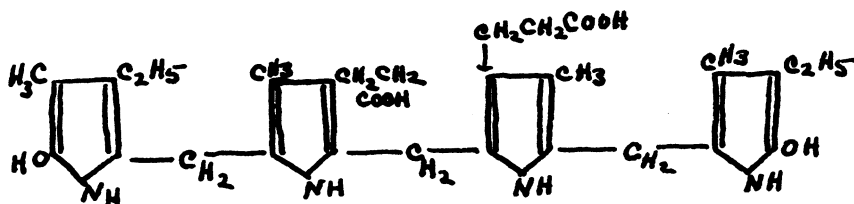
BIBLIOGRAPHY

1. Andrewes, C. H. The Nature of the Difference between the Bilirubin of Obstructive and Hemolytic Jaundice, Br. Jr. Exp. Path. 5:213-219, 1924

2. Barron, E. S. G. Bilirubinemia, Medicine, 10:77-133, 1931

3. Blankenhorn, M. A. On the Absorption of Bile Pigments from the Intestines, J. Exp. Med. 45:195-203, 1927

4. Bloom, William. The Role of Lymphatics in the Absorption of Bile Pigment from the Liver in Early Obstructive Jaundice, Johns Hopkins Hosp. Bul. 34:316-320, 1923

5. Bochner-Mortensen. Bilirubin Tolerance Test, J.A.M.A. 103:1188, Oct., 1935

6. Bodansky, M. Introduction to Physiological Chemistry, 234-245, New York, John Wiley & Sons Inc., 1934

7. Boyd, William. The Pathology of Internal Diseases, 353-368, Philadelphia, Lea & Febiger, 1935

8. Corr, Phillip. Histochemical Evidence Concerning the Site of Formation of Bile Pigment, Arch. Path., 7:84-93, 1928

9. Downey, Hal. Handbook of Hematology, Vol. IV, 2445-2580, New York, Paul B. Hoeber Inc. of Harper & Bros., 1938

10. Elton, N. W. Physiology, Correlations and Technic of the van den Bergh Reaction, Icterus Index, and Quantitative Serum Bilirubin, J. Lab. & Clinic. Med., 17:1-13, 1931

11. Fowweather, F. S. Bilirubin and the van den Bergh Reaction, Biochem. Journ., 26:165-182, 1932

12. Gregory, Raymond, & Andersch, Marie. The Influence of the Quantity of Serum Bilirubin on the Type of van den Bergh Reaction, J. Lab. & Clinic. Med., 23:572-74, 1938

13. Haldeman, K. O. A Histologic Study of the Formation of Bile Pigment, Archives of Path., 7:993-1011, 1929

14. Heilbrun, N., & Hubbard, R. An Experimental Study of the Biphasic van den Bergh Reaction. Am. Jour. of Clin. Path., 8:273-280, 1938

15. Hewlett, Albion W. Pathological Physiology of Internal Diseases, 326-336, New York & London, D. Appleton & Co, 1928

16. Hoover, C. F., & Blankenhorn, M. A. Dissociated Jaundice, Arch. Int. Med., 18:289-308, 1916

17. Horral, O. H., & Carlson, A. J. The Toxic Factor in Bile, Am. Jour. Physiology, 85:591-606, 1925

18. Howell, William H., A Textbook of Physiology, 864-887, Philadelphia, W. B. Saunders Comp., 1936

19. Ivy, A. C., Physiologic Disturbances Incident to Obstructive Jaundice, J.A.M.A., 95:1068-1072, 1930

20. Kuhn, A., Hawkins, W. B., & Whipple, G. H.
        I. Bile Pigment and Hemoglobin Interrelation in Normal Dogs, Am. J. Physiol. 96:449-463
        II. Bile Pigment and Hemoglobin Interrelation in Anemic Dogs, Ibid. 96:463-476.

21. Legg, J. W. On the Bile, Jaundice, and Bilious Diseases, New York, D. Appleton & Co., 1880

22. Lemberg, R. Bile Pigments, Biochem. Journ. Vol XXVIII, No. 3:978-987, 1934

23. Lemberg, R. Transformation of Haemins into Bile Pigments, Biochem. Journ., Vol XXIX, No. 6:1322-1336, 1935

24. McMaster, P. D., & Elman, Robert., Studies on Urobilin Physiology and Pathology.
        I. Quantitative Determination of Urobilin
        II. Derivation of Urobilin
J. Exp. Med., 41:503-534, 1925

25. McMaster, P. D., & Elman, R. Studies on Urobilin Physiology and Pathology.
        III. Absorption of Pigments of Biliary Derivation from the Intestine, J. Exp. Med., 41:719-738, 1925

26. Ibid
        IV. Urobilin and The Damaged Liver, J. Exp. Med., 42:99-122, 1926

27. Ibid
        V. The Relation between Urobilin and Conditions involving Red Blood Cell Destruction., J. Exp. Med., 42:619-640, 1926.

28. McMaster, P. D., & Elman, R.   Studies on Urobilin
Physiology and Pathology,
          VI. The Relation of Biliary Infections to the Genesis
and Excretion of Urobilin, J. Exp. Med., 43:753-783, 1926.

29. McNee, J. W., Discussion on Jaundice, Brit. Med. Journ.,
2:495-498, 1924

30. McNee, J. W. Experiments on Hemolytic Icterus, J.
Path. & Bact., 18:325-340, 1914

31. Mann, F. O., Sheard, C. H., & Bollman, J. L., The
Extrahepatic Formation of Bilirubin, Am J of Physiology, 74:49-60,
1925

32. Mann, F. C., & Bollman, J. L., Jaundice--Review of
Some Experimental Investigations, J.A.M.A. 104:371-374, 1935

33. Mann, F. C., & Bollman, J. L., Jaundice: A Review of
Some Experimental Investigations, JA.M.A. 104:371-374, 1935

34. Mendel, L. B., & Underhill, F. P., On the Paths of
Absorption from the Liver, Am. J. Phys. 14:252-259, 1905

35. Najib-Farah. Defensive Role of Bilirubinemia in
Pneumococcal Infections, Lancet, p. 505, 1937, Feb.

36. Perkin, F. S. Blood Bilirubin Estimation and Clinical
Significance. Arch. Int. Med. 40:195-203, 1927

37. Rabinowitch, I. M., Jaundice and the Application of
the van den Bergh and Urobilinogen Tests, Canadian Med. Assoc.
Journ., N.S.25:255-265, 1931

38. Rich, Arnold Rice, The Pathogenesis of the Forms of
Jaundice, Johns Hopkins Hosp. Bull., 47:338-377, 1930

39. Rich, A. R., The Formation of Bile Pigment, Physio.
Reviews, 5:182-224, 1925

40. Rich, A. R., The Formation of Bile Pigment from
Hemoglobin in Tissue Culture, Bull. Johns Hopkins Hosp. 35:415-435,
1924

41. Rich, A. R., Identity of Hematoiden and Bilirubin,
Bull. Johns Hopkins Hosp. 36:225-238, 1925

42. Rozendal, H. M., Comfort, M. W., & Snell, A. M.
Slight and Latent Janudice, J.A.M.A. 104:374-382, 1935

43. Sackey, M. S., Johnston, C. G., & Ravdin, I. S.,
Fate of Bilirubin in the Small Intestine, J. Exp. Med., 60:189-194, 1934

44. Still, E. U., On the Toxicity of Purified Bile
Preparations, Am. Jour. Phys., 88:729-737, 1929

45. Van den Bergh, Hijmans, Discussion on Jaundice, Brit.
Med. Jour., 2:498-500, 1924

46. Vaughan, J. M., & Haslewood, G. A. D., The Normal
Level of Plasma Bilirubin, Lancet, 1:133-135, Jan., 1938

47. Wallace, C. B., & Diamond, J. S., Significance of
Urobilogen in the Urine as a Test for Liver Function., Arch.
Int. Med., 35:689-725, 1925

48. Watson, C. J. Studies of Urobilinogen, Arch. Int.
Med., 59:196-205, 1937

49. Whipple, G. H., Hemoglobin and Bile Pigment Studies
in the Splenectomized Dog, Arch. Int. Med., 29;711-720, 1923